INTERNATIONAL KILMER CONFERENCE PROCEEDINGS

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Volume VIII

Editors:

John B. Kowalski, Ph.D.
Robert F. Morrissey, Ph.D.
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Preface

The Eighth International Kilmer Memorial Conference expanded on a number of key themes from the previous Conference, including prions and BSE, and introduced a critical new issue, bioterrorism. The state of infection control in the Pacific Rim was thoroughly reviewed as was the area of viral infections, particularly hepatitis. The Conference again benefited from the leadership of Nobel Laureate Joshua Lederberg as General Chairman.

The Conference focused on five themes: Bioterrorism, BSE/Prions, Infection Control: Pacific Rim and Emerging Issues, Viral Infections, and Viral Hepatitis. The program consisted of 29 speakers from six countries. The speakers and participants at the Conference represented a number of diverse groups: the Centers for Disease Control and Prevention, the National Institutes of Health, the Environmental Protection Agency, academia, medicine, and the healthcare industry.

A highlight of the Conference was the presentation of two Kilmer Awards. The first recipient was Dr. Hiroyoshi Kobayashi, Honorary President, NTT Kanto Hospital, *for international leadership in advancing the research and the practice of infection control*. Dr. Kobayashi has made many notable and significant contributions to the field of infection control which have improved not only the safety of patients but that of healthcare practitioners as well. In combining his research with day-to-day hospital procedures for the practice of infection control, he has had a tremendous influence on regulatory bodies, hospital management, infection control practitioners, researchers, and industry.

The second award was presented to Mr. John Masefield, retired Chairman of Isomedix, Inc., *for international leadership in advancing the science and practice of radiation sterilization*. Mr. Masefield pioneered the concept of contract radiation sterilization building a company that processes millions of cubic feet of healthcare products annually. He also provided critical support to the development of the modern methods for validation of radiation sterilization processes. This dual contribution was critical to the acceptance and growth of this method of sterilization.

New Brunswick, NJ

J.B. Kowalski, Ph.D.
R.F. Morrissey, Ph.D.

Conference General Chairman
Joshua Lederberg, Ph.D.
The Rockefeller University, U.S.A.

Opening Ceremony

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Joshua Lederberg, Ph.D.
The Rockefeller University, U.S.A.

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Centers for Disease Control and Prevention, U.S.A.

Welcoming Remarks

Joshua Lederberg, Ph.D.

The Rockefeller University, U.S.A.

May I join Mr. Lenehan in welcoming you to the Eighth International Kilmer Memorial Conference. I am happy to recall my pleasure in participating in the Seventh Conference 5 years ago in Scottsdale, Arizona.

It gives pause to reflect on what's happened in the realm of infection control in this 5-year period. Just on the heels of the influenza H5N1 outbreak in Hong Kong and its exemplary management there, we've had several other viral outbreaks around the globe. Jim Hughes will describe these in some detail. I just remind you that with the last to join the list — severe acute respiratory syndrome (SARS) — leaves us still waiting to see if a new season will bring a recurrence. The deplorable state of AIDS, although somewhat mitigated by antiviral chemicals, is a challenge and a humbling one regarding the limited capacity of medical science to develop remedial vaccines. Prions remain as puzzling as ever in terms of their basic biological mechanisms, not to mention their therapeutic management.

On the positive side, of course, we have achieved astounding leaps in genomics. Scores of pathogens have now been sequenced. We have almost bewildering, but illuminating, new insights into pathogenic mechanisms. We're quite confident these are going to help us generate new tools if we simply apply ourselves to their implementation. A shadow on this is the near abandonment of the development of antimicrobials on the part of most big pharmaceutical companies and I want to commend the very notable exception of Johnson & Johnson. Johnson & Johnson has historically been the prime mover in the universal adoption and implementation of the doctrine of reliable sterilization that we attribute to Joseph Lister.

I have a modest plea for the next century. Perhaps with more knowledge of the intricate ecology of our relationship to the microbial world, we'll think more of domesticating that microbial world than necessarily eradicating it, which is a hopeless enterprise in the long run. I propose enhancing an understanding and utilization of what I call the microbiome. This is the myriad of additional genomes that share our body space — the bugs that inhabit our skin, mucous membranes, and gastrointestinal tract — and which undoubtedly play a significant role in how we combat or succumb to disease.

This harmonization, akin to the globalization of the economy, may well distinguish the 21st Century.

Opening Remarks

James T. Lenehan

Johnson & Johnson, U.S.A.

It is a privilege for me to welcome such a distinguished group of delegates, speakers, and past Kilmer Award recipients to the Eighth International Kilmer Memorial Conference. What a pleasure to be here in Osaka – a vibrant city, noted for its commerce, culture, and fine cuisine.

First of all, I want to thank the Japanese Ministry of Health, Labor, and Welfare and the Japanese Society of Medical Instrumentation for their endorsement of this Conference.

The objective of our time together in Osaka is consistent with prior Kilmer Conferences, namely, to provide a forum for the sharing of scientific information that has a direct bearing on the health and well-being of people around the world, with special emphasis on the prevention of infection through diagnosis and intervention in the disease process.

This week's program consists of 29 invited speakers from 6 countries, representing academia, government, medicine, the healthcare industry, and such diverse organizations as the Centers for Disease Control and Prevention (CDC), the National Institutes of Health (NIH), and the Environmental Protection Agency (EPA).

We thank Professor Joshua Lederberg for agreeing to serve as Conference General Chairman and for the participation of our keynote speaker, Dr. James Hughes, the Assistant Surgeon General and Director of the National Center for Infectious Diseases in the United States (U.S.).

This Conference is actually the tenth undertaking that Johnson & Johnson has been associated with over a span of 30 years devoted to the theme of infection control – a subject that has its roots deep in the history of our enterprise.

Many of you may be wondering why Johnson & Johnson would sponsor such an event. Beginning as it did in 1886, in the infancy of modern medicine, Johnson & Johnson joined physicians in the struggle against disease and infection. It was a time when life itself was fragile and the most modest medical advances reaped huge rewards in terms of lives saved. Probably no area of human progress has made such remarkable gains over the past 117 years as has medical science – going as it has from virtual ignorance to vast knowledge of the human body and now its genetic foundation. Contributing to that progress, Johnson & Johnson developed the first ready-to-use surgical dressings in the mid-1880s which marked the first practical application of the theory of antiseptic wound treatment.

Years later, in the mid-1950s, our Ethicon division pioneered the first industrial application of ionizing radiation for the sterilization of medical products by using an electron accelerator to sterilize surgical sutures. More recently, Ethicon Endo-Surgery advanced minimally-invasive surgical procedures to reduce surgery time and thus the potential for

nosocomial infections. As a corporation, we continue to develop new sterilization technologies and equipment to meet the surgical requirements of the future.

At Johnson & Johnson, we have a long-term outlook in managing the business and are guided in our everyday conduct and social responsibility decisions by a Corporate Credo. The Credo, originally written by General Robert Wood Johnson in the 1940s, is a reminder of our responsibilities to 4 constituencies: our customers, our employees, the communities in which we live and work, and our stockholders.

Against that backdrop you can better understand why we are pleased to be able to help provide this forum for the exchange of knowledge about improved healthcare through infection control.

Around the world, people have come to expect to live longer and healthier lives. Few scientific meetings are capable of having a direct impact on these expectations. This Conference, however, is an exception. As a group, you have great potential to positively impact human health and well-being.

At the last Kilmer Memorial Conference held in 1998 in Scottsdale, Arizona, it was pointed out that infectious diseases are the leading cause of death worldwide. As the new century begins, we continue to be challenged by newly emerging infections and the decreasing effectiveness of our antibiotic arsenal.

In a recent report, “Addressing Emerging Infectious Threats,” the CDC notes that almost 50,000 children and adults die every day from infectious diseases, the world’s leading cause of premature death. Of approximately 52 million deaths from all causes in 1995, more than 17 million were due to infectious diseases, including about 9 million deaths in young children.

New microorganisms capable of causing disease in humans continue to be detected. There is a seemingly endless chain of events that result in the ease of transmission between animals and people and among people. Some of the new agents detected in the past 25 years and the threat of bioterrorism are now genuine public health problems on a local, regional, or global scale.

It is clear that infectious disease agents do not respect geographic or political boundaries. The virus that infects a poor villager in South America or a young child in India can also cause havoc among residents in Los Angeles, Boston, Paris, Moscow, or Osaka.

In just the past year, many parts of the world experienced a new disease called SARS. Severe acute respiratory syndrome (SARS) is a respiratory illness that was first reported in Asia in February 2003. In early March, the World Health Organization (WHO) issued a global alert about SARS. Over the next few months, the illness spread to more than 2 dozen countries in North America, South America, Europe, and Asia. By late July no new cases were being reported and the illness was considered contained. According to WHO, 8,437 people worldwide became sick and 813 died during the course of this outbreak.

The cost of emerging infectious diseases on a global basis is so high that it cannot be calculated accurately. For example, the cost in lives lost and the economic impact of the AIDS pandemic alone is staggering. It is estimated that, since the start of the epidemic, 30.6 million people worldwide have become HIV infected and nearly 12 million have died from AIDS or AIDS-related diseases. Among the countries of China, Russia, and India, it is

estimated that there will be 66 million cases by 2025. The U.S. this year committed 15 billion dollars to help Africa combat AIDS.

According to the U.S. Institute of Medicine, for every U.S. dollar spent on healthcare in poor countries, less than 10 cents currently goes to controlling infectious diseases, although infectious diseases cause nearly 30% of deaths in poor countries. Only 1.5% of the foreign aid received by these countries is targeted toward the control of infectious diseases.

Dr. James Hughes will describe CDC's plan for preventing emerging infectious diseases, part of which will have countries in all regions of the world participating in a global system for surveillance and response to infectious agents resistant to antimicrobial drugs. This effort will be undertaken in partnership with the World Health Organization and other organizations and agencies around the world. In addition, CDC promotes the enhancement of the public health infrastructure, which will help prepare the U.S. and the world to respond to bioterrorism incidents.

Clearly, there is much more work to be done to stay abreast of disease-causing microorganisms and their all too real threat to human health. I hope that this Conference helps meet the challenge, not only from the formal presentations, but also from the opportunity to exchange ideas face-to-face with your scientific colleagues from around the world.

My best wishes for a successful meeting!

Thank you.

Emerging Global Microbial Threats

James M. Hughes, M.D.

Centers for Disease Control and Prevention, U.S.A.

Introduction

Infectious diseases present increasing challenges to public health. The World Health Organization (WHO) estimates that nearly 15 million (26%) of the 57 million deaths that occurred throughout the world in 2002 were caused by microbial agents [24]. Leading the list are lower respiratory infections, responsible for 3.7 million deaths per year, followed by HIV infection/AIDS (2.8 million), diarrhea (1.8 million), tuberculosis (1.6 million), and malaria (1.2 million) [24]. While these statistics clearly highlight the continued threat of infectious diseases, the last half of the 20th century was largely characterized by a sense of complacency toward the risk of such health threats among persons in developed countries. In a 1992 report entitled *Emerging Infections: Microbial Threats to Health in the United States*, the Institute of Medicine (IOM) highlighted this complacency and issued a strong caution on the threats posed by infectious diseases and the need to rebuild the nation's public health system [9]. The recommendations in this report have served as a framework for the Centers for Disease Control and Prevention's (CDC) infectious disease programs for the last decade. Although much progress has been made, especially in the areas of strengthened surveillance and laboratory capacity, much remains to be done.

In the last 10 years, infectious diseases have been emerging and reemerging at alarming rates. The 1993 outbreak of hantavirus pulmonary syndrome in the United States (U.S.), the emergence of West Nile encephalitis in Russia and the U.S. in 1999, and the 2003 global outbreak of severe acute respiratory syndrome (SARS) clearly indicate the ability of microbes to emerge and spread globally. In addition, the emergence of new strains of diseases such as influenza and the increasing problem of antimicrobial resistance are vivid reminders of the ability of microbes to adapt and survive. In today's highly connected world, infectious disease outbreaks can spread globally in a matter of hours, impacting national security and the global economy and requiring a rapid and coordinated response to limit their spread.

In March 2003, the IOM published an update to its 1992 report on emerging infections [17]. The new report, *Microbial Threats to Health: Emergence, Detection, and Response*, cites additional health threats such as bioterrorism that have surfaced in recent years. The report also describes a host of factors – broadly categorized as genetic and biological factors; physical environmental factors; ecological factors; and social, political, and economic factors – that can work singly or in concert to produce global microbial threats. The new IOM report includes a series of conclusions and recommendations to address these threats. Leading the list are recommendations for increased response capacity and strengthened domestic and global infectious disease surveillance.

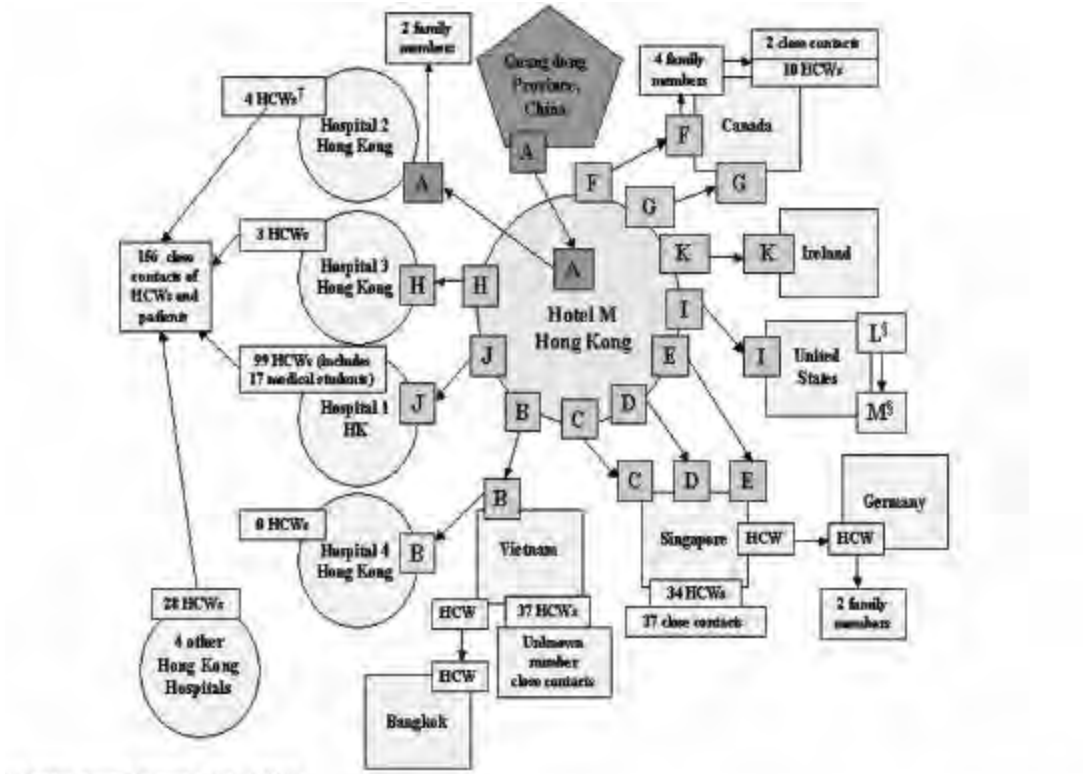
Microbial Threats to Health

Severe Acute Respiratory Syndrome

In the weeks following the release of the new IOM report, the importance of the committee's findings and recommendations was underscored as an unprecedented worldwide effort was launched to determine the cause and contain the spread of a new microbial threat to health: SARS. The first indication of the outbreak came on February 10, 2003, through a report posted on ProMed, an informal, online infectious disease reporting program of the International Society for Infectious Diseases [12]. The following day, Chinese officials notified WHO that 305 cases of severe atypical pneumonia, including 5 deaths, had occurred among persons in Guangdong Province since early November 2002. The global spread of the disease was sparked on February 21, when a Guangdong physician traveling while ill spent 1 night in a Hong Kong hotel. This individual would infect more than a dozen other hotel guests and visitors [4], who would subsequently serve as index patients for outbreaks in Hong Kong, Vietnam, Singapore, and Canada (Figure 1).

On March 12, WHO issued a global health alert and established a network of laboratories to determine the cause of the new disease [25]. In less than a month, 3 laboratories working within this network found evidence of a previously un recognized coronavirus in patient samples [8,14,19]. A fourth laboratory successfully completed studies fulfilling Koch's postulates with the new SARS-associated coronavirus (SARS-CoV) [10]. A few weeks later, 2 laboratories had sequenced the complete genome of the virus [15, 21].

The worldwide response to SARS involved unparalleled collaborations among scientists and health officials throughout the world. WHO's leadership and coordination of the global response enabled CDC and many other organizations and individuals to provide international assistance and share critical information that helped to minimize the spread of SARS. CDC's response to the outbreak involved more than 800 individuals throughout the agency, including nearly 90 individuals deployed to assist internationally. As information about the illness evolved, it was rapidly disseminated through CDC's website, regular press conferences, and a global videoconference held in collaboration with WHO. Frequent, sometimes daily communications and teleconferences were held with state epidemiology and laboratory personnel and with clinicians, the research community, and professional organizations and groups in the U.S. and Canada. Part of this communication effort involved the distribution of more than 2.7 million health alert notices translated in 7 languages to airline passengers traveling to or from SARS-affected areas and to travelers at selected U.S.-Canadian land crossings. These notices informed passengers of possible symptoms of SARS and of the need to contact and inform their physician of their travel should they become ill.



* Data as of March 28, 2003.
† Health-care workers.
§ Guests L and M (spouses) were not at Hotel M during the same time as index Guest A but were at the hotel during the same times as Guests G, H, and I, who were ill during this period.

Figure 1. Chain of transmission among guests at Hotel M–Hong Kong, 2003*

In the U.S., state and local health departments reported a total of 344 suspect and 74 probable cases to CDC. These numbers would subsequently be revised following a change in the U.S. SARS case definition which allowed for exclusion of cases whose convalescent serum specimens (collected more than 28 days after illness onset) tested negative for evidence of SARS-CoV infection [5]. Excluding these SARS-CoV negative cases provides a more accurate indication of the epidemic in the U.S. The revised total of SARS cases in the U.S. through October 1, 2003, was 134 suspect and 19 probable, including 8 confirmed. The 8 laboratory-confirmed cases included 7 individuals who reported having traveled to SARS-affected areas; the eighth case occurred in the wife of one of these individuals. Although the wife had also traveled to a SARS-affected area, she did not have illness onset until 13 days after departing, suggesting that she acquired her illness from contact with her husband.

Although much has been learned about this new illness, many questions about the virus and the illness remain unanswered and have important implications for prevention efforts. Major research concerns include identifying transmission risks, including factors contributing to super-spreading events such as occurred in the Hong Kong hotel; developing rapid, sensitive, and specific diagnostic tests; conducting studies to increase understanding of the pathogenesis of the virus; and developing effective therapies and vaccines.

Similarly, several factors have implications for recurrence and must be considered in SARS preparedness efforts. Although current research suggests an animal reservoir for SARS-CoV [11,16], the exact source of the virus is unknown. Other potential sources for recurrence include laboratory infection, as occurred in Singapore [23] (and more recently in

Taiwan and China), and transmission from persons with persistent and/or missed infections, although studies to date have found no evidence of chronic infection. Additional factors possibly affecting recurrence include the rate of evolution of the virus and whether the illness is seasonal, as is the case for many other respiratory diseases.

Preparing for a possible recurrence of SARS will require a coordinated global effort similar to that undertaken in response to the initial 2003 reports. CDC has established working groups to prepare for the possible return of SARS with active and ongoing consultation and collaboration with other federal partners, state and local health officials, and professional organizations and societies. Through these working groups, CDC has developed a guidance document, *Public Health Guidance for Community-Level Preparedness and Response to Severe Acute Respiratory Syndrome (SARS)*, that provides a framework and strategies that would guide the U.S. response to a SARS outbreak and describes many of the activities needed at the federal, state, and local levels to prepare for and respond rapidly and decisively to a reemergence of SARS [1]. The information in the document is based on lessons learned from the 2003 global SARS epidemic and the advice and suggestions of domestic and international public health and healthcare partners. CDC made the document available in draft form to assist local and state public health and healthcare officials in their preparations for a possible reemergence of SARS during the approaching respiratory disease season and to solicit comments from interested public health partners. The document was revised based on comments received from partner organizations and other federal agencies and to reflect increased understanding of SARS-CoV transmission dynamics and the availability of improved prevention tools.

Monkeypox

In June 2003, as the global health community remained vigilant for SARS, CDC began to receive reports of patients with a febrile rash illness who had been in close contact with prairie dogs and other exotic pets. The first reports came from the Marshfield Clinic in Marshfield, Wisconsin, where laboratory workers had identified a poxvirus in samples taken from skin lesions from one of these patients and from lymph node tissue from the patient's pet prairie dog [2]. Additional testing by CDC determined that the cause of the lesions was monkeypox, an orthopoxvirus that clinically resembles smallpox. These cases would represent the first outbreak of monkeypox in the Western hemisphere.

In collaboration with state and local health authorities in the affected states, CDC began investigations to determine how monkeypox virus was introduced into the U.S. These investigations found that more than 800 small mammals of 9 different species had been shipped from Ghana to a Texas animal importer on April 9, 2003 [3]. Included in the shipment were several African rodents, including Gambian giant rats which are found in regions of Africa where monkeypox is endemic. Laboratory testing of available animals from this shipment found evidence of monkeypox virus in several species including 1 Gambian giant rat, 3 dormice, and 2 rope squirrels. Traceback and traceforward investigations found that the Texas animal importer had sold both Gambian giant rats and dormice to an animal

vendor in Iowa who had subsequently sold some of the animals to a vendor in Illinois (Figure 2). The Wisconsin index patient had purchased a pet prairie dog from an animal vendor in Wisconsin who had obtained prairie dogs from the Illinois vendor. All of the cases in this outbreak would subsequently be linked to contact with pet prairie dogs from the Illinois vendor. In total, 72 cases were reported from 6 Midwestern states; 37 of the cases have been laboratory confirmed.

This outbreak illustrates the serious public health threat resulting from introduction of non-indigenous pathogens from exotic species of animals, such as rodents, from Africa. To help address these threats, CDC and Food and Drug Administration (FDA) issued a joint order on June 11, 2003 prohibiting the importation of any African rodent. The order also prohibits the sale and transport within the U.S. and the release into the wild of prairie dogs and certain African rodents. An interim final rule has been issued and is available for public comment [7].

West Nile Virus Infection

Following its emergence in North America in 1999, West Nile virus (WNV) has continued to spread westward across the U.S. By 2002, surveillance for human and veterinary cases had documented spread to the Pacific Coast (Figure 3). That same year, WNV caused the largest outbreak of arboviral meningoencephalitis ever recorded in North America. Moreover, several new clinical syndromes and 5 new modes of transmission were documented [13,18,20,22]. These included transmission to recipients of transplanted organs and transfused blood, to infants transplacentally and through breastmilk, and to laboratory workers through percutaneous occupational exposure.

The 2003 WNV epidemic has been even more severe than in earlier years, with more than 9,800 cases and 260 deaths reported [6]. More than one-third (>2,800 individuals) of these infections resulted in severe neuroinvasive disease, i.e., WNV meningitis or encephalitis. The 2003 outbreak was concentrated primarily in the Western states, particularly Colorado where the virus caused nearly 3,000 cases, and more than 60 deaths.

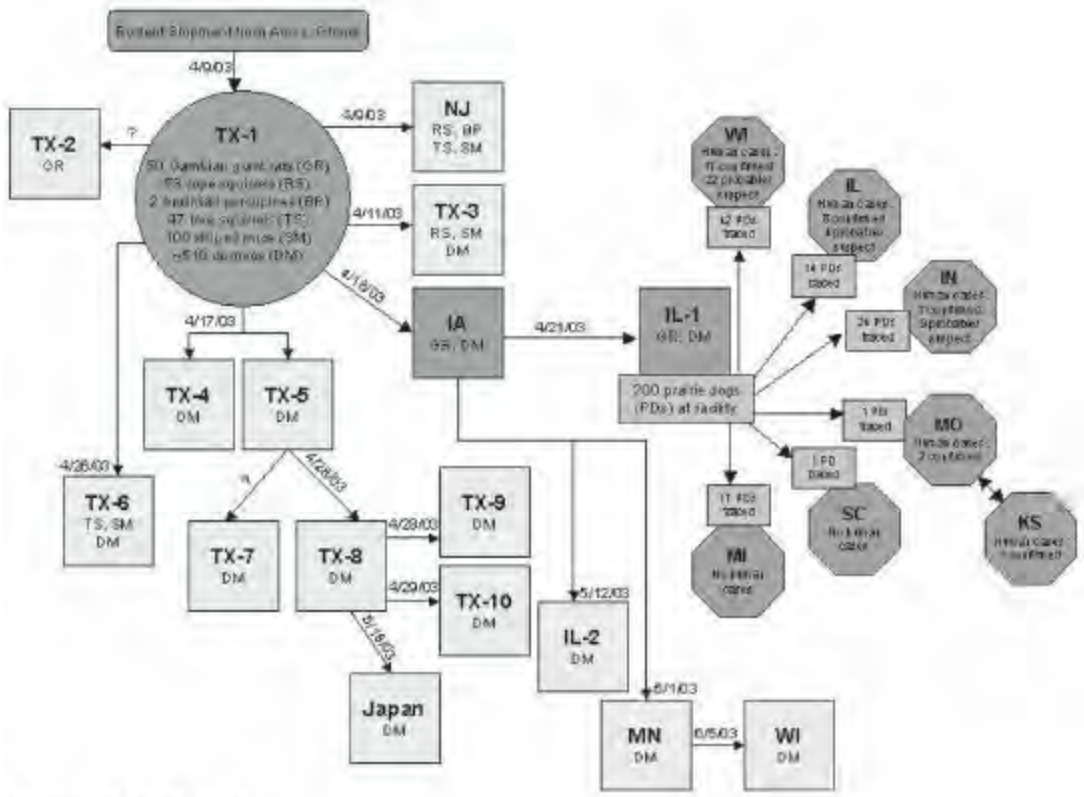


Figure 2. Movement of imported African rodents to animal distributors and distribution of prairie dogs from an animal distributor associated with human cases of monkeypox, United States, 2003*

CDC's Bioterrorism Program

Current efforts to address infectious disease threats must include strong programs to prepare for and respond to potential acts of bioterrorism. CDC's terrorism prevention program, coordinated with the Department of Homeland Security (DHS), addresses biological, chemical, and radiation terrorism, and focuses on 6 main areas: preparedness planning; epidemiology and surveillance; biological laboratory; chemical laboratory; communications; and education and training. Infectious agents receiving the highest priority within CDC's bioterrorism program, termed Category A Biological Agents, include *Variola major, Bacillus anthracis, Yersinia pestis, Franciscella tularensis*, *Clostridium botulinium* toxin, and hemorrhagic fever viruses – the causative agents of smallpox, anthrax, plague, tularemia, botulism, and Ebola-Marburg viral diseases, respectively.

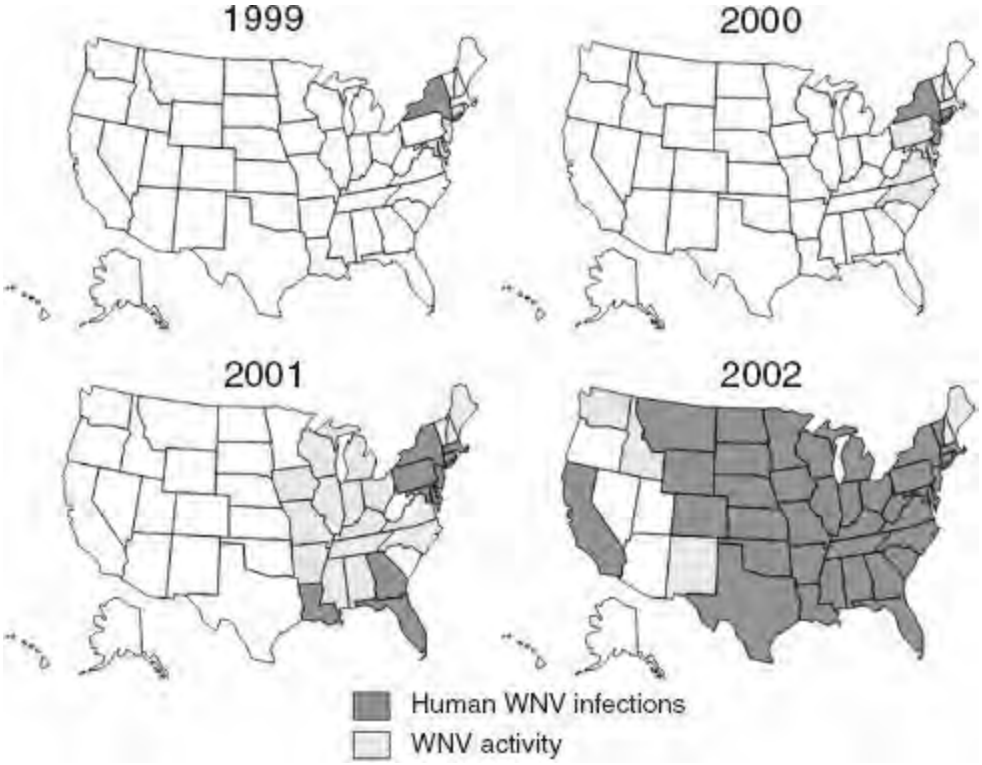


Figure 3. West Nile Virus Activity, United States, 1999-2002.

A critical component in CDC's efforts to detect and respond to bioterrorism is the Laboratory Response Network (LRN). The LRN is a multi-level system that links state and local public health laboratories with advanced capacity clinical, military, veterinary, agricultural, water, and food-safety laboratories. The more than 120 laboratories within the network have progressively higher levels of safety, containment, and technical proficiency that enable them to recognize, rule out, confirm, or definitively characterize highly infectious agents using standardized protocols and reagents and to maintain communication through a secure website.

Another important part of CDC's bioterorrism preparedness and response efforts is the Strategic National Stockpile (SNS). The SNS is a national repository of antibiotics, chemical antidotes, antitoxins, life-support medications, intravenous administration materials, airway maintenance supplies, and medical/surgical items. Under the direction of the Department of

Health and Human Services and DHS, CDC maintains the stockpile to ensure that large quantities of these essential items can be sent to states and communities during an emergency within 12 hours of the federal decision to deploy. The SNS is designed to supplement and re-supply state and local public health agencies in the event of a national emergency.

To further strengthen preparedness and response for naturally occurring and intentional infectious disease threats, CDC has recently joined with the National Institutes of Health to fund extramural programs to enhance biodefense and emerging infectious disease research. CDC's priority areas include innovative research in surveillance, prevention, and control of infectious agents. In October 2003, CDC awarded approximately $9 million for research on animal and human syndromic surveillance, detection of bioterrorist agents, understanding of the immune response, and testing of drug candidates and development of viral vaccines.

Addressing Emerging Global Microbial Threats

The emergence of these diseases and the ensuing efforts undertaken to contain them has provided valuable lessons for responding to future outbreaks. Foremost among these is the need to remain vigilant for signs of new or reemerging diseases. The alert clinician will continue to play an essential role in this process, often serving as the sentinel for the unusual – recognizing the initial signs of both naturally occurring and deliberately inflicted infectious diseases. The highly globalized world in which we live has enabled microbes to emerge and spread into new populations in a matter of hours. Successfully responding to such threats requires strong national and international public and private sector partnerships, active preparedness planning, and proactive communications involving an expansive and multidisciplinary workforce. Increasing linkages among the clinical, public health, research, and veterinary communities worldwide will serve us well in our efforts to meet the challenges posed by emerging global microbial threats.

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Session I

Bioterrorism

Chairman: **Joshua Lederberg, Ph.D.**
The Rockefeller University, U.S.A.

Preparedness for Bioterrorism: Challenges for the Practice of Epidemiology

James L. Hadler, M.D., M.P.H.

Connecticut Department of Public Health, U.S.A.

Introduction

September 11 and the subsequent anthrax mail attacks in the United States (U.S.) made it compelling for those involved in public health to take terrorist threats seriously. It became clear that the world's problems in their worst manifestations had come to our doorstep. In response, we knew that we had to consider what was needed to respond to terrorism, including bioterrorism, and that we had to develop the capability to recognize attacks and respond efficiently and effectively. The U.S. Congress responded in early 2002 with appropriations of more than $1 billion per year for at least the next 2 years. Most of these appropriations have been directed to states and municipalities. This funding has provided an unprecedented opportunity to truly improve the public health infrastructure to support public health needs. How then, are we and should we be using these funds to achieve better preparedness?

This paper will first set the context for bioterrorism preparedness by describing how public health and the initial response to bioterrorism are organized in the U.S. Then, the key components of preparedness for bioterrorism will be outlined, followed by review of the main technical challenges that preparedness poses for public health epidemiologists. Following this, the "dark side" of preparedness for bioterrorism will be discussed. Finally, the paper will conclude with a presentation of the principles and direction that preparedness should take in the future to meet some of these challenges.

State/Local and Federal Roles in Preparedness

Public health is constitutionally a state matter and the basic public health system is state funded. State and local health departments are responsible for disease surveillance, investigation, and response, including outbreaks.

Although there is national disease surveillance, what is to be under national surveillance is jointly determined by the Centers for Disease Control and Prevention (CDC) and the states. National data are usually a collation of data collected at the state level. When outbreaks exceed state capacity or are multistate or international, CDC will usually become involved. For any event within a state, however, CDC has to be invited to participate. Thus, preparedness for bioterrorism must be focused at the level of state and local health departments.

Principles of Public Health Preparedness for Bioterrorism

There are several main principles of preparedness for bioterrorism as listed in the guidance for use of funding prepared by the CDC.

First, each state needs to have systems that will enable early detection of possible bioterrorism events. Then, the steps in investigation and response, and the partners needed to carry these out need to be identified. Finally, response plans need to be developed with these partners, and these plans need to be exercised to be sure that complicated

responses will be coordinated.

Although it is easy to list a series of principles, meeting them is complex, time-consuming, and long-term. This is particularly true for anything that involves engaging partners like hospitals and law enforcement, getting the time commitment from each to participate, and achieving consensus.

Bioterrorism Preparedness and Surveillance Considerations

A major role for epidemiology is surveillance to detect outbreaks due to bioterrorism before they become full-blown and while there is still a chance to intervene to limit the physical and psychological consequences. To appropriately plan for detection and response to bioterrorism, it is necessary to consider how a bioterrorism event would first manifest itself.

Bioterrorism is different from other forms of terrorism. Exposure is likely to be covert or unannounced, and initial illness will not be particularly distinct from the spectrum of illnesses or outbreaks seen every day. Usually, there will be a time lag of at least several days to weeks between the initiation of exposure and onset of symptoms in the first cases. Thus, people are likely to be scattered geographically when they first become ill, they will not become ill at exactly the same time or even all on the same day, and there will not be a distinct terrorism scene as there is after a bomb explosion. In addition, depending on the agent, there is the potential for person-to-person transmission or lingering environmental contamination to amplify the medical and psychological consequences.

Technological Challenges for Epidemiologists

Based in large part on the federal guidance for preparedness of surveillance and epidemiologic response systems [2], there are a number of areas requiring special efforts to assure that the preparedness needs are covered. These include: 1) enhancing existing surveillance systems to make them more sensitive to detection of bioterrorism agents or unusual events; 2) devising new systems to detect outbreaks earlier; 3) developing algorithms to respond to findings from new environ mental surveillance efforts; and 4) responding to bioterrorism scenarios such as a large-scale smallpox outbreak that would require mass clinics and extensive contact investigation efforts. The following briefly elaborate on each of these challenges and how some states are approaching them.

Enhancing Sensitivity and Timeliness of Current Systems

Current surveillance is dependent on physician, hospital, and laboratory reporting. A number of efforts are being made to enhance the sensitivity and timeliness of this reporting system. First, each state has established the legal basis for receiving critical information by expanding disease reporting requirements to include all Category A bioterrorism agents, outbreaks, and individual cases of unusual disease. To be able to provide diagnostic testing to back up the reporting requirements, state laboratories have been given the necessary equipment, technology, and staffing.

Second, each state has been encouraged to develop special systems to assure immediate reporting of any rash illness that could be smallpox and laboratory findings that could be anthrax. As an example of a new rash illness surveillance system, a system is being piloted in Connecticut in which physicians report all cases of suspected severe chickenpox that require hospitalization ***by telephone*** any time of day or week. Usually chickenpox, if reportable at all, is reported using paper forms on weekdays, ***not*** by telephone and not on nights or weekends. Thus, these unusual cases of chickenpox, which could be a first case of smallpox, have been put in the same reporting category as any outbreak of illness, immediately by telephone. The initial experience with this system is that telephone reporting has increased substantially.

Finally, to increase physician awareness of reporting systems and their importance, communication systems between public health workers and clinical providers have been enhanced. E-mail and blast fax lists of hospitals, infectious disease specialists, emergency department directors, and as many clinicians as possible have been developed. These lists are used to communicate special public health alerts. Such systems were invaluable to rapidly establish specific surveillance for severe acute respiratory syndrome (SARS) and West Nile virus as emerging acute infectious diseases. They have also been used to distribute a special CDC-developed algorithm to distinguish between chickenpox and smallpox.

Systems to Detect Outbreaks Earlier

A number of methods are being tried to meet the second challenge of early detection. Foremost among them is syndromic surveillance [1]. New York City has been a pioneer in this area, having established an extensive early warning system based on daily monitoring of visits to emergency rooms by syndrome, calls to the 911 emergency line, and pharmacy prescriptions for selected conditions. In Connecticut, unscheduled acute care hospital admissions for selected syndromes such as pneumonia or fever with rash are monitored on a daily basis.

A second major method many are trying is to move from paper to electronic laboratory reporting. Each state, city, and county health department monitors laboratory reports for trends in the number of positive tests for various infectious agents. However, reports generally come in by mail. Mail can take up to 10 days to traverse laboratory, postal service, and health department postal systems. Once it arrives, each report still needs to be entered into a database for analysis. Direct daily electronic reporting has the potential of providing the data immediately, in real time.

Environmental Surveillance and Response

In addition to detection of illness, which is state-based, another approach to early detection is being tried by the federal government with funding from the Department of Homeland Security. This is environmental monitoring to detect airborne releases of selected agents of concern. In particular, military researchers have developed sensors that can be used to rapidly detect genetic material from anthrax spores, smallpox virus, and other selected microbes such as tularemia and plague from environmental samples. In a program known as BioWatch, these sensors are now being used to test air sampling filters from selected Environmental Protection Agency air quality monitoring sites in certain cities across the U.S. In addition, the U.S. postal system is beginning air sampling for anthrax in selected postal distribution centers. Filters from air sampling are sent to public health laboratories for analysis. The epidemiologic challenge is to develop meaningful algorithms for a graded response to positive signals from rapid screening tests applied to these filters, including when to initiate a full-scale public health response.

Planning Response to Large-Scale Exposures

A final epidemiologic challenge is that each state has been asked to prepare for intentionally-caused, large-scale exposures and outbreaks that require a complex and massive response – such as aerosolized smallpox or anthrax exposure. This challenge involves the state's lead epidemiologists and is largely led by them. Although some of the surveillance challenges previously listed are technologically more challenging, this is a more formidable one. To appreciate the scale of this challenge, one only need consider SARS as a real scenario that totally overwhelmed the often sophisticated local healthcare systems in the countries that were most affected. Could we keep a smallpox outbreak from having the medical, psychological, social, and economic consequences that SARS had in the most affected countries?

During the past year, each state was put on high alert by the Department of Health and Human Services to immediately prepare for smallpox. This was in anticipation of the pending invasion of Iraq and a possible counter-attack with bioweapons. As part of this preparedness, each state developed a comprehensive written smallpox response plan and worked with hospitals and local health departments to implement smallpox pre-event vaccination on short notice. Whether or not we were affected directly by SARS, we all discovered from our smallpox experience that planning for something like smallpox or SARS is indeed a massive task that involves a large number of people and an enormous time commitment.

Progress in Bioterrorism Preparedness

In the first 18 months since large-scale funding was appropriated, there has been a very positive impact. Public health has been able to engage hospitals and laboratories in the joint public health mission as never before. State laboratories are now better equipped and staffed. Epidemiologic investigation and response capacity has been expanded through additional positions and working with new partners, and new types of surveillance systems are being piloted. New and enhanced rapid communications systems are in place that enable public health departments at all levels, from city and county to state and CDC, to be in constant contact with each other and with their disease surveillance and control partners across each jurisdiction. In addition, each state has developed a smallpox response plan that can be applied to other situations. Public health's visibility has never been higher. In all of these ways, we have an extraordinary, ongoing opportunity to enhance the practice of epidemiology and applied public health.

However, this opportunity and the reasons for it have come with their own challenges. This is the "dark side" that public health infectious disease epidemiologists have to negotiate.

“Dark Side” Challenges for Epidemiologists and Public Health

Among the “dark side” challenges are the association of public health science with politics, working in an atmosphere in which there is a rush to preparedness driven by fear of imminent attack, and trying to maintain public health priorities during times of economic downturn.

Impact of Politics on Public Health

Public health scientists are now much more visible and publicly accountable, an appropriate position to be in. However, it means they are also more subject to the whims of politics and the media. For example, there is a lingering perception that the public health response to anthrax was inadequate. In fact, the epidemiologic response to the anthrax mail attacks, and the knowledge it contributed were extraordinary, and all the illness that was preventable was prevented. Ironically, the response driven by political concerns was at times problematic and by association, public health scientists lost some credibility.

Consequences of the Rush to Preparedness on Public Health

Unfortunately, the funding that provides us the opportunity to take a quantum leap forward in public health infrastructure comes from a focus on protecting the American people from imminent terrorist threats at home and with it, a special urgency to preparedness. This urgency to be prepared brings yet more challenges for the practice of epidemiology and public health.

Most of our preparedness objectives are based around known Category A Weapons of Mass Destruction (WMD) agents. Public health officials are asked to do very focused preparedness and develop special surveillance systems to detect these agents. The prevailing message from our government is that we cannot be fully prepared too soon. While there is funding to help achieve this, the available monies cannot simply buy, on short notice, the type of expertise and leadership needed to do the job quickly. As a result, key scientific leadership at the state and local level is being consumed in this effort. Most State Epidemiologists have done virtually nothing else over the last year and a half but try to meet WMD-related preparedness objectives. Before that, state and local health departments were entirely focused on the response to anthrax. Neglected in the meantime are all other infectious disease programs: HIV/AIDS, vaccine-preventable diseases, tuberculosis, sexually-transmitted diseases, foodborne diseases, and global public health problems. Only the most immediate and compelling emerging infectious disease issues such as SARS have received any attention. This is not to say that there are not good maintenance programs in those areas. But it does mean that as of now, nearly 2 years of opportunities for special data-gathering efforts and innovation in these areas have been lost. This is a challenge to public health leadership: we need to re-assess and balance priorities for use of our time.

A second consequence of our haste is burnout. Between November 2002 and May 2003, all states took on the urgent task of smallpox preparedness in preparation for the possibility and then the reality of the invasion of Iraq. Enormous amounts of the energy and goodwill of our public health preparedness partners were consumed. Many were not happy about the fast implementation of potentially morbidity-generating smallpox pre-event vaccination. The challenge now is to reengage our partners in maintaining smallpox preparedness, and to find a pace of preparedness that can be sustained without exhausting everyone involved.

Impact of the Economy

A final challenge for the practice of epidemiology in these times is the economy. Most states are experiencing large-scale cutbacks in state funding to manage the budget crisis. In Connecticut, approximately 10% of our state public health workforce was lost to cutbacks and early retirement programs to deal with the state budget deficit. The only source of significant new funding is in the area of bioterrorism preparedness. If we are not careful about how we use public preparedness funding, we will end up with a public health workforce that is entirely devoted to terrorism preparedness. What is needed is a well-trained, efficient workforce devoted to basic public health that is ***also*** prepared for terrorism. We may never need to exercise our bioterrorism preparedness, but public health remains an everyday concern.

Future of Bioterrorism Preparedness: Meeting the Challenges

It is clear there are many challenges to the practice of epidemiology in preparing to respond to the threat of terrorism – not all of them welcome and not all of them constructive. But they are real and need to be addressed in a constructive manner. If they are not, we are at risk of failing in our efforts at long-term preparedness and could waste the opportunity to enhance public health. How then, should we address these less technical but more daunting challenges to public health preparedness?

Integrated Public Health Preparedness

First, we need a unified approach to public health preparedness that does not focus exclusively on bioterrorism above anything else. The job of detecting, investigating, and responding to emerging infections and "natural" outbreaks like SARS, and the job of preparedness to respond to intentionally-caused outbreaks is 1 job, not 2. They simply cannot be separated. The best way to prepare to deal with the unknown is to deal with it all the time. We cannot create a separate group to deal with bioterrorism and another to deal with other public health infectious disease problems. Public health epidemiologists are experts in dealing with the unknown. We need to take advantage of that expertise and enhance it. The basic job is applying epidemiology to public health problems with an overlay of preparedness for unusual situations of all sorts, including bioterrorism. In other words, "universal preparedness." In Connecticut, we follow this model and have no dedicated bioterrorism epidemiologists. Instead, all of our epidemiologists are trained to recognize and respond to the agents that could be used by bioterrorists.

Sustained and Flexible Preparedness

A new preparedness paradigm of "sustainable and flexible preparedness" is needed. Although preparedness efforts in each state last year were on a fast track in preparation for Iraq, public health preparedness is a long-term venture. We need sustainable levels of preparedness that are built upon and enhance the larger public health structure, not isolated, high-intensity efforts that result in burnout and then a drop in readiness.

This is the right time for sustainable and flexible preparedness. One of the beneficial results of the exhausting drive to short-term smallpox preparedness is that the key infrastructural partnerships needed between public health, hospitals, providers, and law enforcement are now in place. The emergence of SARS and the need to be prepared to limit its impact on public health, the healthcare delivery system, and the economy provide an opportunity to do more integrated, sustainable planning. In Connecticut, the need to be prepared for SARS is currently more compelling to hospitals than the need to be further prepared for smallpox despite the fact that most hospitals are still ill-prepared for smallpox. To resolve this dilemma, we have combined specific preparedness efforts for smallpox and

SARS since the public health response to each is similar and involves the same people. Our combined planning workgroup gets better attendance and more vigorous discussion than the one dedicated only to smallpox received after the immediate concern about smallpox waned.

Balance Between Public Health and Bioterrorism Preparedness

Related to the concept of sustainable and flexible preparedness is the need to assure that a ***balance*** is maintained between attention to existing public health programs and bioterrorism preparedness. The current budget crisis that most states are experiencing means that there will be no state funding for infrastructure in the near future. Thus, we must ensure that available funding for public preparedness is used wisely. The bulk of the available funding should be used first to enhance basic laboratory and technical infrastructures that support existing surveillance and response systems. From a staffing perspective, funding needs to be used as generally as possible for disease investigation and response capacity. If it is too categorical, it may compromise other public health efforts. Investment in new, unproven systems such as syndromic surveillance should be limited until it is demonstrated that such systems are worth their cost. Finally, it is critical that there be enough senior medical epidemiologists to be able to divide the labor of infectious disease program and public health preparedness oversight. Without leadership to meet the ever-changing needs of basic public health programs, we risk causing more morbidity in the name of preparedness than we prevent. We cannot afford to compromise our capacity to monitor, investigate, and creatively respond to the many ongoing infectious disease problems.

Preparedness will only be achieved and sustained if public health leadership is involved as a full partner in an open, scientific dialogue in the process of determining and implementing preparedness initiatives that affect them. Optimally, new but costly surveillance ideas should be taken on by just a few pilot states or cities as demonstration projects that can be properly implemented and objectively evaluated. In the U.S., we have a powerful matrix system of more than 50 individual health departments in which to examine issues, test ideas, and determine which ones really work and in what settings. We must be sure this public health system laboratory is used to its fullest.

Summary and Conclusions

Much progress has been made in preparing to detect and respond to bioterrorism. At the public health level, the response to emerging public health crises like anthrax, West Nile virus, SARS, and monkeypox, and the implementation of smallpox pre-event vaccination, were greatly facilitated by laboratory and epidemiology response infrastructure, communications systems and planning partnerships, and mechanisms established as a result of bioterrorism preparedness funding.

Much remains to be done, however. There will be special evaluations done to assure accountability for preparedness funding that has been spent. And it is likely that no matter how much progress is made and how dedicated the preparedness effort is, there will be reports that will say despite spending more than $1 billion per year, the U.S. is not yet really prepared for anything big. The challenge for public health epidemiologists will not be to try to please all of our critics, but to do what only we can do. We must evaluate the surveillance efforts we have tried and determine which are really worth keeping, and be outspoken in challenging policies and mandates that are scientifically questionable. We must promote the paradigm that the best preparedness is sustainable, flexible, and fully integrated into the more basic public health response system – and that public health preparedness is ongoing – it has no endpoint. And finally, we must ensure that all funding under our control is used in a way that enhances but does not undermine the public health system. These are big challenges. If we can have an open dialogue on them in the next few years, the U.S. will have a reasonable chance of ending up with a stronger public health system and a long-term improved state of public health preparedness.

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Bioterrorism Preparedness and Response at the State and Local Levels

Marcelle Layton, M.D.

New York City Department of Health and Mental Hygiene, U.S.A.

Since the tragic events of the fall of 2001, bioterrorism preparedness planning efforts have accelerated at local and state public health agencies. The local and state public health response to a large-scale, covert bioterrorist event requires rapid recognition that an unusual disease manifestation or a clustering and/or increase in infectious disease illnesses is occurring. This is followed by prompt epidemiologic and laboratory investigations to determine the etiologic agent and whether this finding represents a true outbreak, and, if so, whether the source may have been intentional (e.g., terrorist act) versus a natural cause. If a potential bioterrorist event is suspected, public health authorities will need to mobilize rapidly to identify the time and site of the initial attack and conduct ongoing surveillance and epidemiologic investigations to monitor the extent of the outbreak. At the same time, health authorities will need to address public and provider concerns and provide frequent updates as the outbreak evolves and new information becomes available. There will be a need to determine whether antibiotic or vaccine prophylaxis is indicated, and if so, coordinate with emergency management agencies and the healthcare community to provide medications to potentially exposed persons, which depending on the circumstances of the event, may involve the entire population of the jurisdiction. The enhanced capacities and infrastructure required for a rapid and successful response to a large, covert bioterrorist event will also ensure that public health agencies are able to respond to natural infectious disease threats, such as pandemic influenza or severe acute respiratory syndrome (SARS).

An effective public health response to bioterrorism will also require that local and state public health departments are closely coordinated with the appropriate governmental agencies at the local/state (e.g., Emergency Management, Police, Emergency Medical Services), regional (neighboring state and county authorities, especially public health counterparts), and federal (e.g., Health and Human Services, Department of Homeland Security, Department of Defense, and Department of Justice) levels. Lastly, and most importantly, the successful detection and response to a bioterrorism event relies heavily on linkages that should already be in place between local and state public health officials and the local healthcare provider, hospital, and laboratory communities within their jurisdictions.

Routine Linkages between Public Health and the Healthcare Sector

One of the core missions of public health agencies is the control of communicable diseases. This legal mandate has prompted public health officials to work closely with local healthcare providers and hospital and laboratory communities. Traditionally, disease surveillance activities depend on prompt reporting by healthcare providers and laboratorians concerning suspect or confirmed cases of notifiable diseases to local and state health departments. Case investigations by public health officials often require frequent communication with the reporting clinician or hospital infection control personnel to obtain more detailed clinical and epidemiologic data, or to help facilitate obtaining appropriate clinical specimens for testing at public health reference laboratories (e.g., rabies or botulism). For certain contagious diseases (e.g., hepatitis A or invasive meningococcal disease), public health officials may request assistance in providing prophylaxis to contacts at high risk for secondary transmission. The successful implementation of public health prevention campaigns also relies on close partnerships between health departments and the medical community. Recent examples of this successful partnership include the response to the epidemic of multidrug-resistant tuberculosis in the early 1990s [8], perinatal hepatitis B prevention programs, and HIV counseling, testing, and partner notification programs.

From a surveillance and control perspective, one of the most important linkages at the local level is between public health officials, infection control practitioners, and hospital epidemiologists. Infection control practitioners and hospital epidemiologists serve as the primary points of contact in hospitals for surveillance and disease control activities during both community and nosocomial outbreaks, and as such, serve a critical role in any hospital's ability to respond to a bioterrorist event.

Bioterrorism Preparedness Requires Enhanced Linkages

Bioterrorism preparedness requires building upon the existing linkages between the public health and hospital/medical care sectors that are already in place to confront routine public health problems, such as influenza, tuberculosis and new, or emerging infectious diseases, such as SARS. Key aspects of plans that need to be in place for the public health response to bioterrorism parallel what needs to be in place for naturally occurring infectious disease outbreaks, and include: detection, epidemiologic investigation, active surveillance, laboratory testing, communication, and prophylaxis. The areas in which relationships with the hospital and medical communities are crucial include: the initial detection of the event; provision of care to the victims; isolation of potentially contagious patients; provision of prophylaxis to those potentially exposed; and providing timely and effective education to the public regarding the medical aspects of the event. All clinicians and laboratorians should be familiar with the legal requirements under their local and state health codes, including which diseases are listed as notifiable in their state and mechanisms for disease reporting.

As with any emergency, it is essential that the local medical and public health communities are familiar with each other ahead of time. Medical providers and key hospital staff (e.g., infection control and microbiology staff) should be aware of both their local and state health departments' 24-hour, 7-day/week emergency contact numbers and website information as well as be registered and familiar with any existing public health electronic communication systems (e.g., Health Alert Network, broadcast facsimile, and electronic mail health alert systems) that provide urgent notification of the healthcare community during acute events.

Detection of a Bioterrorism Event: Traditional and Non-Traditional Surveillance Systems

Concern has been raised regarding possible delays in recognizing an outbreak due to bioterrorism, and the subsequent impact this could have on morbidity and mortality if treatment or preventive measures were delayed. The diseases caused by many of the potential bioterrorist agents may not be accurately diagnosed until late in their course since the initial presentations may be nonspecific (e.g, influenza-like prodrome of anthrax), most physicians in the United States (U.S.) have little or no clinical experience with these agents (e.g., anthrax or smallpox), and the laboratory diagnosis for some agents may require days or even weeks for a positive or presumptive identification (e.g., tularemia). Therefore, alternative or early warning surveillance systems for recognizing unusual disease manifestations or clusters may be needed to improve the ability to detect a biologic terrorist event as rapidly as possible. Prompt recognition is necessary to expedite mobilization of an effective public health response, including rapid testing at a public health or other reference laboratory to identify the etiologic agent (along with susceptibility results for bacterial agents); enhanced or active surveillance for additional cases; epidemiologic investigations to determine the cause (natural versus intentional source), the time, and site of a suspected release and event reconstruction to identify those still at-risk; and initiation of treatment and prophylaxis measures.

State and local public health officials need to be alert to potential scenarios that may represent the initial evidence of a bioterrorist event. A possible bioterrorist event, from the health department's perspective, could include any of the following:

(1) A single, definitively diagnosed, or strongly suspected case of an illness due to a recognized bioterrorist agent occurring in a patient without a plausible explanation for his/her illness (e.g., a case of plague in the absence of a recent travel history to a recognized endemic area).

(2) A cluster of patients presenting with a similar clinical syndrome with either unusual characteristics (unusual age distribution) or unusually high morbidity or mortality, without an obvious etiology or explanation.

(3) An unexplained increase in the incidence of a common syndrome above seasonally expected levels (e.g., a marked increase in influenza-like illness during the summer with both rapid and conventional virology tests being negative for influenza or other common respiratory viruses).

In the event that a potential bioterrorist event is suspected, an investigation should be initiated immediately to confirm the suspected diagnosis, ensure that there are no other explanations for the illness(es), and determine the likely source of infection. Since these case definitions are nonspecific for bioterrorism, and would also apply to natural outbreaks of known or new infectious diseases, it is essential that bioterrorism be considered as part of all routine case and/or outbreak investigations, until a natural cause is established. Prior outbreaks of salmonella in a community [24], and *Shigella* among employees of a hospital

laboratory [16] were eventually found to be due to intentional contamination of food items when the initial epidemiologic and laboratory investigation revealed concerning features that would not have been expected if the infections had occurred naturally.

There are several surveillance methodologies for detecting a bioterrorist event that focus on ensuring the prompt recognition of (1) a suspected or confirmed case or cluster due to a recognized bioterrorist agent, (2) community-wide or localized increases in influenza-like illness activity or other non-specific syndromes, or increases in potential markers of early prodromal illness (e.g., over-the-counter drug sales), or (3) detection of select bacteria or viruses in air samples obtained through routine environmental biomonitoring programs.

Traditional Public Health Surveillance

Traditional public health surveillance for bioterrorism relies on enhancing the medical and laboratory communities' awareness of bioterrorism to improve reporting of suspect cases of illness potentially caused by a bioterrorist agent or unusual disease manifestations/clusters. Most local and state health codes require that physicians, hospitals, and laboratories report a defined list of notifiable infectious diseases. With recent concerns regarding the threat of bioterrorism, most state public health agencies have added all Centers for Disease Control and Prevention (CDC) Category A and most Category B agents that were not already included on their reportable disease lists [22]. In addition, recognizing the need to detect newly emergent diseases that are not yet listed on the health code, most states also require reporting of any unusual disease clusters or manifestations.

Early recognition of a bioterrorist event depends in large part on astute clinicians and laboratorians recognizing one of the index cases based on a suspicious clinical, radiologic, or laboratory presentation (e.g., a febrile illness associated with a widened mediastinum on chest radiograph in an otherwise healthy adult has a limited differential diagnosis besides anthrax). Isolated cases presenting at separate hospitals will not be recognized as a potential outbreak, unless each case is reported promptly to the local health department, where the perspective exists to detect population-based aberrations in disease trends. Previous examples of astute clinicians recognizing and reporting unusual disease clusters or manifestations which led to the detection of a more widespread outbreak include an outbreak of Legionnaire's disease associated with the whirlpool on a cruise ship [15], an outbreak of Cyclospora associated with contaminated raspberries imported from Guatemala [12], and the initial outbreak of West Nile virus in New York City in 1999 [21]. Similarly, the initial detection of the intentional anthrax outbreak in 2001 was due to a public health responsive physician who recognized a suspect case of inhalational and meningeal anthrax in Florida after noting large Gram-positive rods in the cerebrospinal fluid of his patient, and promptly reported his concerns to local and state public health authorities [4].

To educate clinicians and laboratorians regarding their essential role in recognizing and reporting a suspected case of bioterrorism or other unusual infectious disease occurrences, public health officials need to promote the importance of disease reporting through ongoing educational efforts. For bioterrorism concerns, targeted outreach efforts should focus on specialists in key areas, such as infectious disease, infection control, microbiology,

emergency medicine, dermatology, and neurology. Educational outreach should emphasize the clinical presentations and diagnostic clues for specific bioterrorist agents (e.g., anthrax, plague, smallpox) as well as unusual illness patterns suggestive of a potential bioterrorist event. One of the lessons learned during the 2001 anthrax attack was the need to maintain awareness of all potential clinical manifestations of the bioterrorist agents, such as cutaneous disease, and not simply focus on illness due to inhalational routes of exposure. Educational materials should emphasize prompt reporting of any unusual disease clusters or manifestations to the local or state health department as paramount to the early recognition of natural and intentional outbreaks. Educational outreach is also needed for key members of the local first responder community (Office of Hazardous Materials Safety [HAZMAT], Police, Emergency Medical Services).

Improving the overall relationship between the health department and the medical community is an important element in ensuring that providers promptly report. Therefore, efforts to improve provider relations and ease the burden of physician reporting should be prioritized (e.g., having a single telephone number {e.g., by implementing 1-800 provider hotlines, such as 1-800-MD-REPORT} for physician reporting to ensure easy access to health department staff – and a dedicated office, with clinically-trained professionals, for handling telephone inquiries from the medical and laboratory communities). The medical and laboratory communities are more likely to report if the process is streamlined, and there is a positive perception of the public health responsiveness to disease and/or outbreak reporting. Routine dissemination of surveillance data and prompt feedback are essential for fostering an ongoing, collaborative relationship between public health and the medical/laboratory communities. These efforts will have the additional benefit of improving all aspects of local public health surveillance.

Recent experiences with the West Nile virus [25] and monkeypox [6] outbreaks have highlighted the need for public health officials to establish and maintain similar active linkages with the animal health community. Since many potential bioterrorist agents cause zoonotic disease (e.g., anthrax and plague), the first indication of an aerosolized release may be illness or death in animals or rodents. Historically, with the exception of rabies-related issues, local and state infectious disease epidemiologists have not had strong relationships with clinical veterinarians and wildlife specialists in their community. With the continued emergence of new zoonotic disease threats, as well as concerns about bioterrorism, this has needed to change. Many state and local health departments have recently added or expanded current disease reporting requirements for animal health specialists to notify public health officials of any suspect or confirmed illness in an animal that may be due to a potential bioterrorist agent. Similar to the list of notifiable diseases in humans, these regulations often require reporting of any unusual disease clusters or manifestations in animals as well.

Non-traditional Surveillance Systems (Syndromic Surveillance)

The recent bioterrorist attacks associated with the intentional release of *Bacillus anthracis* spores have increased interest in the potential value of enhanced public health

surveillance systems for early detection of epidemics caused by biologic terrorism. In the event of a covert, large-scale biologic attack with the potential to cause hundreds to thousands of casualties, rapid detection and characterization of the outbreak would be of the utmost importance. Rapid mobilization of surveillance and epidemiologic resources to determine the place and time of the attack would facilitate targeting preventive measures to those at-risk, speed the epidemiologic and criminal investigation, and reduce public panic. For diseases such as inhalational anthrax that have short incubation periods, the window of opportunity to mobilize a response in order to mitigate morbidity and mortality is quite small, only a matter of days. Therefore, surveillance systems that provide early and enhanced recognition of a covert bioterrorist event are advantageous.

The traditional public health surveillance system, based on passive reporting of a limited number of defined, notifiable diseases, may not be sufficient for early detection of a bioterrorist event. Certain potential bioterrorist agents (e.g., tularemia) have non-specific clinical presentations and/or laboratory diagnosis that may be difficult. Thus, alternative systems that allow prompt recognition of unusual disease manifestations, illness clusters, increases above seasonal levels of common syndromes (e.g., influenza-like illnesses), or deaths due to infectious causes – prior to suspicion or confirmation of the causative agent(s), are potentially useful components of bioterrorism surveillance.

While increasing provider education on bioterrorism and improved communication among public health officials, clinicians, and laboratorians is an essential component of enhanced traditional surveillance, surveillance for non-specific clinical syndromes using data available in existing electronic health databases is also now considered a potentially valuable adjunct system for the timely detection of large-scale bioterrorist events. While many of the most concerning infections (e.g., anthrax, plague, smallpox, and viral hemorrhagic fever) have distinct clinical characteristics and diagnostic criteria once the disease is full blown, the first signs of illness for many of these agents is a non-specific prodrome with respiratory and constitutional symptoms similar to influenza-like illness. Since most medical providers and laboratories in the U.S. have little experience with these pathogens, the diagnoses may be delayed. Therefore, the first indication that a large-scale attack has taken place might be an increase in non-specific symptoms at the community level. Surveillance for these increases in non-specific syndromes (e.g., respiratory or gastrointestinal) constitutes the cornerstone of “syndromic surveillance’ [20].

The ideal features of a syndromic surveillance system for early detection of a covert bioterrorist attack include the ability to detect changes in disease trends that are based on health event information available continuously or at least in 12- to 24-hour increments. Health event information is most timely when it is electronic, gathered routinely for other purposes, and not limited by diagnostic or recording delays. Syndromic surveillance systems based on clinical data have proven most popular, but other sources such as over-the-counter drug sales may also have utility.

Electronic data that may provide a reflection of community-wide illness is increasingly available, including for example, emergency department visit logs [1], ambulance dispatches [19], ambulatory care encounters [17], volume of specimen submissions to commercial clinical laboratories, and sales of prescription and over-the-counter pharmaceuticals. Due to

the challenges in requiring additional efforts by physicians or other providers, the most reliable electronic data sources are probably those that already exist and do not rely on additional collection or reporting of data by medical providers. In many systems, these data include geographic information (e.g. home or work zip code, or location of store), theoretically enabling the detection of localized disease outbreaks.

Important features to consider when evaluating potential syndromic surveillance systems include the presence of computerized data that is already categorized into clinical syndromes, can be made available to health authorities on at least a daily basis (including weekends and holidays), and is geographically representative of the population. Ideally, the operation of these systems for public health surveillance purposes should include at least daily electronic transfer of data that has been stripped of personal identifiers, and that utilizes statistical algorithms to rapidly detect increases in disease syndromes compared to expected seasonal trends [14]. Some systems have the additional sensitivity to detect spatial aberrancies or unexpected geographic clusters [3]. Hospitals and medical care systems may be able to share such data sources with their local or state health departments, including information on emergency department or primary care clinic visits or hospital admissions. If data does not contain confidential information on patients (e.g., limited to age, date of visit, chief complaint, or provider diagnosis) then potential restrictions in the Health Information Privacy and Accountability Act would not apply [18].

When an aberration in a particular syndrome is identified, either a jurisdiction-wide increase or a geographic-specific signal, public health officials need to assess the situation to determine if the finding truly represents worrisome illness in the community, and if so, conduct an investigation. Similar to traditional outbreak investigations, syndromic signal investigations attempt to determine if the aberration represents a common illness with or without a common exposure. To assist in determining if the aberration is a real event versus a statistical anomaly, it is generally assumed that a continued rise in the incidence of the syndrome is evidence of a real event. Interim data from the involved facilities (e.g., a 12-hour chief complaint log), though often difficult to obtain, can be useful in this evaluation. Inspection of the aberrant data will reveal any unexpected coding mistakes and the presence of commonalities in demographic variables. Although anecdotal, calls to emergency department staff, as directed by geographic clustering, can be reassuring. When signals contain features of concern (as determined by the particular syndrome) along with parameters such as size of the cluster (including age clustering), magnitude of the increase, seasonality, and overlap with other syndrome(s), staff can be dispatched to review medical records and conduct interviews or telephone follow-up on discharged patients. Prospective surveillance is implemented with augmented diagnostic testing for newly presenting patients, as indicated by the syndrome of concern (e.g., rapid antigen tests for influenza, chest radiographs, or blood cultures).

One of the challenges to syndromic surveillance has been establishing the definitive microbiologic cause when an increase in an infectious disease syndrome is identified. Limitations in obtaining clinical specimens, particularly in outpatient settings, and in being able to perform full diagnostic testing (especially for viruses) renders interpretation of signals and evaluation of syndromic systems difficult. As the primary objective of syndromic

surveillance is early outbreak detection, there is a need to link the use of rapid diagnostics (especially for viral agents, such as influenza, and ideally, the bioterrorist agents as well) to investigations of syndromic surveillance signals.

Although the initial objectives for many of the current syndromic systems was early detection of a large, aerosolized covert bioterrorist attack, these systems also offer the ability to monitor for both natural infectious disease outbreaks, as well as trends in non-infectious events of public health importance. In New York City, syndromic surveillance has been in place since 2000 and currently includes multiple data sources, including ambulance dispatches, emergency department visits, pharmaceutical sales from a large retail chain, and employee absenteeism. As an aberration in one system may represent an artifact (e.g., an increase in anti-diarrheals being caused by a promotional sale at the pharmacy chain), when multiple systems are in place, an increase in more than one system is considered more concerning and may prompt a more intensive investigation. These New York City systems have provided the earliest warning of influenza-like activity at the start of each season, allowing earlier notification of the medical community of the need to prioritize completion of vaccination campaigns. In addition, New York City's syndromic surveillance systems provided early warning of the 2002 norovirus outbreak, as well as detected a citywide increase in diarrheal illness in the days following the August 2003 blackout.

Health officials in New York City also have found syndromic surveillance useful for providing reassurance that medium to large outbreaks are not being missed during times of heightened concern. During the international outbreak of SARS, the absence of a persistent citywide increase or geographic clustering of respiratory or febrile syndromes in any of the current systems in place in New York City provided some assurance that unrecognized SARS transmission was not occurring in the city. Similarly, when cases of inhalational anthrax [13] and bubonic plague [7] were confirmed in New York City, the lack of signals from these systems suggested that these were isolated, not citywide, events.

Over the past few years, there has been great interest in establishing syndromic surveillance in both the private and public sectors, but these systems remain untested with respect to their ability to detect a bioterrorist event. Although early indications support the potential usefulness of these systems, more formal evaluation is needed to ensure that the significant investment of funding and staff resources in these systems is warranted. Most importantly, these systems complement but cannot replace traditional disease surveillance based on disease reporting from astute medical providers. Most of the major outbreaks of public health importance in recent years have been detected after a concerned physician rapidly notified their local or state public health authorities [7,13].

Integrated syndromic surveillance systems may represent reasonable investments for large metropolitan public health departments, but may not be practical or advisable in smaller jurisdictions. New systems are being developed (e.g., BioSense) that may have the capacity to identify local and regional illness trends from large national datasets, allowing smaller local and state agencies to make use of syndromic surveillance technology. As this interest in non-traditional public health surveillance continues, it will be important to keep in mind its limitations. Syndromic surveillance, generating signals before or at the moment when persons seek medical care, is a public health early warning system suggesting that a

serious public health concern may be evolving. As such, it requires a response, no less than a smoke alarm calls for a rapid assessment and the possible intervention by firefighters. It would be unwise to invest in syndromic surveillance at the expense of core, traditional surveillance and epidemiology infrastructure (the public health epidemiologists or nurses who would investigate natural or intentional disease outbreaks). Similarly, it would make little sense for public health departments or hospitals to focus on syndromic surveillance before concerted efforts had been expended to enhance traditional public health surveillance.

Environmental Monitoring for Covert Biological Releases

First-generation environmental biosurveillance systems have recently been deployed in some urban centers to routinely test for certain select biological agents. This technology currently relies upon air sample collection onto filter media and transport to local or state public health laboratories for polymerase chain reaction analyses. Second-generation systems are in development that will be more automated, thus decreasing the potential impact that biosurveillance systems would have on public health laboratory resources.

While biodetectors have been deployed successfully in combat environments, their use in civilian settings is new. Technical limitations include cost, impact on public health laboratory resources, and the potential for disruptive false positive results. As these barriers are overcome, it will be important to deploy these systems with forethought and care. Not all jurisdictions live under the same level of ongoing threat, and not all locations within a city are likely targets for attack. Threat assessments by local, state, and federal law enforcement agencies can guide a rational strategy for the deployment of environmental biomonitors. Essential to the use of these new systems will be effective and coordinated multidisciplinary planning among local agencies (including public health, law enforcement, emergency management) for the potential response strategies that would be implemented depending on which potential bioterrorist agent is detected and the number and distribution of sites that test positive. These response plans need to include the surveillance, environmental, laboratory, and forensic assessments that would be initiated to determine if a positive environmental finding has implications for human health, as well as whether environmental results alone necessitate communication to the public and clinical interventions, such as antibiotic or vaccine prophylaxis.

Coordination with Law Enforcement

In the U.S., a suspected or confirmed bioterrorist outbreak is a criminal event, and requires the involvement of law enforcement including the Federal Bureau of Investigation (FBI) and local/state police. The primary agency charged with leading the forensic investigation is the FBI, with support from local and state law enforcement. These disciplines have not historically worked closely with the public health community and relationships needed to be established, especially at the local level. One of the lessons learned during the anthrax attack of 2001, was the value of public health and law enforcement officials knowing each other ahead of time as opposed to first meeting at the time of a crisis [5].

At both the local and federal levels, public health and law enforcement officials should establish consensus protocols regarding how they will communicate and coordinate during any investigation of a suspected bioterrorist attack. These protocols should address the mutual importance of early notification to the other discipline when there is concern or suspicion of a potential event. If law enforcement officials become aware that terrorists known or suspected to have access to biologic weapons are present locally, the health department should be notified to be alert to any suspicious disease occurrence, including lowering the threshold for investigating any aberrations in existing syndromic surveillance systems or reports of an unusual disease case or cluster. In certain circumstances, the health department might notify hospitals, so that active surveillance is enhanced. Likewise, if public health officials detect sporadic disease or a small disease cluster potentially caused by a bioterrorist agent, this information may need to be shared confidentially with law enforcement to evaluate whether the victims or close contacts are associated with any known terrorist group. The threshold for providing law enforcement with confidential patient information needs to be high and consistent with local, state, and federal statutes and regulations, and criteria for a potential bioterrorist disease/cluster need to be well-defined. Open communication links with clearly designated points of contact should be established between these respective disciplines so that intelligence or disease-specific information can be shared confidentially and securely.

Once a suspected covert bioterrorist event is detected, public health and law enforcement staff will need to conduct joint epidemiologic and forensic investigations of potential victims and their close contacts to determine the exact site, time, and circumstance of the initial release. This may require joint interviews of patients and families in hospitals, sharing of data, and active participation in each other's respective meetings to discuss findings of both epidemiologic and criminal/intelligence information. Any laboratory specimens obtained as part of the investigation, including both clinical and environmental samples, will be considered potential evidence and will need to be collected with full attention to chain of custody documentation requirements.

Public Health Reference Laboratory Testing of Suspicious Clinical and/or Environmental Samples

A close and active partnership between the public health laboratory and local clinical laboratories is essential to any infectious disease emergency response. Clinical laboratories must be aware of how to reach local or state health departments on a 24-hour basis in the event that a test result of potential public health importance is identified. Protocols on the proper procedures for packaging and transporting clinical specimens must be in place between hospital laboratories and local/state health departments, and between the health departments and CDC. In addition, the current capacity at local clinical microbiology laboratories for diagnosing microorganisms that could potentially be used as bioterrorist agents should be assessed. Some hospital microbiology laboratories may have (or could develop) the capacity for preliminary identification of the bacterial bioterrorist agents.

The CDC and the Association of Public Health Laboratories (APHL) have developed a tiered-response national laboratory system, the Laboratory Response Network (LRN), for handling the testing and confirmation of potential bioterrorist agents. Level A laboratories include many hospital and commercial laboratories and require the use of established protocols for the initial testing of suspicious specimens as well as the ability to "rule out" a bioterrorist agent. Appropriate training materials and slides are available via the LRN to educate hospital laboratory staff regarding the staining properties, growth characteristics on routine media, and preliminary biochemical test results for bacterial agents, as well as the need to immediately report to the local health department and arrange for confirmatory testing if a potential bioterrorist agent is suspected. Suspicious samples must be referred to Level B or C laboratories that involve federal, state, and some local health departments. Level B and C laboratories have protocols and reagents for "ruling in" Select Agents. Level D laboratories include the CDC and Department of Defense laboratories. These laboratories have Biosafety Level 4 capabilities and can provide final confirmatory testing, especially for the initial victims of a suspected bioterrorist event.

Symposiums on the laboratory diagnosis and biosafety precautions for biological weapons of mass destruction and chain of custody requirements are being offered by state and local health departments, with the target audience being clinical microbiologists at local hospitals. All training materials and programs emphasize the critical potential role of the clinical microbiology laboratory in early recognition of a bioterrorist event, and that prompt reporting to the local or state health department is essential to the success of the public health response in the event of an attack.

Protocols for ensuring rapid collection and packaging of specimens from clinical laboratories, and safe transport to public health reference laboratories that meet forensic chain of custody requirements need to be in place and well understood by clinical laboratory staff at hospital-based and commercial laboratories.

If a potential bioterrorist event is suspected or recognized, the medical and public health response will require rapid confirmation of the etiologic agent so that disease-specific recommendations regarding medical management can be provided to clinicians and laboratorians. Since neither hospital nor commercial microbiology laboratories have the technical capability (LRN reagents and protocols are not available to Level A laboratories) to confirm bioterrorist agents (e.g., anthrax, smallpox, tularemia), suspicious bacterial cultures or other clinical specimens will require rapid and safe transport to a public health

reference laboratory for confirmatory testing. Antibiotic susceptibility data for the bacterial bioterrorist agents will also be essential so that the appropriate recommendations regarding antimicrobial therapy and prophylaxis can be provided to medical providers.

Active Surveillance and Epidemiologic Investigations after the Initial Detection of a Confirmed Bioterrorist Event

Once a bioterrorist event has been recognized and confirmed by laboratory testing, public health officials will be primarily responsible for (1) tracking the number of cases to define the scope of the incident and (2) performing epidemiologic investigations to determine the common source(s) and site(s) of exposure. After detection of an unannounced bioterrorist event, it will be essential to determine where and when the attack occurred, and who else may have been exposed (either at the event or due to downwind distribution of the aerosol if an outdoor release occurred) and thus require antimicrobial prophylaxis. The epidemiologic investigation will need to be coordinated with local and federal law enforcement officials, with joint interviews and sharing of collected data. Since a large bioterrorist event in an urban setting will likely result in cases throughout the metropolitan area and potentially in distant states and countries, there will be a need for interstate and international coordination of the epidemiologic investigation. If the attack was covert, outlier cases occurring among residents of neighboring or other jurisdictions may provide valuable information to help identify the site and time of release.

Active surveillance needs to be initiated rapidly once a bioterrorist event is recognized. Pre-readied materials are invaluable to ensure the ability to expedite an investigation, including: (1) surveillance instruments (e.g., ready-to-go generic questionnaires for case ascertainment and risk exposure histories that can be rapidly modified to the specific circumstances under investigation); (2) a sampling strategy to use when conducting a rapid, large-scale epidemiologic investigation; (3) a centralized database system with linkages to the laboratory to facilitate tracking the outbreak and data analysis; and (4) a communication system and protocols for mobilizing and deploying active surveillance teams to area hospitals.

Data management will be one of the highest priorities and challenges during an acute, high profile epidemiologic investigation of a potentially large outbreak. Effective outbreak data management requires the linking of clinical and epidemiologic data with laboratory information, including whether appropriate specimens have been obtained, test results, and the patient's case status (i.e., suspect or laboratory-confirmed). Appropriate public health decisions will depend on having up-to-date, accurate information about the evolving outbreak, and political leaders, the media, and the public will need accurate information describing the impact of the event. This requires having flexible, tested databases that can be modified to the specific event. These systems should be exercised during routine outbreak investigations to facilitate efficient use during emergencies.

Guidance for Hospitals and Medical Providers

Most medical and laboratory professionals in the U.S. have had minimal clinical experience with the most worrisome bioterrorist agents (e.g., anthrax, smallpox, or plague). In the event of a bioterrorist event, public health authorities will need to provide timely information on the medical management of these diseases and how to coordinate with local, state, and federal public health partners.

Disease-specific protocols should be prepared ahead of time for the biologic agents of greatest concern (e.g., anthrax, smallpox, tularemia, Q fever, botulism, or plague). These protocols should address clinical presentations, diagnosis, therapy, patient isolation and waste disposal, biosafety issues for handling clinical specimens, and preventive therapy. These draft protocols can then be modified to the specific circumstances of the event and rapidly distributed. Additional information that should be included in guidelines for hospitals and medical providers include: clear criteria for reporting suspect cases (including clinical and epidemiologic features that meet the public health case definition); instructions for submitting laboratory samples for testing at the public health reference laboratory; details regarding mass prophylaxis plans being coordinated by the local or state authorities, and mechanisms to obtain current patient information materials being prepared by the health department. These protocols will need to be updated as new information becomes available. Electronic and paper copies of these disease specific protocols should be readily accessible in several sites for rapid distribution in the event of an emergency. Frequent public health alerts to provide updates on the evolving outbreak and any changes in public health recommendations should be provided as often as needed.

Health departments also need to be able to rapidly mobilize medical hotlines using clinically-trained staff who would be available to triage calls regarding suspect cases as well as answer questions regarding the medical management of cases, their close contacts, and potentially exposed persons who are not yet symptomatic. The staffing, training, and telephone equipment needs for this unit should be pre-defined. Ideally, pre-existing provider hotlines that are already in place for routine public health issues should be used. Given the potential for a marked increase in calls to this hotline during times of emergency, however, planning should address the need for enhancing staff and telephone line capacity as well as the hotline staff's training needs when an event is rapidly unfolding and information and guidance may change frequently.

Planning for Mass Medical and Mortuary Care and Mass Prophylaxis

Local and state public health authorities should play an active role in planning for how mass medical care will be addressed and coordinated from a jurisdiction-wide perspective, in coordination with area hospitals, emergency medical services, and emergency management agencies. Essential to planning efforts will be up-to-date information on existing bed capacity, including isolation capacity, as well as key equipment inventories, such as ventilators, for all acute care facilities within the jurisdiction. Local public health officials should work with area medical care facilities to test response plans using tabletop

and/or field exercises to assess institutional response to a bioterrorist disaster, with a focus on addressing those areas where close coordination between hospital and public health authorities are key to an effective response.

In the event of a successful release of an aerosolized bioterrorist agent, there is a potential for mass casualties on a scale that could easily overwhelm local hospital capacity. Therefore, contingency plans for how mass casualties will be handled, with special attention to isolation and infection control issues if an agent with potential person-to-person transmission is involved, will need to be developed. Individual hospitals or hospital networks will need to develop institutional-specific plans for how they will respond to a large infectious disease outbreak such as activating the hospital's incident management system, triaging massive numbers of visits to and admissions from the emergency department, canceling all nonemergent admissions, transferring non-acute patients, calling in additional staff, rapid re-opening of wards that have been closed due to decreasing census, and establishing emergency isolation units.

Difficult issues that need to be addressed ahead of time include whether and how specific hospitals will be designated to care for victims of a bioterrorist event, how limited resources will be distributed if insufficient supplies are available (e.g., ventilators), establishing alternate sites for triage of patients with less severe symptoms, and emergency credentialing procedures to allow non-affiliated staff to work in local hospitals. Mass care planning should be coordinated with local relief agencies, such as the American Red Cross, as well as public health authorities in neighboring states and counties. Pre-established memoranda of understanding for sharing resources are useful to have in place prior to emergencies, as has been done among fire departments and other traditional first responders. Finally, since there will likely be a need to call in federal support (e.g., Disaster Medical Assistance Teams), pre-determination of how and where these teams will be deployed needs to be planned.

Mass mortuary issues (including tracking, storage, and disposal of victims) need to be addressed by the local and state medical examiners, in coordination with local public health officials, emergency management, and hospital associations. Guidance for the handling and disposal of potentially infectious remains should be developed. For most potential bioterrorist agents, routine infection control practices should be sufficient to protect pathologists, medical examiner staff, and persons involved in preparing the body for burial or cremation. In the event of a smallpox attack, however, these professionals should be prioritized for receiving smallpox vaccine if not already vaccinated. Reducing the potential for disease transmission should also be a priority. Certain procedures (e.g., embalming) should be ended temporarily and recommendations for use of sealed caskets or cremation should be considered by local authorities. As a bioterrorist event is a criminal act, an efficient mechanism needs to be established urgently to assure that all deaths due to the outbreak are reported to the appropriate local authorities, such as the medical examiner or coroner's office.

In addition to medical care for ill patients, there may be a need to provide mass prophylaxis to potentially exposed persons and/or close contacts for certain bioterrorist agents (e.g., anthrax, smallpox). Planning for the rapid provision of antibiotics or vaccines to

large populations requires the involvement of public health and emergency management officials, with input from the local medical community. Efforts should focus on (1) pre-determination of which antibiotics and vaccines may be needed (including recommendations for special populations, such as children and pregnant women) and (2) how these medications will be mobilized and distributed rapidly in the event of a bioterrorist event. Although there is currently a federal stockpile of medications and supplies (the Strategic National Stockpile), local and state officials should consider whether to maintain a smaller stockpile locally to ensure supplies are available in the first hours or days after an attack is detected, given cost issues and limited shelf-life of many pharmaceutical agents. Specifically, contingency plans for setting up community-based, mass prophylaxis clinics that address staffing resources and equipment/space requirements, as well as procedures that outline patient flow need to be developed ahead of time [2]. Many jurisdictions are creating a reserve corp of medical volunteers to help support these clinics, and providing training on expected roles and responsibilities in the event that such clinics are required.

Multi-lingual medical information sheets and vaccine informed consent forms should be prepared, and multiple mechanisms for rapid mass reproduction identified. Risk communication strategies should be developed to ensure that persons at-risk understand the need for compliance with prevention messages, and as importantly, that those not at-risk understand the need to avoid over whelming hospitals and clinics if they do not have an exposure or symptoms of concern. Mass prophylaxis plans need to consider the specific challenges in distributing antibiotics and vaccine to difficult-to-reach populations, such as the homeless and homebound.

Interagency and Intersector Coordination and Communication

The response to a large biologic disaster, whether a bioterrorist event or natural outbreak such as pandemic influenza or SARS, will require successful coordination and communication between public health agencies, other relevant local/state (e.g., emergency management, police, fire/HAZMAT) and federal (e.g., CDC, FBI) agencies and the healthcare sector (both in-patient and out-patient). A centralized emergency operating center is essential to facilitate coordination and communication. In the event of an emergency, pre-designated representatives from all involved agencies and any local or state hospital associations should be assigned to this center to ensure effective coordination of the overall response.

The public health sector's communication, transportation, and other equipment or infrastructure needs for disaster response should be assessed ahead of time. Essential resources include reliable and redundant communication capacity (e.g., cellular telephones, laptop computers with modem, 2-way and 800-megahertz radios or satellite telephones); broadcast facsimile and electronic mail capability; secure internet sites (e.g., Health Alert Network) to rapidly notify and inform the healthcare sector regarding events of public health concern; and computer systems that are networked between the local/state health department, the local emergency management command center, and appropriate state/federal agencies. Back-up generators should be available as well as alternative locations to meet if the primary emergency operations center is damaged due to the disaster.

Ongoing training for public health staff should not just include the clinical, laboratory, and epidemiologic features of the bioterrorist agents, but also focus on the agency's emergency response command system, with an emphasis on the staff's expected roles and responsibilities during the health department's response to a bioterrorism event. Among the more effective training tools are tabletop and field exercises, with involvement of representatives from all key local, state, and federal agencies, as well as representatives from the local medical and laboratory communities. These exercises provide the opportunity to test assumptions in existing plans, and work out issues related to decision-making authority and respective roles and responsibilities among the various disciplines that would be involved in responding to a bioterrorist attack. A successful exercise will highlight gaps in preparedness that should be addressed through follow-up meetings and revision of written plans, if indicated, and re-evaluated with repeat exercises.

Legal Issues Related to the Public Health Response to Bioterrorism or other Infectious Disease Emergencies

Many state and local public health laws have not undergone major revisions since the middle of the last century. Although current regulations are adequate for routine public health concerns, the response to a bioterrorist event may require emergency powers beyond existing legislative or executive authority. In 2002, the CDC, in collaboration with Georgetown University School of Law, developed a model state public health law for jurisdictions to use to assess their current regulations and implement modifications to those areas that were not yet adequately addressed [11]. In addition to ensuring sufficient authority to collect disease surveillance data, conduct contact tracing, and provide preventive measures to those at risk, public health laws need to provide health officials with the authority to implement isolation and quarantine measures if needed to control a severe and virulent contagious communicable disease outbreak. Isolation and quarantine regulations should include the components needed to establish and enforce a large-scale quarantine including who has this authority, what criteria need to be met, the legal mechanism for rapid implementation in the event of an emergency, and who will be responsible for enforcement as well as providing for due process measures to protect those affected. In addition to the legal aspects of isolation and quarantine measures, public health officials need to ensure that plans address the operational aspects of implementing and enforcing these regulations. Specific issues include developing criteria for the use of home quarantine versus removal of contacts to separate facilities, identification of potential facilities that might be used to isolate contagious patients or quarantine close contacts, determination of how detained persons will be fed and cared for, as well as addressing mechanisms to compensate detainees for lost wages.

Environmental Issues

Public health agencies need to have plans and staff expertise to assess the environmental impact of an attack and determine if remediation efforts are needed to decontaminate the affected site(s). Environmental health specialists should work closely with their counterparts in infectious disease epidemiology to plan for how epidemiologic and environmental investigations will be coordinated, especially if the attack was covert and these investigations are needed to identify the initial site of release.

The anthrax attacks of 2001 revealed the lack of knowledge regarding the environmental impact of weaponized bioterrorist agents in indoor settings. At that time, no public health agency at the local, state, or federal levels had sufficient expertise or experience regarding methods for collecting and testing environ mental samples of weapons grade anthrax in workplace-type settings, interpreting these findings to assess ongoing risk, or optimal methods for decontamination and remediation of affected environments. Fortunately, this experience did lead to the development of federal guidelines for assessing environmental contamination due to weaponized anthrax. Similar efforts now need to address the other potential bioterrorist agents, especially regarding whether there are any ongoing environmental risks when agents that do not have a spore form are released.

Mental Health Preparedness and Response

Both the World Trade Center attack and the outbreak of intentional anthrax due to contamination of the mail highlighted the dramatic psychological effects that a terrorist event can have on the public, even in areas far removed from the actual events. One of the primary targets of terrorism is the public's mental health, with the potential impact lasting beyond the immediate event and affecting persons far from the area affected. The media often plays an unwitting role in facilitating this with constant replays and graphic images shown frequently on television and in newspapers in the immediate aftermath of an event.

In New York City, soon after the World Trade Center attacks, a telephone survey revealed that between 7.5 and 40% of Manhattan residents had symptoms consistent with post-traumatic stress disorder; the prevalence was higher among those closer to the site or among those who had witnessed the attack [9]. As impressive, a similar survey conducted nationwide within a week of the attack revealed that 44% of adults and 35% of children had one or more stress symptoms [23]. The tremendous subsequent number of "powder incidents" illustrated that one does not need sophisticated weapon delivery systems to cause public panic. In many of the affected jurisdictions, it was not the outbreak response at the affected worksite locations that overwhelmed local public health and emergency response authorities, but the hundreds to thousands of calls regarding concerns about potential "powder threats". This illustrated the impact that the "worried well" can have on the public health and medical care systems, and many jurisdictions did not anticipate nor were they prepared for the response needed to manage these calls.

Unfortunately, mental health preparedness is an area that many local and state public health agencies have minimal staff expertise or experience addressing. It is essential that a community's bioterrorism response plan address the community's mental health response to terrorism both before and after an event. Pre-planning efforts for mental health preparedness should include development of a risk communication strategy with training of all potential public health spokespersons, as well as establishing surge capacity for mental health services after an event occurs [10]. Ideally, the public and media should be educated ahead of time about the risk of bioterrorism and relevant details of their local government response plans so that they know what to do in the event of an attack and what steps can be taken to improve personal, family, and community preparedness.

Planning for the potential demands for mental health counseling should not be limited just to the actual victims of the attack, but also should address the needs of the victims' families and friends, those responding to the event including traditional first responders, the medical provider community, and the general public. Strategies could include plans for rapidly establishing crisis hotlines and referral sites, and for mobilizing additional assistance through creation of a mental health reserve corps. Involvement of community-based organizations, religious leaders, and local government officials in both pre-planning and response efforts will be essential. Given the potential for the "worried well" to overwhelm medical care services, as was seen in the immediate days after the anthrax attack was first recognized, a pro-active, clear, and effective risk communication strategy should be prioritized to ensure that the public understands what is known about the event; who is at risk; which symptoms

suggest the need for medical evaluation; the importance of not seeking medical care if one does not have these symptoms to avoid overwhelming local hospitals and clinics; and who needs antibiotic prophylaxis and where and when to go to obtain it if indicated.

Communication with the General Public

As with any major disaster, one of the most important components of the government response is a pro-active, effective communication strategy. An essential component of a communication strategy is having pre-existing and effective communication links with the media (including local and national print, radio, and television outlets). The multidisciplinary involvement in the response to a bioterrorist event will require coordination of media outreach through a joint information center that includes local, state, and federal officials. Public affairs staff at hospitals should coordinate any public messages with their counterparts at the local and state health departments.

There should ideally be one primary pre-designated spokesperson to provide consistent messages throughout the disaster response. This spokesperson should be clearly in charge (e.g., the top elected official), be an effective, clear, and concise communicator, and be available for frequent press briefings. Although the primary spokesperson does not have to be a medical or public health official, it will be essential that persons with such expertise be present to answer or clarify health-related questions or issues. One of the most difficult risk communication challenges following a bioterrorist event will be communicating uncertainty, given that it may take days or weeks before the full circumstances of the event are known. It will be important for spokespersons to be ready to admit what they do not know, yet also be able to reassure the public by providing detailed information regarding what is being done to answer all key questions. Frequent updates should be provided to the news media and public when new information becomes available.

Although the most efficient mechanism for communicating to the general public is through the news media, public health officials also need to be prepared to provide additional information through other mechanisms to be able to address the numerous questions and concerns that the news media stories will generate among the public. Fact sheets on the likely bioterrorist agents should be prepared ahead of time for the general public, posted on the agency's public website and then tailored to the specific events. During the emergency, these informational sheets should be widely distributed to multiple venues. In most parts of the country, translations into one or more languages will be needed.

In addition to written materials, contingency plans for establishing a hotline for the general public should be a key component of the public health response planning efforts. Hotlines will likely need surge capacity for the immediate hours and days after an acute event, with respect to both staff and telephone infrastructure. Staff used for hotline support need to be trained for handling acute calls from a concerned public, and mechanisms need to be in place to provide ongoing training and updates, as information about the outbreak and public health recommendations will likely evolve rapidly over the course of the event.

Summary

After the terrorist events of 2001, there was nationwide recognition of the importance of improving and maintaining the public health infrastructure at local, state, and federal levels as a primary defense against bioterrorism. On January 31, 2001, the U.S. Department of Health and Human Services announced the availability of $1.1 billion in federal funding that was made available to all states, and 4 large urban areas (Chicago, District of Columbia, Los Angeles, and New York City), in Federal Fiscal Year 2003. This level of funding was continued in 2004. This has provided an unprecedented opportunity to address critical gaps in current bioterrorism public health preparedness plans.

Jurisdictions have used these funds to address the following key areas: emergency planning and response for biologic or chemical terrorism events, as well as natural disease outbreaks; enhancing surveillance and epidemiologic capacity; expanding reference laboratory services, especially for confirmation of the CDC Category A and B agents; developing or enhancing environmental health expertise; planning for large-scale antibiotic and vaccine distribution clinics; developing or expanding secure electronic communication links with key partners at the local level; establishing or enhancing local and state legal authorities for implementing and enforcing isolation and quarantine; ensuring that communication mechanisms and strategies are in place to provide up-to-date information to the medical community and general public; providing risk communication and media training for key public health staff; training of medical providers on the clinical aspects of the potential bioterrorist agents; and mental health preparedness planning.

Integration of bioterrorism-related surveillance, laboratory, environmental, and communication efforts into routine public health activities will improve the core public health functions, which in a crisis need to function well. This will also have the dual benefit of improving local and state public health responses to natural disease disasters, such as would be required to respond to pandemic influenza or SARS. Significant advances have been made in the past 2 years. Enhancing our public health infrastructure is a long-term investment, one that will have long-lasting impact in protecting the public's health from natural, as well as intentional disease threats.

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Lessons Learned: Remediation of Anthrax Contamination

Dorothy A. Canter, Ph.D.

U.S. Environmental Protection Agency, U.S.A.

Introduction

The 1999 Consensus Statement on Anthrax as a Biological Weapon of the Working Group on Civilian Biodefense concluded that “decontamination of large urban areas or even a building following exposure to an anthrax aerosol would be extremely difficult and is not indicated” [7]. Although the prevailing opinion at that time, events since then have demonstrated that civilian structures contaminated with aerosolized anthrax spores can be successfully remediated.

In the Fall of 2001, 3 known terrorist attacks occurred in which *Bacillus anthracis* (*B.a.*) spores were transmitted through the United States (U.S.) mail system. In the first attack, letters mailed from New Jersey to media outlets in New York City passed through the Hamilton Processing and Distribution Center (P&DC) in Trenton, New Jersey on September 18. The second attack involved a letter or package sent in late September to American Media Incorporated (AMI), a publisher of weekly newspapers, in Boca Raton, Florida. In a third wave, letters to Senators Tom Daschle and Patrick Leahy entered the Hamilton P&DC on October 9. The Federal Bureau of Investigation subsequently recovered 4 letters; the letter to Tom Brokaw of NBC, the letter to the *New York Post*, and the letters to Senators Daschle and Leahy. It is believed that there were at least 7 such letters.

Twelve cases of cutaneous anthrax and 11 cases of inhalational anthrax resulted from these attacks [3,4]. Five of the persons with inhalational anthrax died. The cases of inhalational anthrax were reported only after the second and third attacks. Seven of the 11 cases occurred in postal workers in New Jersey and the Washington, D.C. area, while 2 occurred in AMI employees. All 9 of those persons are believed to have been exposed to letters or packages known to contain *B.a*. spores [10]. Two of the workers at the Washington, D.C. facility as well as one AMI employee died. The route of exposure to the other 2 cases, a woman who worked in a New York City hospital and an elderly woman who lived in Oxford, Connecticut, is unknown, since no *B.a*. spores were identified in any environmental sampling performed in connection with these cases. Both of these women died.

Numerous sites were contaminated either directly or through secondary (cross) contamination. Among these were media offices, postal facilities, the Hart Senate Office Building, and residences. The contaminated postal facilities included large P&DCs such as the Hamilton P&DC, the Morgan P&DC in New York City which processes all mail into and out of Manhattan, and the Curseen-Morris facility (name changed from Brentwood to Curseen-Morris in memory of the 2 deceased workers) in Washington, D.C., which handles all mail to and from the U.S. government in the D.C. metropolitan area. Numerous smaller U.S. Postal Service facilities also experienced contamination, as did a number of federal government mail facilities downstream of the Curseen-Morris facility.

Remediation of Anthrax-Contaminated Sites

The remediation process for anthrax-contaminated sites has consisted of up to 8 steps: site assessment including environmental sampling to characterize the contamination, isolation of contaminated areas, artifact and critical item removal for off-site treatment, source reduction, remediation of contaminated areas, post-remediation environmental sampling, further remediation and sampling if the initial post-remediation sampling indicated continuing areas of contamination, and disposal of decontamination waste.

Environmental sampling is a key activity at a number of phases of the remediation process, from confirming the existence of contamination, through identifying the nature and extent of the contamination (characterization sampling), to assessing the usefulness of specific source reduction activities prior to implementing the main remediation, to ultimately evaluating whether the remediation has been effective (clearance sampling) and the site is ready for re-occupancy.

Environmental sampling for *B.a.* spores has evolved significantly since the initial sampling events in the Fall of 2001. Consensus exists that in-depth environmental sampling should be performed to characterize the nature and extent of contamination before any cleanup activities are undertaken. Further, wipe samples should be used for sampling large surface areas, and wet sampling techniques are more effective than dry techniques. In addition, air sampling of specific areas should be incorporated into the sampling plan, both before and after decontamination, particularly for sites at which primary aerosolization events have taken place. Finally, sampling results should be reported in as quantitative a form as possible [27].

The U.S. Environmental Protection Agency (EPA) has 2 important roles in the remediation process. First, EPA has the responsibility to ensure that the cleanups are performed in accordance with the Comprehensive Environmental Restoration Compensation and Liability Act (Superfund Law), either by performing the cleanups or by providing technical assistance to the organizations conducting the cleanups. The extensive experience that staff in the EPA waste program have gained over a quarter of a century from cleanups of chemical spills and hazardous waste sites has been an important asset in planning and implementing the remediations of anthrax-contaminated sites. Second, EPA has granted crisis exemptions for treatments with pesticidal agents not registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). No chemical agent has ever been registered specifically to kill *B.a.* spores. Hence, organizations responsible for the cleanup of sites and chemical manufacturers have had to submit to EPA crisis exemption requests under FIFRA that are supported by data on the expected efficacy and safety of the remediation process, in order to receive approval to use the chemical agents proposed for that process. The expertise of the staff in the EPA Antimicrobials Program in treating microbiological contamination with antimicrobial chemicals has added substantially to the efficacy of the remediation processes. As of September 2003, EPA had received 52 crisis exemption requests, issued 23 exemptions, rejected 29 requests, and withdrawn 2 exemptions that were initially approved [12].

Chemical agents that have been approved for cleanups of non-porous surfaces are

chlorine bleach (sodium hypochlorite), aqueous chlorine dioxide, and mixtures of hydrogen peroxide and peroxyacetic acid. Three chemical agents have received crisis exemptions for use in fumigating contaminated sites – gaseous chlorine dioxide, formaldehyde generated from paraformaldehyde polymer, and vaporized hydrogen peroxide. Of these, formaldehyde has a long history of usage [30].

Fumigation of Sites

When all remediation activities have been completed, at least 6 of the contaminated sites will have been fumigated. They are portions of the Hart Senate Office Building of the Capitol Hill Anthrax Site, the Curseen-Morris P&DC, the Hamilton P&DC, the Department of Justice (DOJ) postal facility, the General Services Administration (GSA) Building 410, and the Department of State (DOS) Annex-32 (SA-32) in Sterling, Virginia. A seventh site, the building previously owned by AMI but purchased in the Spring of 2003 by White Palm Real Estate, Inc., is currently being evaluated as to the remediation approach that will be used. Aerosolization of *B.a.* spores within the facility is known to have occurred, however, since 2 AMI employees developed inhalational anthrax. Moreover, experiments performed in the Daschle suite of the Hart Building by scientists in personal protective equipment prior to the fumigation demonstrated secondary aerosolization of viable *B.a.* spores in the suite [29]. Therefore, unless it can be demonstrated by extensive environmental sampling, including aggressive air sampling, that re-aerosolization of the *B.a.* spores remaining in the AMI building will not occur, the site will probably need to be fumigated. Four other sites were known to have experienced primary aerosolization events, as demonstrated by the opening of the highly contaminated letter in the Daschle suite and by the occurrence of inhalational anthrax in workers at the Curseen-Morris and Hamilton P&DCs and at DOS SA-32.

Gaseous chlorine dioxide (ClO_2) was used for the first 3 sites, formaldehyde generated from paraformaldehyde was used at the DOJ mail facility, and vaporized hydrogen peroxide (VHP) was used to remediate GSA Building 410 and SA-32. At 2 of the sites, fumigation was/will be performed of the entire site at one time (Curseen-Morris, Hamilton), while at 2 other sites (GSA Building 410, SA-32), the entire site was fumigated, but in sub-sections treated one at a time. At two of the sites (Hart Senate Office Building, DOJ postal facility) only a portion of the entire facility was fumigated; other areas with secondary contamination received surface treatments; and still other areas judged not to be contaminated with *B.a.* spores received no treatment at all. The volume of space fumigated at one time in these facilities ranged from 8,300 cubic feet (ft^3) to 14.5 million ft^3. The time required to complete fumigation remedies has ranged from 3 months for the Hart Senate Office Building to over 2 years for the Hamilton P&DC. The remedial approach for the Florida office building has not yet been selected. Five of the sites are mail facilities, while 2 are office buildings. Only the Florida site is in the private sector. Table 1 summarizes information on the 6 sites with fumigation remedies.

The selection of the fumigant for a particular facility results from a consideration of the following factors: effectiveness of the agent, both historically and in anthrax attack cleanups to date; toxicity; penetration capability; materials compatibility; generation of the agent;

post-fumigation aeration of agent and potential by-products from absorbing materials; nature of the site to be treated; cost; and duration of cleanup. Each agent has its advantages and disadvantages, there is no silver bullet [24]. For example, formaldehyde generated on site from paraformaldehyde has been routinely used for years to decontaminate biosafety hoods and research laboratories, as well as to fumigate buildings used in the U.S. biowarfare program prior to its termination. A standard has been issued by the National Science Foundation/American National Standard Institute for the use of (para)formaldehyde to decontaminate Class II biosafety cabinetry [17], and the National Institutes of Health recommended formaldehyde as the chemical of choice for space disinfection in its 1979 Laboratory Safety Monograph [21]. VHP is routinely used in the pharmaceutical industry [19] for isolator decontamination and in the biodecontamination of rooms up to 7,000 ft^3. Prior to the 2001 anthrax attacks, VHP had not been used to fumigate sites with *B.a.* contamination. Chlorine dioxide is used mainly as an alternative to chlorine for the disinfection of drinking water and in the bleaching of paper. Gaseous ClO_2 also has applications as a sterilizing agent for biomedical and pharmaceutical applications [13]. Similar to VHP, the gaseous form had not been used to treat anthrax contamination prior to the 2001 attacks.

Formaldehyde is an animal carcinogen and probable human carcinogen and is also genotoxic [9]. Neither ClO_2 nor VHP have been tested for carcinogenicity in long-term animal bioassays. Chlorine dioxide, however, is the most acutely toxic of the 3 fumigants with a permissible exposure limit (PEL) of 0.1 parts per million (ppm) and an Immediately Dangerous to Life or Health (IDLH) value of 5.0 ppm. The PELs for formaldehyde and hydrogen peroxide are 0.75 ppm and 1.0 ppm, respectively, while the IDLHs for these compounds are 20 ppm and 75 ppm, respectively [15]. Table 2 contains information that should be considered in evaluating the 3 agents for fumigation of a civilian site.

Table 1: Sites with Fumigation Remedies

Sites with Fumigations	Nature of Contamination	Fumigant	Volume of Space Fumigated	Fumigation Approach	Duration of Remediation*
Daschle Suite/Hart Building/Capitol Hill Anthrax Site	Aerosolized	Chlorine dioxide	90,000 ft^3 on 2 floors	All at one time	3 months
Dept. of Justice mail facility	Secondary	Paraformaldehyde	8,300 ft^3 (tented space containing mail sorting machine)	All at one time	5 months
GSA Building 410	Secondary	VHP**	1.6 million ft^3	Fumigation of 9 zones individually	Not completed yet – more environmental sampling needed
Curseen-Morris P&DC	Aerosolized	Chlorine dioxide	14.5 million ft^3 on 2 floors	All at one time	Approximately 24 months
SA-32	Aerosolized	VHP	1.4 million ft^3	Fumigation of 10 zones individually	Approximately 24 months
Hamilton P&DC	Aerosolized	Chlorine dioxide	5.8 million ft^3	All at one time	Greater than 24 months

* Remediation times do not include time required to renovate sites following remediation processes.

** Vaporized hydrogen peroxide

Table 2: Factors to be Considered in Selecting Fumigant for *B. Anthracis* Contamination

Chemical (Molecular Formula)	Chlorine dioxide (ClO_2)	Formaldehyde (HCHO)	Vaporized Hydrogen Peroxide (H_2O_2)
Chemical/physical properties	Yellow-green gas, single electron oxidizing agent, unstable in UV light, explosive at [ClO_2] >10%	Flammable, colorless gas at room temperature, pungent, suffocating odor; very reactive, combines readily with many substances, polymerizes easily	Nonflammable, colorless, nearly odorless vapor, which is heavier than air; powerful oxidizing agent; very water soluble
Workplace exposure limits	PEL*:0.1 ppm; IDLH**: 5.0 ppm	PEL: 0.75 ppm; IDLH: 20 ppm	PEL: 1.0 ppm; IDLH: 75 ppm
History of usage	Used in water disinfection, and in pulp and paper industry; first use as fumigant in civilian building was at Hart Senate Office Building	Routinely used to fumigate research biosafety hoods and labs, and buildings used in U.S. biowarfare program	Used in pharmaceutical industry to clean manufacturing and animal toxicology study rooms; first use as fumigant for *B.a.* was at Building 410
Penetration capability	Good	Good	Limited
Generation of fumigant	On-site by reaction of sodium chlorite with other chemical(s)	On-site by heating paraformaldehyde	On-site from concentrated 35% H_2O_2 solution
Destruction of fumigant after treatment	Scrubbing through reaction with reagents that destroy chlorine dioxide, can be followed by passage through carbon absorption bed	Vaporizing of ammonium bicarbonate to neutralize HCHO	Closed system with passage of return vapor through palladium/platinum catalyst throughout process to convert H_2O_2 to water and oxygen
Toxicity	Highly acutely toxic to skin, respiratory and gastrointestinal tracts, and eyes. No animal data on inhalational carcinogenic potential.	Animal carcinogen/probable human carcinogen; genotoxic	Repeated exposures to vapor may cause chronic irritation of respiratory tract; no data on carcinogenic potential

* PEL: permissible exposure limit promulgated by Occupational Safety and Health Administration as safe for 8 hour time weighted average workplace exposures throughout employment

** IDLH: Exposure level considered to be immediately dangerous to life or health for exposure periods of 15 minutes duration.

Addressing the Safety and Efficacy Issues for Fumigations

Before EPA will issue a crisis exemption for the use of a particular fumigant to remediate *B.a*. contamination, the organization with responsibility for the cleanup must submit a site-specific remediation action plan that describes how the fumigation can be performed both safely and effectively.

There are several critical safety issues. First is the containment of the space to be fumigated. Containment may be achieved by maintaining negative air pressure within the facility or by tenting the facility. Prior to fumigation it is important that studies be performed to test the effectiveness of the containment. This testing is particularly crucial for sites at which the entire facility is to be fumigated at one time. During fumigation, it is also necessary to monitor for leakage of fumigant from the facility. For each fumigant, action levels for ambient concentrations of the fumigant need to be set in the event of significant leakage or of releases due to equipment failures or other emergencies. The action levels, both for temporarily pausing the fumigation and for terminating it, will depend on the acute toxicity of the fumigant and the site being fumigated. In addition, an emergency response plan addressing worst case and reasonably expected failure scenarios, and potential acts of terrorism, needs to be developed and approved by both EPA and the responsible local governmental agencies prior to the start of fumigation. It is important to run pre-fumigation

tests of the equipment to be used during the fumigation process, including the fumigant generation equipment, the fumigant removal equipment, and the monitoring equipment for key process variables, so that any needed safety modifications can be made before the actual fumigation takes place. At the end of fumigation, it is important to remove/destroy the fumigant and process by-products quickly and efficiently. The nature and extent of equipment used for this purpose will depend on the fumigant and the volume of space fumigated at one time.

In terms of the efficacy of the fumigation process, it is critical to maintain the key process variables – concentration, exposure time, relative humidity, and temperature – within the ranges specified for each phase of the fumigation process, as approved in the EPA crisis exemption. For each fumigant, specified ranges exist for each of the variables. For example, the relative humidity for chlorine dioxide fumigations should be in the range of 70 to 95% throughout the process, while the relative humidity prior to introduction of VHP into the space to be fumigated should be no greater than 40%.

To maintain the key process variables within the prescribed ranges throughout the space being fumigated, it is important to ensure appropriate distribution of the fumigant. This can be achieved by the usage of an adequate number of fans located and directed to deliver the fumigant to hard to reach locations. The presence of materials that serve as absorbers of the fumigant within the space to be treated also must be taken into account to ensure the maintenance of the specified fumigant concentration in all regions throughout the treatment process. Where possible, all such materials (e.g., carpets, draperies, ceiling tiles) should be removed prior to the fumigation. Adequate numbers of monitors to measure fumigant concentration, relative humidity, and temperature on a real time basis need be placed in locations that are hard to reach for the fumigant. Experience to date emphasizes the need for redundancy of key equipment, especially monitoring equipment, given the equipment failure rates that have been observed.

Biological indicators placed throughout the space prior to fumigation are collected after the fumigation and used to measure the effectiveness of a fumigation process. To be fully successful, a fumigation must kill the prescribed numbers of spores of a surrogate species on all biological indicators placed within the space prior to the fumigation. Spores from a species within the *Bacillus* family that is not pathogenic to humans, but that is both genetically similar to, and at least as resistant as, *Bacillus anthracis* spores, are placed on carriers such as paper strips or metal coupons. The species utilized will depend upon the fumigant being used. For example, *Geobacillus stearothermophilus* is considered the indicator species of choice for fumigations utilizing VHP, since it is the most resistant to VHP of the surrogate *Bacillus* species which have been validated for FDA sterilization processes [2]. For ClO_2, *Bacillus subtilis* is the most resistant species, followed by *Geobacillus stearothermophilus* [24]. The U.S. Army Medical Research Institute of Infectious Diseases' (USAMRIID) issued regulations for formaldehyde fumigations which specify the use of both *Bacillus subtilis* and *Geobacillus stearothermophilus* spore strips [20]. In general, strips containing 1 million spores of the surrogate species are used.

The number and placement of the spore strips within the space to be fumigated are important decisions. A number of the organizations performing fumigations have followed

the USAMRIID procedures, which specify the use of at least one spore strip for every 100 ft^2 fumigated [23]. Spore strips are placed in locations that are hard for the fumigant to reach, to ascertain whether high enough fumigant levels are reached in those locations for sufficient periods of time to kill all the surrogate spores. They are also placed in locations identified or suspected to have *B.a.* contamination based upon the characterization environmental sampling.

It is useful to select a laboratory to analyze growth of the biological indicators that is independent of the organization conducting the fumigation to prevent potential conflicts of interest. The laboratory should have sufficient expertise and experience in analyzing biological indicators, so as to decrease the potential for adventitious contamination that will compromise interpreting the results of the fumigation.

The decision to fumigate all of the facility at once rather than to fumigate smaller subsections one at a time has ramifications on both the potential safety and efficacy of the process. The larger the space to be fumigated at one time, the greater the need to assure effective containment and to have a safe, reliable, and efficient system for removing the fumigant after the process. Moreover, the larger the space, the greater the challenges to achieving adequate distribution of the fumigant throughout the space and to maintaining the process parameters in the specified ranges, particularly the relative humidity, throughout the entire process in all regions within the space. Further, such a decision may have impacts on the cost and time frame of the overall fumigation process.

A readiness demonstration is a highly recommended step prior to the fumigation(s) for both safety and efficacy purposes. During such a demonstration, the fumigant is introduced into the facility at a lower concentration and for a shorter duration than in the actual fumigation. The functioning of the equipment generating, monitoring, and destroying the fumigant is measured. Distribution and circulation of the agent are also checked, as is the adequacy of the containment in preventing leaks. The demonstration may be performed without obtaining a crisis exemption, as long as biological indicators are not used to monitor the effectiveness of the process. Results from the readiness demonstration are used to make necessary modifications to the process and can make the difference between a successful and failed fumigation.

Judging the Effectiveness of Fumigations

Two criteria should generally be met for a fumigation process to be considered fully successful. First, the key process variables of gas or vapor concentration, relative humidity, and temperature, as measured by real-time monitoring equipment, if available, should be within the specified ranges for the prescribed time periods for each phase of the fumigation process. Second, there should be no growth of spores from the biological indicators placed in the space. At the SA-32 facility, the DOS required that both of these criteria be met for the fumigation of each zone; otherwise, the fumigation had to be repeated. At GSA Building 410, which was remediated prior to the DOS facility, real-time monitoring of VHP concentration was not available. However, all spore strips had to be negative for growth of the indicator species in each zone; otherwise the zone had to be re-fumigated until that

condition was reached. There were 2 fumigations in several zones.

These criteria are derived from usage in the biomedical sterilization field, as regulated by the FDA [5], and from the regulation of USAMRIID for the decontamination of containment areas, which specifies that all spore strips used in a fumigation must be negative for growth of the simulant spores, or the fumigation must be repeated [20].

Judging the Effectiveness of the Overall Remediation Process

The criterion currently being used for judging the effectiveness of the overall site remediation process is zero growth of *B.a.* spores from all post-remediation environmental samples. This applies to all sites, regardless of whether the contamination occurred through a primary aerosolization event, such as in the Daschle suite or at the DOS mail facility, or as the result of secondary contamination [16]. Thus, clearance environmental sampling is performed following fumigations, even when the fumigations have been judged to be fully successful, and negative results therefrom constitute the ultimate criterion for a fully successful remediation. In those instances in which one or more clearance environmental samples yield positive growth of *B.a*. spores, further remedial work will generally need to be undertaken. The nature and extent of such additional remediation activities will depend upon the results of the clearance environmental sampling.

This criterion does not guarantee that all *B.a*. spores will have been killed by treatment, nor that there is zero risk of disease, but it does indicate that the risk of developing anthrax is negligible. It is a public health-protective criterion which is based upon the inability, given the current state of science, to estimate the minimum number of *B.a*. spores necessary to induce inhalational anthrax in exposed persons. Although values have been developed from experiments in laboratory animals for the number of inhaled spores that generate inhalational anthrax in 50% of the animals, the values vary from study to study [11,14]. Moreover, it is necessary to extrapolate to disease incidences that are significantly lower than 50% of the population and to consider potential differences in response between laboratory animals and humans. Further, susceptibility of sensitive sub-populations (e.g., immuno-compromised patients) needs to be taken into account. The occurrence in 2001 of fatal inhalational anthrax in 2 women with unidentified sources of exposure to presumably very low numbers of *B.a*. spores provides support that the number of *B.a.* spores needed to cause disease may be quite low [1,10].

Clearing Sites for Re-Occupancy

After all environmental samples are demonstrated to be negative for growth of *B.a.* spores at sites with fumigation remedies, the organization responsible for the remediation reviews the data to determine if the overall remediation has been effective. Thereafter, that organization will generally submit the totality of the remediation data to a multi-disciplinary group of experts for independent evaluation of the data. Such an external group, which has been designated as an Environmental Clearance Committee (ECC), makes recommendations on the appropriateness of re-occupancy of the site. The first such

clearance committee, an ad hoc group which consisted solely of federal governmental experts, evaluated the remediation of the Capitol Hill Anthrax Site. After reviewing the relevant data, it recommended additional environmental sampling. Upon determining that the additional samples were all negative, the committee recommended that the site be opened for re-occupancy. Formal ECCs have since been established for the Curseen-Morris and Hamilton P&DCs, the SA-32 mail facility, and GSA Building 410. Except for GSA Building 410, which is located on a military facility, all ECCs have had representation by experts from the responsible local governments. Variations exist among the ECCs in how the experts are selected, in the process used for evaluating the data, and in the nature of the report prepared, but all ECCs serve as an additional source of review of the cleanup process, which adds to the confidence of the persons who must re-occupy the site that it is safe to re-enter and work in.

Representative Sites with Fumigation Remedies

The highly contaminated letter to Senator Daschle was opened on October 15, 2001 by a member of the staff in the mail handling area of his suite in the Hart Senate Office Building. Upon reading the enclosed note, the staffer dropped the letter on the carpet, and the bomb squad was called to remove the letter and test it for *B.a*. contamination. Upon confirmation that the letter contained *B.a.* spores, the employees were promptly placed on antibiotics. The building was sealed off, and EPA Region 3 was requested by the Capitol Hill Police Board to perform the remediation under its direction. Initially, EPA staff proposed fumigating the entire Hart Building at one time with gaseous ClO_2. EPA submitted this concept for external peer review in early November 2001. Based upon the input provided by the reviewers and additional EPA evaluations, both of which supported using ClO_2 as the fumigant, EPA decided to take a tiered approach to remediating the Hart Building, fumigating only the Daschle suite with gaseous ClO_2 as the first step. On December 1, 2001, the Daschle suite, which consists of about 90,000 ft^3 on 2 floors, was fumigated. The target ClO_2 concentration throughout the suite during the decontamination phase of the fumigation process was 750 ppm, with an overall product of ClO_2 concentration and time (CT clock) of 9,000 ppm-hr. Target temperature and relative humidity were ≥70° F and ≥70%, respectively. These values were derived from experiments performed prior to the fumigation in a trailer located on the parking lot of the Curseen-Morris facility. The fumigation significantly reduced the load of spores. Thereafter, surface treatment with aqueous chlorine dioxide was performed in the suite, and all post-remediation environmental samples performed thereafter were negative for growth of *B.a.* spores. Two air handling units that service the tier of the building containing the Daschle suite were also fumigated later in December 2001.

EPA performed additional environmental sampling throughout the rest of the building based upon following the “mail trail” to determine the appropriate next steps in the remediation process. Based upon a review of the results obtained in the remainder of the building, surface cleanups with aqueous ClO_2 were performed in areas demonstrated to

have secondary contamination. No cleanup activities were undertaken in other areas in which the environmental sampling was negative. Following issuance of the written clearance recommendation by the ad hoc clearance committee and concurrence by the Capitol Hill physician, the entire Hart Building was re-opened for productive re-occupancy on January 22, 2002 [26].

The Curseen-Morris P&DC contains 14.5 million ft^3 of interior space on 2 floors. The letters to the 2 Senators passed through the facility late on October 11 or early on October 12, 2001. Four workers at the facility contracted inhalational anthrax, 2 of whom died. Following its closure on October 21, 2001, the site was demonstrated to have widespread *B.a.* contamination. Most of the contamination, however, was clustered on the first floor at 0 to 6 feet above the floor level in the immediate vicinity of mail sorting machine #17, the sorter through which the 2 letters passed [25]. The U.S. Postal Service (USPS) decided to fumigate the entire site at one time, using ClO_2 gas. That fumigant was chosen to be consistent with the remedial process for the Capitol Hill Anthrax Site. All openings to the exterior of the building were sealed, and all windows covered to prevent the entrance of light into the facility during the fumigation. Surface cleaning of specific parts of, and equipment in, the facility using chlorine bleach and High-Efficiency Particulate Air (HEPA)-vacuuming was performed prior to the fumigation. Two on-site ClO_2 generators were used during the treatment process. Fifteen emitters were installed in the facility to distribute the ClO_2 gas throughout the facility; 50 monitors for ClO_2 concentration, temperature, and relative humidity were placed at key positions in the facility; and 2 negative air units with scrubbing systems using sodium hydroxide and sodium bisulfite to neutralize the ClO_2 gas were assembled on the exterior of the building on its North and South sides [28].

The USPS had to resolve a number of key safety and efficacy issues prior to the fumigation, given the large volume of the facility and the decision to fumigate the entire facility at one time. Toward this end, the USPS performed several tracer gas tests using sulfur hexafluoride and 2 tests of the scrubber systems, including a test of the carbon bed at the end of that system in case of catastrophic failure of the scrubber. It also performed a low level performance test of the entire system in early December 2002. The fumigation of the facility was performed on December 14, 2002. As at the Capitol Hill Anthrax Site, the target ClO_2 concentration throughout the building during the decontamination phase of the fumigation process was 750 ppm, with a target CT clock of 9,000 ppm-hr at each of the 50 ClO_2 monitors. The clock for measuring the decontamination phase only started when the gas concentration reached 500 ppm at each of the ClO_2 monitors. Target temperature and relative humidity were ≥75° F and ≥75%, respectively, at each of the temperature and relative humidity monitors. Approximately 1.5% of the nearly 4,900 *Bacillus subtilis var niger* spore strips were positive following the fumigation, but all surface and air environmental samples were negative for growth of *B.a.* spores. The ECC established by the USPS reviewed all relevant spore strip and environmental sampling data and requested clarification of some of the data. It gave final clearance for re-occupancy of the facility in late September 2003 [6].

The leased DOS diplomatic pouch and mail facility, SA-32, in Sterling, Virginia is believed

to have become contaminated as a result of the letter to Senator Leahy being misdirected there from the Curseen-Morris P&DC. The contamination was discovered after one worker developed inhalational anthrax. The facility, which was closed in late October 2001, consists of 1.4 million ft^3 on one level. Limited characterization environmental sampling was performed at the facility following its closure. The DOS established a multi-disciplinary Technical Working Group (TWG) to provide ongoing expert guidance on its remediation activities. The TWG created a number of subcommittees to assist in planning for the remediation; namely, the Environmental Sampling, Fumigation, Emergency Response and Ambient Air Monitoring, Risk Communication, and Waste Disposal Committees.

The Fumigation Committee invited vendors of the 3 available fumigants (ClO_2, VHP, and formaldehyde) to make presentations and then evaluated the agents using the following criteria: industry experience, technical complexity, demonstrated efficacy in treating anthrax in buildings, risk to community, toxicity (acute/chronic), ease of monitoring, price, emergency response, special liability, availability, and vendor experience. As noted above, advantages and disadvantages were identified for each agent. Based upon the recommendation of the Fumigant Subcommittee and the TWG, the DOS selected VHP as the fumigant for the facility and decided to subdivide the facility into 10 zones ranging from 40,000 to 200,000 ft^3 and perform separate fumigations of each zone. Prior to the start of the fumigation phase of the project, all material capable of being removed from the facility, including the mail and package sorting equipment, air handling units, ceiling tiles, carpeting and room partitions, were treated with bleach and then appropriately discarded.

Two overarching criteria were established, both of which had to be met, for a successful fumigation in each zone. First, specific ranges for each of the process variables were established which had to be met during all 4 phases of the fumigation process (dehumidification, conditioning, decontamination, and aeration) in every zone. Second, every *Geobacillus stearothermophilus* growth biological indicator placed in each zone prior to the fumigation of that zone had to be negative for growth of that organism following the fumigation. For example, during the decontamination phase of the process, the VHP concentration had to be ≥216 ppm for a minimum of 4 hours at all 8 VHP monitors, with the temperature ≥70° F and the saturation level ≤80%, during that same time period [22]. The fumigation process was successfully conducted, with both criteria met in each zone. In one zone, one of the 110 biological indicators placed in a hard to reach location was positive for *Geobacillus stearothermophilus* after the fumigation; all others were negative. The fumigation of that zone was therefore repeated, and all the biological indicators were negative after the second fumigation. For the 10 fully successful fumigations, a total of 773 biological indicators were analyzed for growth of simulant spores and demonstrated to be negative [18].

The clearance environmental sampling was completed in October 2003, and all samples were negative for growth of *B.a.* spores. In early November 2003 the DOS submitted all relevant protocols and results to the ECC, which consists of experts who did not have any role in the cleanup. In mid-November of 2003, the ECC met and determined that the DOS mail facility was safe for re-occupation [18].

Discussion

As a result of EPA's continuing contributions to, and oversight of, numerous anthrax remediation activities, EPA staff have learned a number of lessons that will assist the remediation process, should there be additional attacks in the future. Key among these lessons are the following:

- The emergency response experience that EPA has gained in cleaning up accidental releases of hazardous chemicals and performing time critical removals of such chemicals has been important in the Agency's ability to respond to the anthrax attacks, but expertise in microbiology, sterilization science, and biosafety has been equally important.
- A multi-disciplinary team should be assembled by the responsible agency or building owner to assess and remediate sites contaminated with biological agents of terrorism. Expertise in sterilization science is critically needed to respond to attacks with *B.a.* spores. For sites at which fumigations are planned, chemical engineering support should also be brought to bear.
- For sites at which fumigations are to be performed, it may be very useful to establish a TWG comprised of scientists with expertise in building restoration following a bioterrorism attack. The role of the TWG is to advise the responsible agency or building owner on all facets of remediation.
- All contaminated sites have unique features that need to be evaluated on an individual basis.
- Environmental sampling is important throughout the characterization and remediation processes and is key at the end of the clean-up process. The advances in sampling approaches and techniques since 2001 need to be incorporated in future sampling activities. Further, accurate records need to be kept of all sampling locations, dates, and methods for later use in judging the effectiveness of the remediation process.
- Existing epidemiological data for each site (e.g., following the mail trail to confirm source of contamination, disease distribution data) should be evaluated in conjunction with the characterization sampling results to help delineate the cause and effects of the contamination.
- For all sites at which primary aerosolization of *B.a.* spores has occurred, fumigation of all or a part of the site is the default remediation process, unless extensive environmental sampling indicates negligible potential for secondary aerosolization of spores.
- A number of chemical fumigants are available. More data are needed on their safety, efficacy, optimal use parameters, and costs. They should all be evaluated in the context of the site needing fumigation before determining the appropriate fumigant for that site.
- Real-time ambient air monitoring is needed during fumigations, particularly when businesses and residential areas are adjacent to the site. In conjunction with the relevant local governmental authorities, 2 separate concentrations of fumigant in ambient air need to be established prior to the start of the fumigation. If the first level

is reached or surpassed, the fumigation process will be temporarily paused; if the second level is attained, the fumigation will be terminated.

- Emergency response plans need to be developed to address worst case and reasonably expected failure scenarios, and potential acts of terrorism.
- Coordination with relevant State and local health and environmental agencies is crucial throughout the remediation process.
- The organization responsible for the cleanup should provide continuing outreach to involved workers and the public, and keep them informed as the remediation activities progress.
- Long down times occur when fumigation is needed to achieve effective remediation of the site, especially for large facilities. Even under best case conditions, cleanups currently take months to complete.
- The ECC provides an external review of the adequacy of the cleanup process, particularly at sites with fumigation remedies, and adds confidence that the site is safe for re-occupancy.

Based upon the above lessons learned, it is clear that remediation processes for *B.a.* contamination which entail fumigations of an entire facility currently are complex, costly, and time-consuming actions.

Future Remediation Needs and Challenges

Given the long lead times and the considerable costs of fumigations of entire facilities, it is key to develop optimized fumigation processes that can be performed in a rapid manner, even at very large facilities, without causing damage to sensitive elements of infrastructure. This is particularly critical for certain types of critical infrastructure for which substitute locations or modalities are not available. For such facilities, it is also critical to validate and install reliable, reproducible, and real-time detection methodologies. Further, validated consensus environmental sampling methodologies are needed, as well as approved laboratory analytical techniques for such samples. The overriding need is to be able to return critical sites and facilities to safe and productive use as soon as possible, particularly in the event of future attacks with *B.a*. spores, which could involve the release of larger numbers of spores than was the case for the 2001 attacks.

An issue commanding the attention of a number of organizations is whether they need to implement prevention and preparedness plans for biological and chemical agents of terrorism. Key to such considerations is a determination of the types of organizations that would benefit from these plans. Organizations which have already been targets (e.g., media offices and the USPS) might benefit from implementing such plans. As an example, the DOS will be installing real-time detection equipment for *B.a.* spores in SA-32 as part of the renovation process, once the building has been cleared for re-occupancy [18]. Organizations with known vulnerabilities to such attacks could also benefit from having such plans.

Challenges exist for those organizations that decide to develop a prevention and preparedness plan in terms of the number of agents that should be included in the plan. Should they include only biological agents, chemical agents, or both? How many of the selected agents should be monitored by real-time detection systems? Are the currently available detection systems sufficiently reliable, sensitive, and specific to be utilized on a routine basis? Do they have the ability to detect multiple agents at the same time? What guidance should be put in place for responding to an attack with one or more of the agents? How much will these activities cost? These are important issues; a number of them do not have simple answers at this point in time. Research is being conducted by numerous governmental and other organizations that may provide needed information to make better informed decisions.

Conclusions

After completion of the remediation of the Capitol Hill Anthrax Site in early 2002, the Working Group on Civilian Biodefense updated its Consensus Statement on Anthrax as a Biological Weapon to reflect that remediation of civilian sites with anthrax contamination can be carried out effectively. Since remediation of contaminated buildings or parts of buildings is technically difficult, the revised document advises that “decisions about methods for decontamination following an anthrax attack follow full expert analysis of the contaminated environment and the anthrax weapon used in the attack and be made in consultation with experts on environmental remediation” [8]. This advice is consistent with the lessons learned from remediations performed to date.

In this new millennium, terrorism with weapons of mass destruction is a reality. Organizations will need to be prepared to prevent, detect, mitigate, and respond to attacks with various and multiple biological and chemical weapons, not just *Bacillus anthracis*. One thing is clear, it is crucial to be prepared for the next attack, not just the last one.

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Discussion

Opening Ceremony and Bioterrorism

Question for Dr. Hughes, Centers for Disease Control and Prevention, U.S.A.: What is the probability of a possible reappearance of SARS?

Answer by Dr. Hughes: I think that it's likely that SARS will reappear. As we've heard, SARS presumably has an animal reservoir and therefore, I think it's quite likely that it will be reintroduced into the human population. Certainly whether the virus reappears or not, we will be dealing with the specter of SARS this year because SARS now needs to be part of the differential diagnosis of community-acquired pneumonia. We clearly learned that the earlier SARS is recognized and aggressively dealt with, the better off we are.

Question for Dr. Hughes: Is enough research and development going into the discovery of new antibiotics for drug-resistant bacteria?

Answer by Dr. Hughes: In my opinion, in addition to the threat of pandemic influenza, the challenges posed by antimicrobial resistance are right near the top of the list of serious problems that we face. This is a national and global clinical and public health problem. We need to use the available agents carefully and prudently as the valuable resources that they are. Yet at the same time, we desperately need new antimicrobial agents developed.

Question for Dr. Canter, Environmental Protection Agency, U.S.A.: What was the cost, in dollars, of the fumigation effort at the Brentwood Post Office?

Answer by Dr. Canter: The cost for the entire Capital Hill anthrax site was 27 million USD. I am not at liberty to say what the actual cost of the Brentwood fumigation was, but I will say that before it's over, it will be hundreds of millions of dollars and I personally estimate that it will run half a billion USD.

Question for Dr Canter: Is there any effort to get volume analysis by taking thousands of liters of air and seeing if any anthrax spores actually remain airborne?

Answer by Dr. Canter: Yes. Actually most of this is being done after the fact; after you fumigate, you go back and do aggressive air sampling. There was a paper published in the *Journal of the American Medical Association* in December of 2002 where some of our people on Capital Hill went into the Daschle suite before the fumigation and took air samples. They initially didn't find very many spores. They then went back and stirred up the air. This time they found quite a few spores which indicated that these spores could be re-aerosolized. Similar types of experiments were done at the Trenton facility, again before the clean-up. We know that in those 2 instances the spores could be re-aerosolized. Some of the data from the USAMRIID studies from the actual letters given to them by the FBI

showed that the spores were very easy to get into the air as opposed to spores that are released out into the external outdoor environment.

Comment by Dr. Lederberg, The Rockefeller University, U.S.A.: You're raising a very important point regarding this specific anthrax attack. Based on historical work at USAMRIID, there has been long established dogma that re-aerosolization was very difficult. That was just taken for granted all the way through. That may have applied to the brands of anthrax spores that people knew how to produce prior to the recent attacks. It was really quite a jolt to realize how easily the grade of anthrax used in the recent attacks could be re-aerosolized. So we've had to rethink our basic postulates on that question.

Comment by Dr. Peters, University of Texas Medical Branch at Galveston, U.S.A.: I've talked to Bill Patrick who did some of the studies in the old days at Fort Dettrick and all their work was done with liquids. They did not work with these powders outside and they did not work with powders in the re-aerosolization experiments. That was a real defect in the design of their original studies.

Comment by Dr. Hadler, Connecticut Department of Public Health, U.S.A.: In Connecticut, there was an elderly woman who died from anthrax after receiving mail from a postal facility that was contaminated. I would like to comment on the re-aerosolization issue from the point of view of observational studies. It is very interesting when you look at each of the postal facilities, it took a week to 10 days to close down after the initial cases occurred. Yet, the people who got infected developed anthrax only early on. There weren't cases later on that occurred just prior to antibiotic prophylaxis. This suggests that even though the environments were very heavily contaminated, there wasn't much re-aerosolization occurring, at least at a level that was re-infecting people. This is an important observation in showing that the real risk was in the first day or so after the contaminated letters went through, and didn't appear to be a persisting effect, at least a high level.

Comment by Dr. Lederberg: We have a public that has become accustomed to zero risk. That's often mitigated by having limits of sensitivity that let you live in a practical world. We have such highly efficient methods at detecting even single spores that it's a little bit like worrying about pico curies of radiation.

Question for Dr. Peters and Dr. Hughes: What are the relevant contributions of larger droplets, small particle aerosols, and fomites in SARS transmission? Can we say anything about ordinary patients versus super spreaders?

Answer by Dr. Peters: I'm mystified. I think most of the spread appears to be droplets, I don't think there's much question about that, but I think that some of the things that have happened in hospitals suggests that fomites are important. The other support for this is the finding of considerable copies of virus in the stool – one-to two-thirds of these patients have diarrhea. Of course, the issue of aerosols is extremely important and some of the anecdotes are very suggestive of aerosol transmission. But it's a minority. On the other hand it has a great deal of significance because if you have aerosol transmission, then you

need negative pressure rooms and aerosol respiratory protection for the worker. Negative pressure rooms are very expensive. The aerosol respiratory protection for the worker is theoretically solved by N95 masks that are fit tested. If any of you have ever been fit with an N95 mask, you will probably go out and buy a positive air purifying respirator, because it is very difficult to achieve a satisfactory fit with a N95 mask and have it remain tight over a period of several hours.

Answer by Dr. Hughes: I agree with Dr. Peters that the vast majority of SARS transmission appears to have been in the form of droplets. There is, however, at least one notable airplane incident, raising the possibility of true airborne transmission. In addition to all the respiratory protection that we've been talking about, hand hygiene is clearly an important component of the overall infection control approach for SARS.

Question for Dr. Hughes: Was the importation of exotic animals legal? Domestic pets have to be quarantined and I'm surprised that a license was not required to import the animals which were subsequently implicated in the U.S. monkey pox episode. What are the risks to the national U.S. fauna?

Answer by Dr. Hughes: The importation of animals and their inter-state shipment was legal at the time that it happened. There is currently an order in place that suspends the importation and the inter-state shipment of these animals. The risk to the national U.S. fauna is a concern because prairie dogs are susceptible to monkey pox and as we saw, did become ill and some died. There are some capture studies that have been done and the analysis of that data is in progress. We certainly hope that it did not get into wildlife populations. I am not aware of any evidence that it did, but it is certainly a possibility.

Comment by Dr. Peters: I think that it's worth mentioning that plague was imported into the western hemisphere and established itself into the prairie dog population quite nicely. It's more than just a speculative possibility.

Comment by Dr. Hadler: Just a comment from a state perspective on the inter-state shipment of animals. Each state can ultimately set its' own laws with regards to what is legal to import and sell. Prairie dogs and plague are one of the issues where some states have been successful at passing laws that prevent prairie dogs from being imported and sold as pets.

Comment by Dr. Layton, New York City Department of Health and Mental Hygiene, U.S.A.: Dr. Hughes referred to the 1999 West Nile virus outbreak. When we first began investigating this in New York City, the initial impression was that it was St. Louis Encephalitis based on initial laboratory tests. Unbeknownst to those of us initially investigating the human outbreak, there was a simultaneous outbreak or epizootic affecting birds in New York City, especially crows. The veterinary community, especially the wildlife community, became actively interested and their investigation was occurring without us in the public health sector realizing it, until rather late into the investigation. That was eye-opening to us about the need to establish those linkages on the local level. Since then, we

have hired a public health veterinarian whose primary job is to build relationships, not just with the clinical veterinarians who see domestic animals, but with the wildlife community as well. We have an alert system that connects us with clinical vets. When outbreaks of potential veterinary importance happen, such as monkey pox, we try to outreach to that community specifically with information that is relevant to them. In addition, we made animal diseases reportable. In the past, only rabies was reportable in animals, but we have now made all the potential bioterrorism agents legally reportable and included a phrase about unusual disease manifestations that we felt was important, similar as it is in human health. As Dr. Hughes mentioned earlier about the need to build partnerships, we've recognized the need to build relationships and not just to focus on human disease.

Session II

BSE / Prions

Chairman: **Robert G. Will, M.D.**
National Creutzfeldt-Jakob Disease Surveillance Unit, U.K.

The World of Prions and Prions in the World

Robert G. Will, M.D.

National Creutzfeldt-Jakob Disease Surveillance Unit, U.K.

Introduction

Prion diseases are fatal and untreatable degenerative disorders of the central nervous system (CNS) which affect both humans and animals (Table 1). The causal agents are thought to be host-encoded proteins or prions, which adopt an abnormal structure, act as a template for self-replication, and accumulate to eventually result in neurological disease. Human prion diseases are rare, but have been the subject of intense public interest and concern following the identification of bovine spongiform encephalopathy (BSE) and the probability that this animal disease has been transmitted to the human population in the form of variant Creutzfeldt-Jakob disease (vCJD). Prion diseases have a number of characteristics that pose major challenges to public health: incubation periods that may extend to years or decades, no practical test for the presence of infection until the development of clinical disease, and prions are remarkably resistant to sterilization. One implication is that infected animals or humans may pose a risk of infection during the incubation period but cannot be identified. This has resulted in the introduction of precautionary policies to protect human and animal health that are expensive in terms of both economics and natural resources. This article summarizes current data on vCJD and BSE and outlines continuing concerns and uncertainties.

Table 1. The Spongiform Encephalopathies

Disorder	Species
Sporadic Creutzfeldt-Jakob disease	Human
Inherited Creutzfeldt-Jakob disease (includes Grestmann-Sträussler-Scheinker and fatal familial insomnia)	Human
Iatrogenic Creutzfeldt-Jakob disease	Human
Kuru	Human
Variant Creutzfeldt-Jakob disease*	Human
Scrapie	Sheep/Goat/Moufflon
Transmissible mink encephalopathy	Mink
Chronic wasting disease	Deer/Elk
Bovine spongiform encephalopathy*	Cattle
Feline spongiform encephalopathy*	Cat/Cheetah/Puma/Ocelot
Spongiform encephalopathy of captive exotic ungulates*	Kudu/Nyala/Oryx/Gemsbok/Eland

* These disorders are associated with the same agent of infectivity.

Variant Creutzfeldt-Jakob Disease

A causal link between vCJD and BSE was first raised as a possibility in 1996 [23] and since then evidence has accrued that strongly supports this hypothesis. The clinical and pathological features of vCJD are distinct from sporadic CJD (sCJD) [25,26]. The mean age at death in vCJD is 29 years, the median duration of illness is 14 months, and patients usually present with predominantly psychiatric symptoms [24]. The electroencephalogram (EEG) shows non-specific abnormalities, and in about 90% of cases, magnetic resonance brain imaging (MRI) shows high-signal changes in the posterior thalamus [7]. In contrast, in sCJD the mean age at death is 65 years, the median illness duration is 4 months, and patients usually present with dementia, ataxia, or focal cortical deficits. With sCJD, the EEG shows characteristic periodic complexes in about 70% of cases and, in the majority of cases, the MRI scan shows high signal in the caudate and putamen. There is, however, some clinical overlap between vCJD and sCJD and discrimination between the 2 conditions in life can be difficult in individual cases.

A definite diagnosis of vCJD depends on neuropathological examination of the brain, usually after autopsy. In vCJD there is widespread deposition of aggregates of disease-associated prion protein (PrP^{Sc}) surrounded by a rim of spongiform change, so-called florid plaques [15], in addition to the paradigm neuropathological features of CJD, spongiform change, neuronal loss, and astrocytic gliosis. These appearances are remarkably consistent between cases of vCJD and are distinct from previous experience in human prion diseases, including sCJD. The novelty of the neuropathological phenotype in vCJD was a critical argument in favor of the hypothesis that this was a new disease which might imply a novel risk factor (BSE) and no case with a similar neuropathological phenotype has been identified following review of archival material in the United Kingdom (U.K.), systematically in continental Europe [6], and elsewhere. Retrospective studies involving identification of potential cases of vCJD from death certificates [14,17], with review of clinical details and, in some cases, neuropathology, have been carried out in the U.K. in order to identify previously unrecognized cases of vCJD. No such case has been identified and current evidence indicates that vCJD is indeed a new disease.

All tested cases (121/143) in the UK are methionine homozygotes at the polymorphic site at codon 129 of the prion protein gene (*PRNP*) indicating that this is a risk factor for the development of disease (as in sCJD). There remains the possibility, however, that individuals with alternative codon 129 genotypes may develop vCJD in the future because of a relatively prolonged incubation period. One uncertainty is whether the clinical and pathological phenotype in such cases would be the same as that seen in vCJD with a methionine homozygous background.

As of March 1996, the only identified cases of vCJD were in the U.K., consistent with the hypothesis that exposure to BSE might be a potential risk factor for the development of disease. BSE was identified first in the U.K. and the size of the BSE epidemic in the U.K. was far greater than in any other country and probably started earlier. Since then the numbers of cases of vCJD have increased in the U.K., but cases have also been found in other countries (Table 2). It is important to stress that there is an international agreement

that cases of vCJD will be classified according to the country of normal residence at the time of disease onset and this does not necessarily imply the country in which exposure to BSE took place. Except for the cases of vCJD in France and Italy, all cases of vCJD had a history of residence in the U.K. during the period 1983-1995 when human exposure to BSE was greatest. It has been suggested that the identification of vCJD was solely a reflection of intensive surveillance leading to the discovery of a prevalent and already existing disease [21]. If so, it is a remarkable coincidence that the first cases of vCJD identified in Canada and the United States had a history of residence in the U.K.

Table 2. vCJD Worldwide – October 2003

Country	Number of Cases
U.K.	143
France	6
Republic of Ireland	1
Italy	1
United States	1
Canada	1

In the absence of a significant history of travel to other countries, the cases of vCJD in France and Italy must have been exposed to BSE in their country of residence. This may have been to indigenous cases of BSE, but it is perhaps more likely that the source of infection in these cases was from U.K. exports of bovines incubating BSE or human food products contaminated with BSE. The proportion of cases of vCJD in the U.K. and France is roughly consistent with one estimate of the consumption of beef of U.K. origin in the 2 countries [2], a surrogate marker for exposure to BSE infectivity. The geographic association between BSE exposure and the incidence of vCJD assumes accurate identification of cases in relevant countries. A coordinated system for the surveillance of CJD and vCJD, with shared protocols for case ascertainment and classification, has now been in place since 1993 and currently includes all member states of the European Union together with other countries such as Canada and Australia. Annual mortality rates for sCJD approach or exceed 1 case/million population in nearly all of these countries and this suggests that adequate surveillance for CJD is in place and that cases of vCJD are likely to be identified.

It is likely that there was significant exposure of the U.K. population to bovine food products containing CNS tissues with high levels of infectivity, particularly in the 1980s [9]. The favored hypothesis is that the patients with vCJD were infected through oral exposure to BSE infection, although there is currently no firm evidence to support this hypothesis. The human population could have been exposed to BSE by a range of mechanisms, but there is no evidence to suggest an increased risk through occupational exposure to cattle or cattle tissues, through exposure to bovine-derived medications, or through an iatrogenic route such as blood transfusion. None of the cases have a history of exposure to human pituitary

hormones, a known cause of CJD in younger people, nor do any of the great majority of tested cases have mutations in the *PRNP* to suggest a hereditary cause. A case-control study of dietary history in vCJD does suggest an increased risk from consumption of products containing high titre bovine tissues but this study is confounded by inadvertent bias by respondents. The mortality rates from vCJD are approximately double in northern U.K. compared with southern U.K. [12], which may correlate with bovine dietary exposures, and a cluster of cases have been linked to specific butchering practices. Although not proven, infection through dietary exposure to BSE remains the only credible hypothesis for the causation of vCJD.

The U.K. population was potentially exposed to large amounts of BSE infectivity in the food chain and there have been fears of a major epidemic of vCJD. Early mathematical models estimated that there was a wide range of potential future numbers of cases from less than a hundred to over 80,000 or more [11], but recent estimates are more conservative (Figure 1), with central predictions of a maximum of hundreds of cases in some studies [1,20]. All of these estimates are dependent on assumptions that cannot be verified and all have assumed that only methionine homozygotes will be affected. Up until recently, analysis of short-term trends has indicated a statistically significant increase in the number of deaths from CJD with time [3], again raising the possibility of a major epidemic. However, recent analyses of both deaths and clinical onsets in vCJD have indicated a down turn [4] and the most recent analysis in July 2003 showed, for the first time, a significant decline.

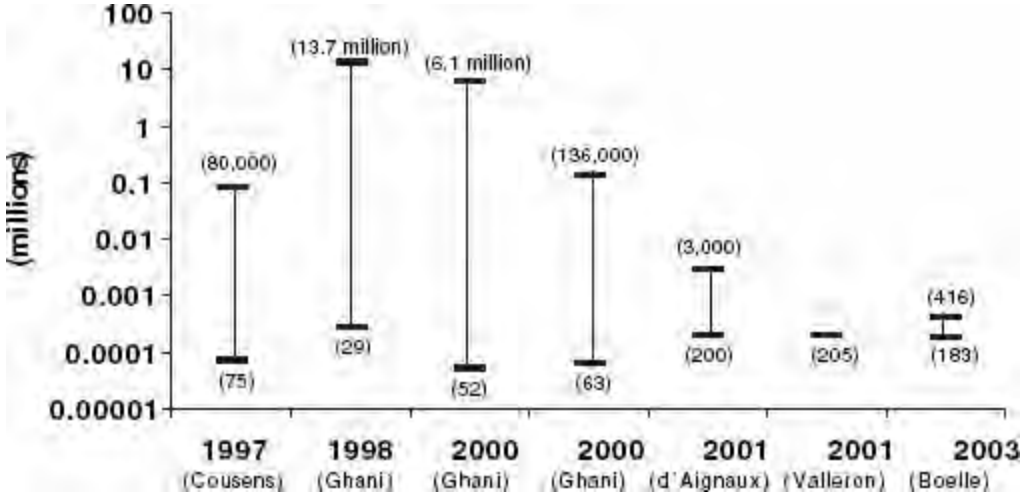

Figure 1. Predictions of the size of the vCJD epidemic.

The number of deaths peaked in the year 2000 while the number of onsets peaked in 1999 (Figures 2 and 3), indicating that the apparent decline in vCJD has been sustained for some years. There remains uncertainty about whether there may be further waves of cases of vCJD related to variation in the temporal pattern of historical dietary exposure to BSE [10], through the occurrence of BSE infection in individuals expressing a valine allele at codon 129 of *PRNP*, or through secondary iatrogenic transmission of vCJD. However, the current downward trend in the numbers of cases of vCJD must suggest that a major epidemic is less likely than originally feared.

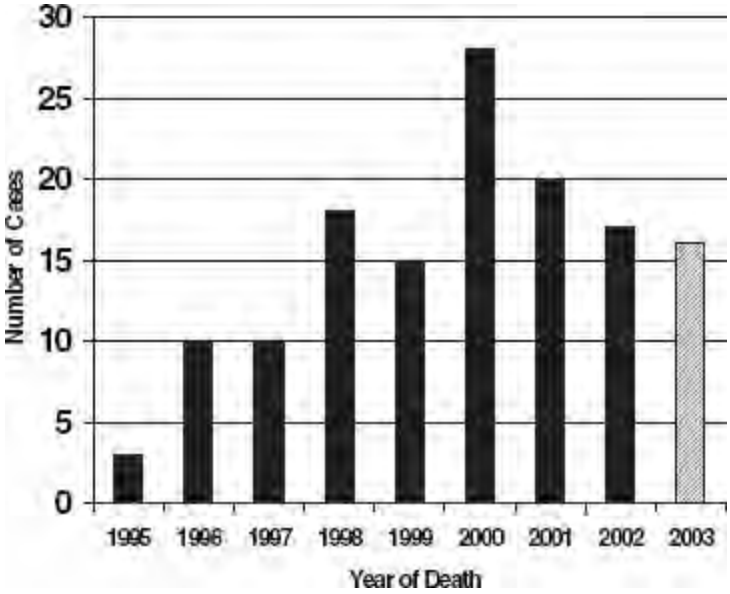

Figure 2. Number of deaths per annum of vCJD (U.K.)

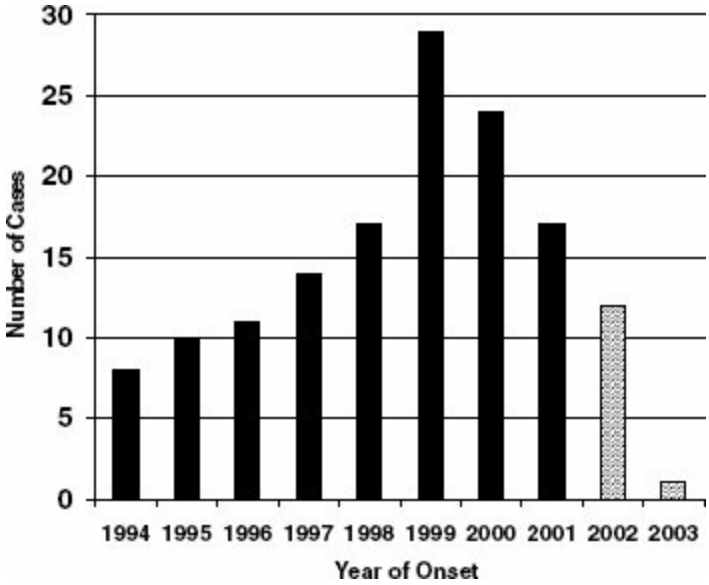

Figure 3. Number of onsets per annum of vCJD (U.K.).

The correlation between the geographical occurrence of BSE and vCJD cannot, in itself, be regarded as strong evidence of a causal link between the 2 conditions. Laboratory studies, however, have provided strong support for the hypothesis that the BSE agent is the cause of vCJD. Macaque monkeys inoculated with BSE show florid plaques in the brain, similar to those found in vCJD [16]. The type of prion protein deposited in the brain in vCJD can be distinguished from that in sCJD on Western blot analysis and this protein type has similar properties, including a relative excess of the diglycosylated band, compared with that in transmitted BSE [8]. Transmission studies in laboratory mice have been the foundation of strain typing in scrapie and latterly in other prion diseases. In these studies there is a remarkable strain dependent consistency in the incubation period and the distribution of the neuropathological changes, i.e. the lesion profile, if parameters such as route of inoculation and dose of inoculation are kept constant. In both wild type [5] and transgenic mice [13], including one study in a bovine transgenic line [19], the transmission

characteristics, including incubation period and lesion profile, in vCJD and BSE are virtually indistinguishable and contrast with similar studies in sCJD. These studies have provided powerful support for the hypothesis that BSE is the causal agent in vCJD.

Even if the link between BSE and vCJD is accepted, many uncertainties remain. Why are the vCJD cases young? Why has only one member of each family with a vCJD case been affected despite presumed similarities in dietary exposures? Will individuals expressing valine at codon 129 of *PRNP* be affected? Will there be secondary transmission of vCJD through blood transfusion or contaminated surgical instruments, a concern raised by the presence of PrP^{Sc} and infectivity in lymphoreticular tissues in vCJD and not in sCJD? Will the numbers of cases of vCJD in the U.K. rise in the future and will vCJD be identified in other countries as a consequence of human exposure to BSE?

Bovine Spongiform Encephalopathy

BSE was first identified in the U.K. in 1986 [22] and by 1991 small numbers of indigenous cases had also been found in Ireland, Portugal, Switzerland, and France through a passive system of veterinary reporting and surveillance. There was then a gap of 6 years before cases of BSE were identified in Belgium, the Netherlands, and Luxembourg, but the true extent of the spread of BSE did not become apparent until the introduction of abattoir testing of animals in the years 2000/2001. Since then all member states of the European Union except Sweden have identified cases of BSE and cases have been found in central European countries (including Poland, the Czech republic, and Slovakia), in Israel, Japan, and most recently, in Canada (Figure 4). The development of sensitive post-mortem tests utilizing samples of brain tissue has been important in improving understanding of the true epidemiological pattern of BSE. One implication of the upsurge of identified cases, both in terms of countries affected and the numbers of cases within individual countries, is that the passive surveillance for BSE was relatively inefficient. Even today there are variations between countries in the proportion of animals tested in different risk groups, but through active testing it is possible to assess trends in the evolution of BSE epidemics by country. In the U.K. and in many, but not all, Western European countries there is an apparent decline in the number of cases per annum.

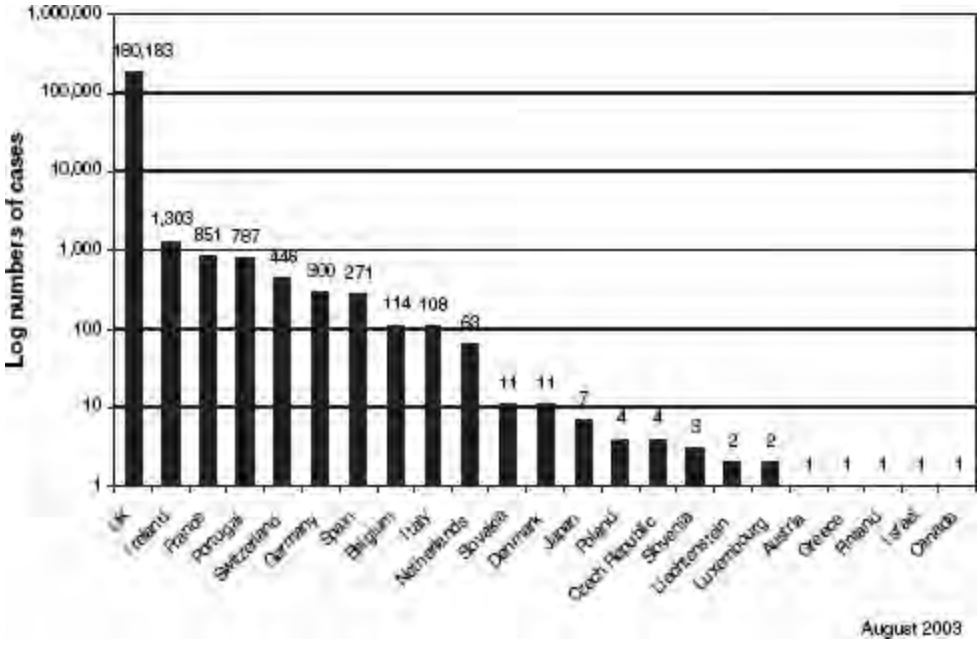

Figure 4. Number of BSE cases per country.

BSE is thought to have been caused by feeding meat and bone meal containing prion infectivity to bovines, probably initially scrapie and in later years BSE infectivity itself, recycled through the animal food chain. One important piece of evidence in support of this hypothesis is that the epidemic of BSE started to decline in the U.K. about 5 years after the introduction of a ban on the feeding of ruminant protein to ruminants. The mean incubation period of BSE is probably about 5 years and the occurrence in the U.K. of affected animals born after this feed ban was probably due to the incomplete effectiveness of the ban, for example with cross-contamination in mills producing meat and bone meal. One implication

of this hypothesis is that animal feed exported from the U.K. since the early 1980s led to exposure of bovines in other countries to BSE and that recycling of infectivity in these countries in animal feed may have led to amplification of infection. The levels of exports of animal feed and living bovines has been made available by U.K. Customs and Excise and using this information, together with other parameters such as the risk of recycling of infection, a risk assessment for BSE was carried out by the European Commission for member states and some other countries. This indicated that the risk of BSE was appreciable in many countries, including some that at the time were BSE free. It is of note that although exports of animal feed from the U.K. to Europe virtually stopped in 1989, some of this material was exported to other countries in the early 1990s, including southeast Asia, and may then have been re-exported. It is also important to note that recent assessments of BSE risk have taken into account the export of animal feed from Western European countries that were thought to be BSE-free and have subsequently identified cases of BSE with active testing to other countries, for example in central and eastern Europe. The true international distribution of BSE is not known and it has been recommended that all countries carry out a BSE risk assessment [18].

From the perspective of human health the relative risk of BSE exposure in the U.K. was higher than in any other country and should the numbers of vCJD cases in the U.K. be limited, it is unlikely that other countries will have significant human epidemics. However, an important variable in assessing human risk is the timing and application of measures designed to minimize the risk of human exposure to BSE, particularly through the food chain. Although measures to protect public health were not fully implemented in the early years, a range of specified bovine tissues that might contain significant infectivity were banned in the U.K. from the human food chain in 1989 and additional measures were introduced over the years, including a ban in 1996 on human consumption of bovines aged over 30 months. In other countries measures were taken to minimize human exposure to BSE, notably in Switzerland, but in some countries these measures were introduced relatively late, particularly in countries that were thought to be BSE-free before the introduction of the active testing systems in abattoirs. Although the absolute numbers of cases of BSE are low in all other countries in comparison with the U.K., the numbers of infected but unidentified bovines that may have entered the human food chain in these countries, for example in the 1990s, is unknown. There may also be countries in which cattle may have been inadvertently exposed to BSE and in which there is limited veterinary surveillance or active testing for BSE. One lesson of the BSE crisis is that it is important to consider measures to protect the public from BSE in at risk countries before cases of this condition are identified in the cattle population.

Conclusion

Although BSE and vCJD have occurred with a higher incidence in the U.K. than any other country, these diseases have had international implications for animal feeding practices, public health policy, pharmaceutical production, and trade. The incidence of BSE is currently dropping in the U.K. and active testing suggests that major epidemics of BSE are now unlikely in other affected countries. The possibility of a major epidemic of vCJD in the U.K. is receding. Because of the potentially protracted incubation periods in prion diseases, however, the full extent of the international risk of BSE is uncertain and the full impact of human BSE infection may not be seen for years or perhaps decades. The relaxation of precautionary measures, which are often highly expensive, may be next on the agenda, but such actions will be a major challenge for risk communication and should be considered in the context of continuing scientific vigilance.

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Inactivation of Prions

David M. Taylor Ph.D.

Consultant, SEDECON 2000, U.K.

Introduction

During the past few decades, a number of unusual and fatal transmissible neurological diseases of animals and humans that were previously disparate have coalesced into a single group. These are now collectively described either as transmissible degenerative encephalopathies (TDEs) or transmissible spongiform encephalopathies (TSEs), as listed in Table 1. A unifying feature of these diseases is that, despite the ongoing debate regarding the precise molecular structure of their causal agents (*vide infra*), they appear to be associated with the post-translational modification of a normal host protein (PrP^{C}) into a disease-specific form (PrP^{Sc}) that occurs as a consequence of infection. PrP^{Sc} resists catabolic digestion by proteolytic enzymes and accumulates to varying degrees in a variety of tissues, particularly those of the lymphoreticular system (LRS), long before clinical disease becomes apparent. By the time that clinical neurological disease occurs, the pathological accumulation of PrP^{Sc} becomes evident within the central nervous system (CNS). This is usually accompanied by spongiform encephalopathy and therefore these diseases are commonly referred to as TSEs. Spongiform encephalopathy, however, is not universally detectable although there is always histopathological evidence of neurodegeneration. For these reasons, it has been considered more appropriate to describe these diseases as TDEs [42].

Table 1. Transmissible Degenerative Encephalopathies

Disease	Species Affected
Scrapie	Sheep, Goats, Moufflon
Transmissible mink encehalopathy (TME)	Mink
Chronic wasting disease (CWD)	Elk, Mule-deer*
Bovine spongiform encephalopathy (BSE)	Cattle, Captive exotic ruminants
Feline spongiform encephalopathy (FSE)	Domestic cats, Captive exotic felids
Kuru	Humans
Sporadic Creutzfeldt-Jakob disease (sCJD)	Humans
Familial Creutzfeldt-Jakob disease (fCJD)	Humans
Variant Creutzfeldt-Jakob disease (vCJD)	Humans
Sporadic familial insomnia (SFI)	Humans
Fatal familial insomnia (FFI)	Humans
Gerstmann-Straussler-Scheinker syndrome (GSS)	Humans

*Primarily in the United States and Canada but some cases in Korea through importation of infected animals.

The Nature of the Causal Agents

The remarkable resistance of the scrapie agent to inactivation by formalin that was reported more than 50 years ago [26] was the first indication that the TDEs are caused by transmissible agents that are unlike conventional microorganisms. Nevertheless, their precise molecular nature has still not been determined. The possibility that the TDEs might be caused by viruses has been largely excluded because no agent-specific nucleic acids have been detected, and agent-specific antibodies have not been found in infected hosts. The prion hypothesis espoused by Prusiner in 1982 [31] proposed that TDE agents consist of nothing more than PrP^{Sc} that, initially, is either created spontaneously within the host by a stochastic process, or is introduced into a naïve susceptible host. Regardless of the origin of the PrP^{Sc}, the prion hypothesis considers that it acts as a template for the conversion of PrP^{C} to PrP^{Sc} [32,33]. More recently, the prion hypothesis has been modified to take into account evidence that a secondary molecule may be needed to form an infectious moiety [54]. The proposed secondary molecule is also considered likely to be a protein, and is described as protein X, but it is acknowledged that it may not necessarily be a protein [S. B. Prusiner, personal communication]. In general, there is no fundamental disagreement with the idea that PrP^{Sc} is at least a component of TDE agents. Models for these agents that involve only host-derived proteins as their constituent molecules, however, do not explain the variety of distinctive phenotypic characteristics of different strains of scrapie agent that are displayed in mice of the same *PrP* genotype, or the remarkable phenotypic stability of the BSE agent in mice, regardless of whether transmission is directly from cattle to mice or via intermediate species such as kudu, nyala, domestic cats, pigs, sheep, goats, and even humans [8,9]. Consequently, some consider that the transmissible agents may contain non-host informational molecules such as small (but as yet undetected) nucleic acids to explain these strain-specific phenomena [1,8,12,13,20].

Accidental Transmission

Despite the ongoing debate regarding the molecular nature of TDE agents, it has been recognized for more than 50 years that they are relatively resistant to inactivation by procedures that are effective with conventional microorganisms. This has resulted in a number of instances in which accidental transmission has occurred as a result of using inappropriate measures to inactivate the causal agents. The first recorded instance occurred when approximately 1,800 of 18,000 sheep developed scrapie after vaccination against louping-ill virus. The vaccine had been unsuspectedly contaminated with scrapie agent, which survived the exposure to 0.35% formalin that inactivated the louping-ill virus [26]. In somewhat similar circumstances, more recently sheep and goats in Italy became infected with scrapie after the administration of a vaccine against *Mycoplasma agalactiae*. The circumstantial evidence suggests that this vaccine was also contaminated with the scrapie agent that was not inactivated by exposure to formalin that inactivated the mycoplasma [11].

The use of decontamination methods retrospectively recognized as being inappropriate has resulted in the accidental transmission of CJD. In one instance, 2 electrodes used for insertion into the brain for diagnostic purposes caused iatrogenic infection. After use on a patient with suspected CJD, the electrodes were washed in benzene and then treated with ethanol and formaldehyde. Because the inadequacy of these processes for inactivating the CJD agent was not recognized, the electrodes transmitted CJD through their insertion into the brain of 2 subsequent patients requiring similar neurological investigation [4]. Convincing evidence that these electrodes caused iatrogenic CJD was provided by the production of a CJD-like disease in a chimpanzee in which the suspect electrodes were implanted into the brain [25]. A standard hot air sterilization process (180°C for 2 hours) was also considered to have failed to decontaminate CJD-contaminated surgical instruments that subsequently transmitted the disease to a patient during brain surgery [22].

It is thought that BSE was likely to have been initially caused by the transmission of the scrapie agent to bovines via feed [17,58]. Prior to the ruminant feed-ban in the United Kingdom (U.K.) in July 1988, it was common practice to incorporate ruminant-derived meat and bone meal into the diets of dairy cattle. This product was prepared by a variety of cooking methods, sometimes accompanied by solvent extraction.

With regard to the failure of the different procedures described above to inactivate TDE agents, the following observations are pertinent. The lack of any beneficial effect by using benzene or alcohol is not surprising given that organic solvents generally have little effect. Experimental exposures have included 1 hour in acetone [29], 2 weeks in 5% chloroform [15], 16 hours in ether [24], 2 weeks in 4% phenol [15], and 2 weeks in ethanol [16]. With regard to the survival of infectivity after exposure to 0.35% formalin or formaldehyde vapor, these agents can survive even more rigorous treatments, e.g., immersion of infected brain-tissue for 1 to 6 years in 10% formol saline [10,23,39] or 974 days in 20% formol saline (D.M. Taylor and A.G. Dickinson, unpublished data). In the one study where titer reduction was measured, only 1.5 logs of infectivity were lost when infected brain was exposed to 10% formol saline for 48 hours [6].

The absence of effective sterilization when using dry heat at 180°C is compatible with the data that has been accumulated. A small amount of infectivity was recoverable after a homogenate of hamster brain infected with the 263K strain of scrapie agent was exposed to dry heat at a temperature of 360°C for 1 hour [6]. However, the brain homogenate had been lyophilized before heating. As with conventional microorganisms, drying of scrapie infectivity is known to enhance its thermostability [2,3]. In contrast, when 7-mg samples of (non-lyophilized) macerated mouse brain infected with the ME7 strain of scrapie agent were exposed to dry heat, there was no detectable infectivity after an exposure to 200°C for 1 hour, even though some infectivity had survived exposure to 160°C for 24 hours or 200°C for 20 minutes [48]. In subsequent studies with 263K and the 301V strains of mouse-passaged BSE agent, however, a significant amount of infectivity survived exposure to hot air at 200°C for 1 hour [37]. The survival of lyophilized infectivity after exposure to 360°C in the study of Brown *et al.* [6] led to speculation that the effectiveness of incineration for inactivating scrapie-like agents might need to be questioned. In more recent studies carried out by Brown *et al.* [7] it was reported that traces of scrapie infectivity could be detected after 263K-infected brain-tissue had been exposed to 600°C for 15 minutes in a muffle-furnace. Such a process would be expected to reliably destroy all forms of organic material and therefore it was hypothesized that an inorganic "fossilized" skeleton of PrP^{Sc} might retain sufficient structural integrity to trigger the conversion of normal PrP into the disease-specific form. Although this is a matter of speculation, the survival of infectivity after exposure at 600°C has been confirmed in a second round of experiments (P. Brown, personal communication). It is now known that the heating process in many of the traditional rendering procedures used to manufacture ruminant-derived meat-and-bone meal do not completely inactivate BSE or scrapie agents [36,47,49]. In studies relating to the historical use of organic solvents by the rendering industry to enhance the yield of tallow from the raw materials, it was found that even hot organic solvents provided a very small amount of inactivation of mouse-passaged BSE and scrapie agents. This was not significantly enhanced by the subsequent exposure of the raw materials to dry heat and steam [51]. The solvents tested included hexane, heptane, perchlorethylene, and petroleum.

Regarding the iatrogenic transmission of CJD, a survey has indicated that individuals who had been subjected to neurosurgery were at a somewhat higher risk of developing CJD in later years compared with the controls [55]. This might have resulted from the use of inadequate sterilizing procedures for the neurosurgical instruments that were involved but the study did not provide any information regarding which procedures had been used. The emergence of variant CJD (vCJD) in the mid-1990s [56], and the subsequent occurrence of more than 140 definite or probable cases in the U.K. by October 2003, has escalated the general degree of concern regarding the potential person-to-person transmission of CJD-like diseases through the inadequacy of the procedures used to sterilize surgical instruments and devices. This enhanced concern is partly because it is not possible at present to predict how many more cases of vCJD will occur, but also because many more tissues (especially those of the LRS) become infected in vCJD-infected, compared with CJD-infected, individuals [27]. Preliminary evidence from the study of vCJD-infected materials from humans, and from the much more wide-ranging studies of scrapie in sheep

and laboratory animals, indicates that infectivity is likely to be present in LRS tissues for a considerable time before CNS involvement which is when the clinical disease manifests itself. Surgeons therefore could perform procedures involving deliberate or incidental invasion of LRS tissues in individuals with no clinical signs of neurological disease but who are incubating vCJD.

Inactivation Studies

Although the precise molecular nature of TDE agents is unknown, this has not prevented the conduct of meaningful studies relating to their inactivation under conditions that are directly relevant to everyday practice in hospitals, laboratories, rendering plants, and facilities for manufacturing biopharmaceutical products. Despite the recognized relative resistance of TDE agents to inactivation, 2 major studies carried out during the 1980s identified a small number of reliable procedures. The work of Kimberlin *et al*. [30] showed that 2 strains of mouse-passaged scrapie agents were completely inactivated by exposure for 30 minutes to solutions of sodium hypochlorite containing 13,750 ppm of available chlorine. It was therefore proposed that exposure for 1 hour to a solution of sodium hypochlorite containing 20,000 ppm available chlorine should be a reliable inactivating procedure. In the same study, previously described differences in the thermostability of mouse-passaged strains of scrapie agent were confirmed. Although strain 139A was completely inactivated by exposure to gravity-displacement (GD) auto claving at 126°C for 2 hours, strain 22A was not. A 4-hour exposure was required to inactivate 22A under these conditions [15]. However, the studies of Kimberlin *et al.* [30] also showed that porous-load (PL) autoclaving at 136°C for 4 minutes was completely effective with both of these strains of scrapie agent. These latter data were used to formulate the recommendations in the U.K. that PL autoclaving at 134-138°C for 18 minutes should be used to inactivate CJD-contaminated materials [14]. Nevertheless, it was recommended that instruments used in surgery involving the brain, spinal cord, or eyes of known or suspected cases of CJD be discarded rather than recycled after autoclaving. This advice was later extended to include other categories of patients recognized as having a higher risk of developing CJD. Specifically, these were defined as blood relatives of families with a known predisposition to TDEs and individuals who had been recipients of a) hormones derived from the pituitary glands of human cadavers; b) dura mater graft material derived from human cadavers; or c) human corneal grafts. The continuing advice not to recycle surgical instruments after they had been used in neuro surgical or ophthalmological procedures was no doubt based upon the knowledge that, despite the apparent reassurance regarding the effectiveness of PL autoclaving in the 1983 study, doubt remained concerning the general effectiveness of autoclaving with TDE agents. It was clear that future studies might reveal inadequacies in the standards adopted in 1984 for inactivating CJD agent by PL autoclaving and, as will be discussed, this proved to be the case.

The main conclusions from the second major inactivation study carried out in the 1980s [5] were that rodent-passaged CJD and scrapie agents could be inactivated by exposure to 1M sodium hydroxide for 1 hour, or GD autoclaving at 132°C for 1 hour. These procedures were incorporated into formal recommendations on how to deal with TDE infectivity [21,35]. Further studies have shown, however, that GD autoclaving at 132°C for 1 hour does not completely inactivate scrapie agent [19,28,43]. As will be discussed, this is also true for the recommended 1M sodium hydroxide treatment.

Although the incidence of BSE in the U.K. during the mid-1980s was relatively modest, the notification rate rapidly increased as time progressed and became a matter of concern.

It was therefore considered prudent to compare the resistance to inactivation of the BSE agent with that of scrapie agents. These studies showed that the already recommended procedure for achieving inactivation by exposure to sodium hypochlorite solutions containing 20,000 ppm available chlorine was effective with the BSE agent [46]. Solutions of sodium dichloroisocyanurate containing the same range of concentrations of available chlorine that proved effective when sodium hypochlorite was used, however, were not effective because they were more reluctant to release their available chlorine content [46].

In the late 1980s, Brown *et al*. [5] reported that treatment for 1 hour with 1M sodium hydroxide inactivated CJD and scrapie agents. The sensitivity of the bioassays in this study, however, was reduced because it proved necessary to dilute the samples to render them non-toxic for the recipient animals. Other reports have recorded the detection of residual scrapie infectivity after treatment with 1M sodium hydroxide for either 1 hour [18,19] or 24 hours [34]. Others have also reported on the survival of CJD infectivity after exposure to 1M or 2M sodium hydroxide [40]. More recent work with sodium hydroxide involving the BSE agent and 2 strains of scrapie agent has demonstrated that, if the pH of the samples is carefully neutralized, they can be injected into the assay animals without further dilution, thus enhancing the sensitivity of the bioassay [46]. Under these circumstances, infectivity can be shown to survive exposure to 2M sodium hydroxide for up to 2 hours. With the 263K strain of scrapie agent, although more than 5 logs of infectivity were inactivated during such treatments, approximately 4 logs survived [46].

Newer studies with PL autoclaving cast doubt on the reliability of the existing standard (134-138°C for 18 minutes) because the BSE agent and 2 strains of rodent-passaged scrapie agent survived exposure to such PL cycles [46]. The average mass of the infected brain macerates used in the more recent study, however, was 340 mg [46], compared with 50 mg used in the earlier study [30]. The decision to use the larger volumes of macerates of infected brain tissue was based upon the knowledge that, with similarly sized samples of intact (but not macerated) brain tissue, inactivation had been previously achieved by the 134-138°C PL autoclaving procedures [41,43,45]. In addition, it was considered that the larger sample sizes might more realistically represent the actual mass of TDE-infected tissue that may need to be disposed of by autoclaving during human and veterinary healthcare; however, no official advice has ever been issued in this respect.

In view of the uncertainties relating to PL autoclaving introduced by the studies of Taylor *et al*. [46], further experiments were carried out to assess the effectiveness of PL autoclaving cycles at 134, 136, and 138°C for times ranging from 9 to 60 minutes using samples of infected brain macerates weighing either 50 or 375 mg. The agents used were a) 22A, a mouse-passaged strain of scrapie agent that is known to be more thermostable than other strains of mouse-passaged scrapie agent [16,30]; b) 263K, a hamster-passaged strain of scrapie agent that had more recently been shown to survive PL autoclaving [46]; and c) 301V, a mouse-passaged strain of BSE agent that is the most thermostable strain characterized to date [53]. The data from these experiments indicated that 301V could survive exposure to 138°C for 1 hour. In accordance with earlier data, 50-mg macerates of 22A-infected brain-tissue in which the infectivity levels were around 7 logs per gram were inactivated by all of the 136°C processes [30]. The same was true for the 50-mg

macerates exposed for 4 different time periods at 134°C. Paradoxically, one case was observed in mice injected with material from a 50-mg sample autoclaved at 138°C for 9 minutes. This might have been rejected as an experimental aberration had it not been that positive cases were also detected in mice injected with material from 375-mg macerates autoclaved at 136°C or 138°C (but not at 134°C). These data suggest that the thermostability of the 22A strain became enhanced as the autoclaving temperature was increased, and the difference between the 134°C and 138°C samples was statistically significant ($p<0.01$). With 263K, the starting titer was $10^{8.3}$ ID_{50}/g, and there was a similar degree of survival of the agent whether autoclaving was performed at 134,136, or 138°C. This also supports the above hypothesis concerning the relationship between thermo stability and autoclaving temperature. For 301V which had a starting titer of $10^{8.6}$ ID_{50}/g, the data are even more convincing in this respect. Sixty percent of the animals that were injected with material autoclaved at 134°C developed disease; the ratio for similar samples exposed at 138°C was 72%. This difference was statistically significant ($p<0.05$). These data indicate that simply increasing PL autoclaving temperatures and holding times would not necessarily be effective in achieving a reliable decontamination standard for inactivating TDE agents by autoclaving. These data could have been open to question had it not been that the efficiency of the autoclave and the steam generator were checked by an independent agency during and after the experimental autoclaving cycles, and each experimental cycle was monitored independently by this agency, using thermocouples.

In an effort to explain the enhanced survival of infectivity as the autoclaving temperature was increased, it is pertinent that some smearing and drying of the infected brain macerates onto the glass surfaces occurred before autoclaving. It has been previously reported that this enhances the resistance of infectivity to inactivation by autoclaving [2,3]. It has been suggested that the PrP^{Sc} in the smeared and dried areas of the samples would become rapidly heat-fixed by steam at the beginning of the cycle, and become paradoxically more resistant to destruction by the subsequent autoclaving process. The proposal is that the rapidity and efficiency of this process would be enhanced by increasing the temperature, and this would explain the enhanced survival at the higher temperatures. Support for this concept comes from an experiment in which smeared infected brain-tissue was made much more resistant to autoclaving by prior heat-fixation in hot air at 160°C [44]. It is also known that prior fixation of infected tissue in formalin [45] or ethanol [43] considerably enhances the resistance of infectivity to inactivation by autoclaving.

Although autoclaving or exposure to sodium hydroxide *per se* do not completely inactivate TDE agents, inactivation can be achieved by combining these procedures. Taguchi *et al.* [38] and Ernst and Race [19] described the successful inactivation of CJD and scrapie infectivity, respectively, by a sequential process involving exposure to 1M sodium hydroxide followed by GD autoclaving at 121°C for 30 or 60 minutes, respectively. Complete inactivation of 263K has also been reported after GD autoclaving at 121°C for 90 minutes in the presence of 1M sodium hydroxide [34]. More recently, it has been observed that inactivation can be achieved if 22A is autoclaved at 121°C for 30 minutes in the presence of 2M sodium hydroxide (without a prior holding period in sodium hydroxide) [49].

The effectiveness of inactivation by hot sodium hydroxide is demonstrated by an experiment in which high levels of 301V, an extremely thermostable agent, were inactivated by boiling for 1 minute in 1M sodium hydroxide [52].

Inactivation Procedures Recommended by the World Health Organization

In recognition of the well-known difficulty of inactivating TDE agents, the World Health Organization (WHO) has adopted a pragmatic and practical approach in its relatively recent recommendations for decontaminating TDE-contaminated materials [55]. While it regards disposal in efficiently-functioning incinerators to be the most reliable method, it recognizes the practical and financial impracticalities of applying incineration universally. It has, therefore, described a number of methods that are listed in their order of effectiveness (Table 2), with the first method listed considered the most effective.

Table 2. Inactivation Procedures Recommended by the World Health Organization

1. GD autoclaving at 121°C for 30 minutes in NaOH*. Clean, rinse, and subject to routine sterilization.
2. Immerse in NaOH* or NaOCl** for 1 hour. Rinse. Immerse in water and expose to GD autoclaving at 121°C for 1 hour. Subject to routine sterilization.
3. Immerse in NaOH* or NaOCl** for 1 hour. Rinse, and transfer to an open pan. Expose to PL autoclaving at 134°C for 1 hour. Subject to routine sterilization.
4. Immerse in NaOH* and boil for 10 minutes. Rinse and subject to routine sterilization.
5. Immerse in NaOCl** (preferred) or NaOH* for 1 hour at ambient temperature. Rinse and subject to routine sterilization.
6. PL autoclaving at 134°C for 18 minutes.

* = 1 M sodium hydroxide solution.

** = sodium hypochlorite solution containing 20,000 ppm available chlorine.

In the first method, the items to be decontaminated are immersed in 1M sodium hydroxide, and are exposed to GD autoclaving at 121°C for 30 minutes. From the data presented earlier in this chapter, it can be seen that this process *per se* would be expected to achieve complete inactivation. As a matter of caution, however, the recommended WHO procedure is to follow this with washing and then to proceed with routine sterilization. The method deliberately does not define “routine sterilization” because the meaning of this will vary from laboratory to laboratory. The objective is simply to add an additional, but undefined, layer of safety to the first stage of the process that should be effective in itself. A similar philosophy applies to the recommended method that involves immersion in a sodium hypochlorite solution containing 20,000 ppm of available chlorine, followed by autoclaving, washing, and routine sterilization. Again, the first stage of the process (hypochlorite) is considered to be highly effective, as has been discussed, with the additional stages providing extra layers of safety. In contrast, the final method listed in Table 2 consists only of autoclaving at 134°C for 18 minutes. Although data presented earlier indicate that this is not a reliable method, its inclusion reflects the philosophy that it would

be better to use this method rather than nothing if the use of other methods listed in the table is precluded.

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‘Extreme’ Inactivation Methods for Transmissible Spongiform Encephalopathy Agents

Paul Brown, M.D.[1], Edward H. Rau, M.D.[2], Richard Meyer, Ph.D.[3], Paul Lemieux, Ph.D.[4], Franco Cardone, Ph.D.[5], and Maurizio Pocchiari, M.D.[5]

[1]National Institute of Neurological Disorders and Stroke, U.S.A.;
[2]National Institutes of Health, U.S.A.;
[3]Washington Farms, U.S.A.;
[4]National Homeland Security Research Center, U.S. Environmental Protection Agency, U.S.A.;
[5]Istituto Superiore di Sanità, Rome, Italy

Introduction

The notorious resistance to inactivation of pathogens that cause transmissible spongiform encephalopathy (TSE) is attested by numerous studies over the years, the results of which are summarized in Table 1. The present-day consensus is that exposure to 5% hypochlorite or 1 N NaOH solutions for at least 1 hour followed by autoclaving at 134°C for at least 20 minutes is the most effective method for re-usable materials [15].

The epidemic of bovine spongiform encephalopathy (BSE) and its species-crossing human consequence, variant Creutzfeldt-Jakob disease, have reawakened interest in the adequacy of inactivation methods for types of materials that are designated for destruction (such as infected cattle carcasses and their meat and bone meal by-product) or for consumption (bovine organs and processed meat products). Historically, incineration has been deemed adequate for the destruction and inactivation of infected tissues, but has not been studied under carefully controlled laboratory conditions that simulate actual incinerators. Moreover, inactivation methods for TSE-contaminated foodstuffs have never been investigated. We have recently acquired data on both types of situations.

Decontamination of Disposable Materials: Incineration

Although steam heat (autoclaving) inactivation has been far more extensively studied, enough is known about the exposure of TSE agents to dry heat to be confident about their resistance to temperatures well above the 150 to 160°C upper stability limit for biological macromolecules. They even partially resist temperatures that inactivate so-called 'extremophilic' archaeal species, which have been shown to survive brief exposures to temperatures between 270°C and 340°C, and bacterial spores, which may remain viable after exposure to 370°C [2].

Dickinson *et al.* reported that a mouse-adapted strain of scrapie agent (ME7) was not fully inactivated by baking at 160°C for 24 hours [7], and Steele *et al*. documented limited survival of several TSE strains after exposures to dry heat at temperatures up to 200°C [14]. During the last few years, we have performed 3 separate dry heat experiments using a hamster-adapted strain of scrapie (263K) which is known to be highly resistant to a wide array of physical and chemical disinfection methods. In the first experiment, we showed that a substantial amount of infectivity in freeze-dried brain tissue or purified brain extracts survived 1-hour exposures to 360°C [4].

Table 1. Physical and Chemical Methods Tested for the Inactivation of Transmissible Spongiform Encephalopathy Agents

Ineffective	Partially effective	Effective
	Chemical methods	
Alcohol	Chlorine dioxide	Hypochlorite (1-5%)
Ammonia	Gluteraldehyde	NaOH (1-2 N)
ß-propiolactone	Iodophores	Formic acid (100%)
Detergents	Guanidinium thiocyanate	
Ethylene oxide	Sodium dichloroisocyanurate	
Formaldehyde	Sodium metaperiodate	
Hydrochloric acid	Phenol (saturated)	
Hydrogen peroxide	Urea (6-8 M)	
Peracetic acid		
Permanganate		
	Physical methods	
Boiling (100°C)	Steam heat (121°C)	Steam heat (≥132°C)
Microwave radiation	Dry heat (300°C)	Dry heat (>600°C)
UV radiation		
Ionizing radiation		

In the second experiment, using crude brain tissue macerates, we verified the 360°C observation, and showed that a very small amount of infectivity could even survive ashing at

600°C. The ash from one sample of fresh brain tissue heated for 15 minutes transmitted to 5 of 18 animals; another sample heated for 5 minutes did not transmit to any of 15 animals; and one formalin-fixed sample heated for 5 minutes transmitted to 1 of 24 animals [5]. As no transmissions occurred from any sample heated to 1000°C, the infectivity extinction point was somewhere between 600°C and 1000°C, most probably very close to 600°C, approaching the operating temperature of some incineration units.

All of these experiments were conducted using simple laboratory ovens with precisely controlled temperatures. Our third experiment was designed more closely to simulate incinerator conditions. Gases flowed through a burner apparatus across a removable open crucible containing infectious tissue, oxidizing and/or pyrolyzing the tissue, and then exited through an impinger train to entrap gas emissions from the burner (Figure 1).

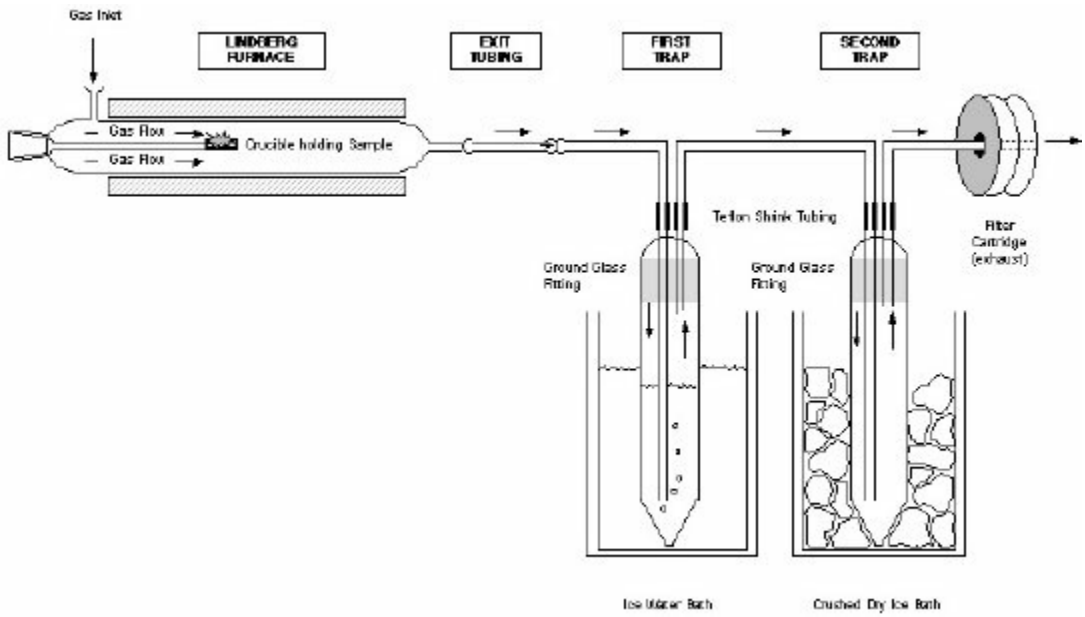

Figure 1. Schematic view of incineration simulation apparatus showing, from left to right, the gas inlet, Lindberg furnace surrounding a removable combustion chamber (quartz reactor tube), quartz exhaust tube, emission impingers (ice water bath followed by dry ice bath), and exhaust through filter into fume hood.

Infectious brain tissue samples were either burned in air or pyrolyzed in N_2 at 600°C or 1000°C for 15 minutes, and parallel tests were performed on uninfected brain tissue. Post-burn sample residues were collected separately from the crucible, the exit tubing, and the impinger train. All tissue ash and gas trap residues were prepared as 1-mL samples in distilled water, and the entire sample volumes were inoculated intra-cerebrally into of groups of 20 to 25 healthy weanling hamsters that were kept under observation in individual cages for 1 year. The brains of all animals, whether surviving or dying during the observation period, were examined for the diagnostic presence of proteinase-resistant 'prion' protein (PrPres).

We found that, once again, despite the nearly total destruction of over 10^9 mean lethal doses (LD_{50}), an ashed sample of 263K scrapie-infected tissue transmitted disease after having been exposed to 600°C for 15 minutes, and we found no survival after exposure to 1000°C (Table 2) [6]. We also showed that no infectivity escaped into air emissions at

either 600°C or 1000°C.

Table 2. Bioassay Results for Combustion Products from Infected Hamster Brain Tissue Macerates and Controls Heated for 15 Minutes

Tissue	Test Conditions		Bioassay Specimen[a]		
	Gas	°C	Crucible	Exit Tube	Traps
Normal	Air	Ambient	NA	NA	NA
Normal	Air	600	0/20	NT	NT
Normal	N_2	603	0/21	0/18	
Normal	Air	1015	0/23	NT	NT
Normal	N_2	1000	0/20	0/18	
Infected	Air	Ambient	NA	NA	NA
Infected	Air	612	2/21	0/22	0/24
Infected	N_2	598	0/20	0/19	0/26
Infected	Air	996	0/15	0/26	0/23
Infected	N_2	996	0/23	0/18	0/23

[a] For each test group, fractions represent number of PrPres-positive animals over total number of inoculated animals. Residues from the exit tubes and emissions from the impinger traps were combined for bioassays of the uninfected control samples subjected to 600°C and 1000°C under N_2.

NA = not applicable; NT = not tested.

We conclude that at temperatures approaching 1000°C under the air conditions and combustion times used in this experiment, contaminated tissues can be completely inactivated. Our conditions resembled those commonly used for incineration of cattle carcasses and by-products, and for the disposal of hospital wastes (Table 3). Whether or not these results can be realized in actual incinerators and other combustion devices will depend upon equipment design and operating conditions during the heating process. If properly operated and subjected to quality control inspections, however, incinerators should totally inactivate TSE-contaminated tissues and materials and should not pose any environmental risk from burial of residual ash in landfills or from gaseous stack emissions.

Table 3. Operating Conditions of Medical Waste Incinerators (MWI) and Meat and Bone Meal (MBM) Furnaces

Type of Unit	Chamber Air Temperature (°C)	
	Primary	Secondary
Controlled Air MWI	760-980	980-1095
Express Air MWI	Variable	870-980
MBM Disposal	800-1000	No data

Disinfection of Consumable Materials: Ultra-High Pressure

Ultra-high pressure has been investigated as a method of food preservation for over a century [11], but did not become popular until about 10 years ago when advances in equipment design led to commercially reliable high pressure processing machines. Ultra-high pressure has a 2-fold effect on the preservation of food: inhibition of enzymatic activity that degrades food flavor and quality and inactivation of conventional pathogens (bacteria and fungi) that may be present in food sources, or may enter in the course of food processing [12,13].

The mechanisms by which ultra-high pressure acts on protein molecules are only imperfectly understood, but clearly involve several different (and sometimes competing) effects on intra- and inter-molecular bonds that produce unpredictable changes in different protein species [3]. One consistently observed effect, however, is the disaggregation of polymeric and aggregated forms of protein, and 'prions' consisting of aggregates of an abnormal β–sheet-rich protein conformer of a normal protein. Reversible disaggregation of transthyretin and amyloid A subjected to pressures of 350 MPa and 1200 MPa, respectively, has been reported [8,9], and in another study, the normal form of the yeast prion (URE 2) suffered limited structural change under pressures as high as 600 MPa [17].

We prepared an infectious brain tissue pool of the same 263K strain of hamster-adapted scrapie used for the incineration experiments and mixed it with homogenized hot dogs, and then aliqoted 2 to 3 gram samples into heat-sealed pouches. The samples were pre-heated to 80 to 95°C, depending on the chosen pressure, to achieve test run temperatures of 121 to 134°C (adiabatic heating is pressure dependent). Samples were subjected to 3 or 10 1-minute pressure pulses at each temperature/pressure combination (Figure 2 shows a representative tracing of one test run). Treated and untreated tissue samples were compared with respect to PrPres (Western blots) and infectivity (bioassays).

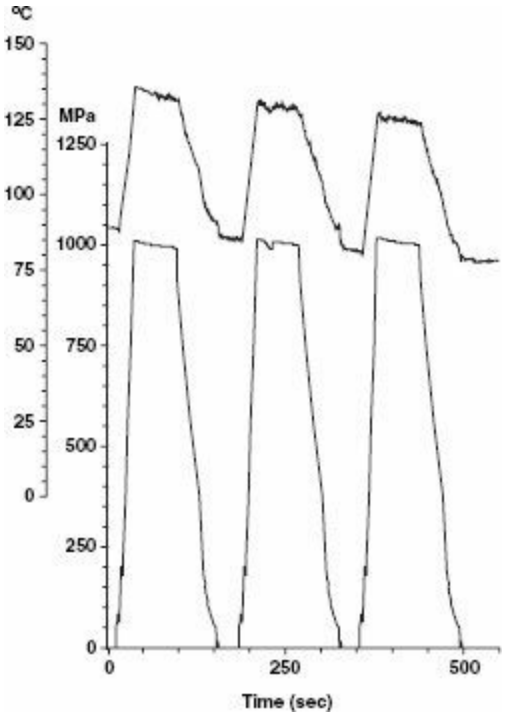

Figure 2. Example of pressure/temperature test run recording: 3 pulses of 1000 MPa at a starting temperature of ~85°C. Upper tracing shows temperature; lower tracing shows pressure. Adiabatic heating during pressure application raised the temperature by 50°C to ~135°C during the first pressure pulse; heat loss through the chamber lowered the temperature of each succeeding pulse by ~5°C.

As summarized in Table 4, the lowest pressure/temperature combination of 690 MPa (100,000 psi) at 120 to 125°C reduced PrPres by 1.5 logs, and infectivity by approximately 3 logs. Higher pressure/temperature combinations produced greater reductions (nearly 6 logs of infectivity reduction at 1200 MPa and 135°C). Comparison of results from the 3 and 10 pulse samples, and from 1 sample that was continuously pressurized for 5 minutes (rather than repeatedly pulsed), suggested that maximum effect could be achieved in 5 minutes or less in a single exposure. Also, one sample that was autoclaved for 5 minutes reduced infectivity by a comparable amount, suggesting that pressure was not a major factor in the inactivation process.

Table 4. Proteinase-Resistant Protein (Log_{10}PrPres) and Infectivity ($Log_{10}LD_{50}$) Reductions Under Various Pressure, Temperature, and Exposure Time Conditions

Pressure (MPa)[a]	Temperature (°C)	Number of 1-minute pulses	Log_{10}PrPres Reduction	$Log_{10}LD_{50}$ Reduction (8 log input)
690	125	3	1.5	~3.0
690	120	10	1.5	~3.0
1000	135	3	≥3.0	3.8
1000	135	10	≥3.0	5.7

1200	135	3	≥3.5	5.8
1200	135	10	≥3.5	5.6
1200	142	1 (5 min)	≥3.5	6.3
0.2	120-135	1 (5 min)	≥3.5	6.7

[a]690 MPa = 100,000 psi.

Bottom row shows results of a precisely controlled autoclave exposure

It might be asked why we should go to the trouble of using high pressure when simple autoclaving would provide an equally satisfactory infectivity reduction. The answer is that meat and meat products cannot be autoclaved and retain their ‘aesthetic’ characteristics of texture and flavor. Also, as it turns out, subsequent experiments have shown that pressure does in fact contribute to the inactivation process, as shown in Table 5.

In further ongoing experiments, we have established that similar results can be obtained using a variety of different meat product substrates (hamburgers, paté spreads, canned corn beef, baby food, and cat food), and most importantly, using a variety of different TSE strains, including BSE. A great deal more work still remains to be done: validation of the reproducibility of infectivity reduction; demonstration of total inactivation of the much lower levels of infectivity that might realistically be present in contaminated meat products; and optimization of conditions for practical commercial application of the methodology, but the results to date are encouraging.

Table 5. Titers of PrPres (Western blots) in 263K Hamster-Adapted Scrapie Brain Tissue Subjected to Different Pressure/Temperature/Time Conditions

Test Conditions			Weight equivalent brain (µg) in sample							
MPa	°C	Min	1000	330	100	33	10	3.3	1	0.3
Untreated control sample					+	+	+	-		
Varying pressure series										
600	134	5			+	+	±	-		
800				+	+	±	-			
1000			+	±	-					
1200			-	-	-					
Varying temperature series										
1200	115	5	+	+	+					
	118		+	+	±					
	125		+	+	-					
	130		+	±	-					
	134		-	-	-					
Varying time series										
600	127	1			+	+	+	+		
		2			+	+	+	±		
		3			+	+	±	-		
		4			+	+	±	-		
		5			+	+	±	-		

Independent of, but coincident with, our own study, 2 other groups have also been investigating the effects of ultra-high pressure on ‘prion’ species. Working with a recombinant form of the protein, Balney *et al.* reported that at ambient temperature under pressures as high as 600 MPa, a type of reversible denaturation occurred that was distinct

from that seen after exposure to heat [1,16]. Fernández Garcia *et al.* claim to have obtained progressive loss of resistance to proteolytic digestion of PrPres and parallel infectivity reductions in 263K brain homogenates exposed for 2 hours at 60°C to pressures from 100 to 1000 MPa [10]. If this result can be confirmed, it would open the way to applications of the technology to materials even more difficult to preserve than food, such as therapeutic plasma proteins and contaminated medical and surgical instruments that cannot withstand disinfection by autoclaving.

In conclusion, we believe that the ensemble of studies on ultra-high pressure inactivation of TSE pathogens, still at a very early stage of development, have the potential both to elucidate the molecular biological mechanisms by which the normal protein is transformed into its pathological conformer, and to add a significant new dimension to strategies designed to minimize the risk of environmentally-acquired TSE infections.

Acknowledgement

We are indebted to a number of people who assisted us in each of the two separate inactivation method studies: Dr. Bruce Johnson, Mr. David Liles, and Mr. Al Bacote for incineration studies; and Dr. Huub Lelieveld and Mr. Jan Hjelmqwist for high pressure studies.

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Infectious Prion Diseases in Japan

Takeshi Sato, M.D., Ph.D.

Higashi-Yamato Hospital, Japan

Introduction

Prion disease or transmissible spongiform encephalopathy (TSE) is comprised of a number of fatal neuroinfectious diseases in humans and animals. The animal prion diseases include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, and transmissible encephalopathy in mink. In humans, the prion diseases include sporadic Creutzfeldt-Jakob disease (CJD), familial CJD, and infectious prion disease [10].

Sporadic CJD is the most common prion disease accounting for approximately 85% of human prion diseases. The mean age at onset is 63 years of age (range: 30 to 92 years). The clinical features are characterized by rapidly progressive dementia, myoclonus, and periodic synchronized discharges (PSDs) in the EEG.

The infectious prion diseases include kuru in Papua New Guinea transmitted by cannibalism, variant CJD in the United Kingdom (U.K.) transmitted by BSE [17], and iatrogenic cases.

Iatrogenic Prion Disease

As of September 2003, 158 cases of iatrogenic prion disease were reported worldwide due to prion-contaminated dura mater grafts; 107 of these cases were reported in Japan. In addition, 154 patients received cadaveric human pituitary growth hormone and gonadotropin, and worldwide 3 received corneal grafts, 2 of them in Japan. Two cases were reported to be transmitted from intra cerebral stereotactic electrodes used to record EEG in the cerebrum [1,5]. Patients thought to be infected during neurosurgery were 5 worldwide, one of which was from Japan [11].

CJD Associated with Dura Mater Grafts

Epidemiology and Inactivation Procedure

The first case of iatrogenic CJD transmitted through cadaveric dura mater grafts was reported in 1987 [9], and since then the number of cases has increased steadily, reaching 158 cases in 18 countries as of September 2003. More than two-thirds of these cases were reported in Japan. A nationwide survey documented 107 dura-related cases during the period between 1979 and 2003. Most cases had received dural grafts between 1982 and 1987 [11-13] (Figure 1).

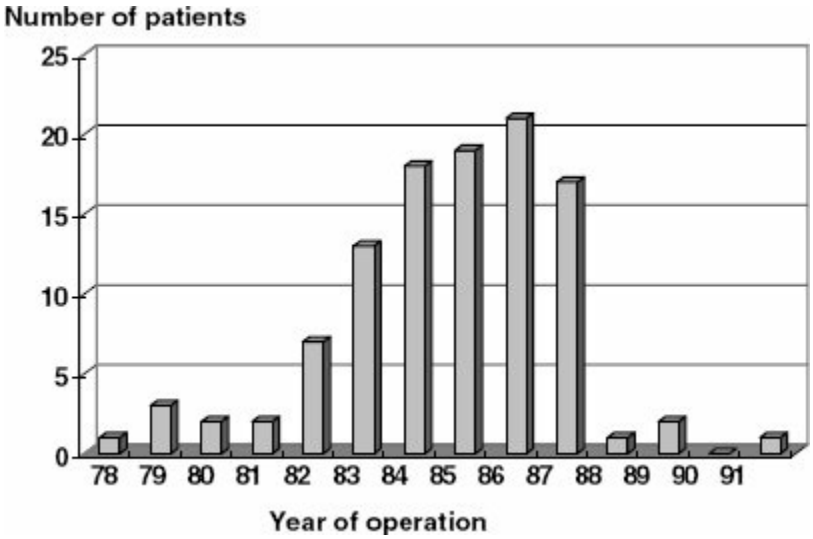

Figure 1. Number of Creutzfeldt-Jakob disease (CJD) cases from dural grafts and year of operation.

Figure 2 shows the occurrence of 107 cases with dura-related CJD during the period from 1985 to 2003. The incubation period from graft implantation to the appearance of first symptoms varied from 16 months to 23 years (Figure 3). The number of confirmed cases was seen to increase concomitantly with an increase in the length of the incubation period from 1 to 15 years. As seen in Figure 4, however, the number of new CJD patients decreased markedly when the incubation period was 16 years or longer.

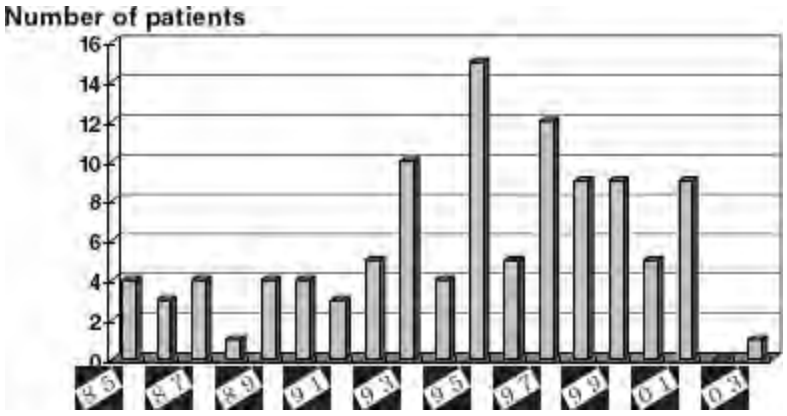

Figure 2. Number of dura-related CJD patients in Japan, 1985-2003.

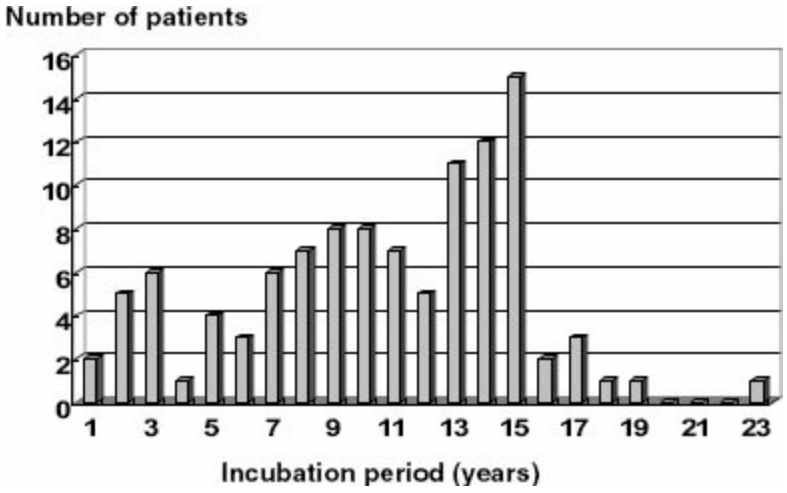

Figure 3. Number of dura-related CJD patients by incubation period (from graft to onset of CJD symptoms).

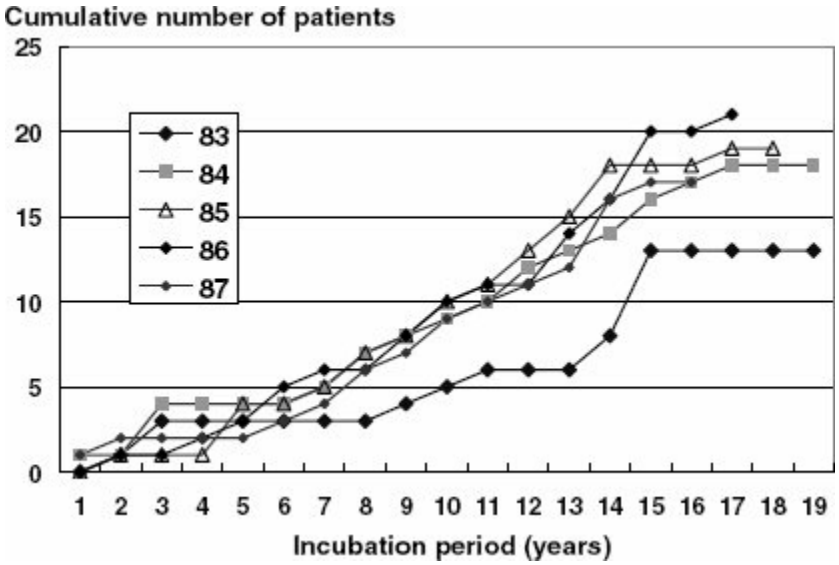

Figure 4. Cumulative number of CJD patients versus years to symptom onset for patients receiving dural grafts between 1983 and 1987. Number of patients between 1- to 15-year incubation period shows gradual increase. Number of new CJD patients increases only slightly for incubation periods longer than 15 years.

A number of patients in Japan received dura mater during neurosurgical and orthopedic procedures (36 meningioma or other brain tumors; 30 aneurysm, subdural, or intracerebral bleeding; 12 acoustic neurinoma; 22 Jannetta's operation for decompression of facial nerve spasm or trigeminal neuralgia; 4 Arnold-Chiari malformation; 2 spinal tumor; 1 ossification of the posterior longitudinal ligament). The mean age of onset for dura-related CJD was younger (54.8±14.0 years) than for sporadic CJD (64±10 years).

Incidence of sporadic CJD was 0.6 to 1 per million inhabitants in Japan. It is estimated that approximately 12,000 to 20,000 grafts/year were used in Japan. Two thirds of imported dura mater was Lyodura®. Of the 107 dural patients, 100 were identified as receiving the same type of dura mater. The source of the dura mater for the other 7 patients could not be identified (1, 3, 2, and 1 in 1985, 1986, 1987, and 1991, respectively).

In the peak years between 1984 and 1987, 17 to 21 patients were present among 8,000 and 13,000 Lyodura recipients. Risk calculation of dura-related CJD revealed that the incidence was very high, as 0.15 to 0.25% in neurosurgical patients receiving dura mater during the peak years of 1984 to 1987.

Until April 1987, Lyodura was sterilized with 10% H_2O_2 and gamma irradiation without NaOH treatment. Beginning in May 1987, the supplier began supplementing the standard sterilization procedure with immersion in 1N NaOH for 1 hour. Following this change in procedure, no new CJD cases were seen among patients receiving dura mater grafts treated with 1N NaOH, except one case that received a dura mater graft from another supplier [3]. However, even when treated with 1N NaOH, the infectivity of cadaveric dura mater does not disappear completely [16]. Therefore, the recommendation that all existing cadaveric dura mater products be banned was finally endorsed in 1997 by both the World Health Organization (WHO) and the Japanese Ministry of Health and Welfare.

Spread of Infectious Prion Protein from the Site of Graft

We recently cared for a 74-year-old housewife who received a dura mater graft (Lyodura) in September 1986 during the course of neurosurgical treatment for meningioma in the right parasagittal cortex. In April 2003, she developed a tremor in the upper left extremity and on June 3, she visited our out-patient clinic and complained of a rapid development of gait disturbance and left-sided hemiparesis associated with myoclonic jerks.

Neurological examination revealed left hemiparesis, myoclonus, and mild rigidity in both extremities. She had normal mental activity and no cerebellar ataxia. Examination of the cerebrospinal fluid was positive for 14-3-3 protein. Magnetic resonance diffusion-weighed imaging (DWI) revealed a clearly increased signal surrounding the postoperative site in the right parietal cortex. These findings suggested that the abnormality associated with the prion protein originated in the region surrounding the dura mater implantation. The patient rapidly deteriorated to akinetic mutism with bilateral myoclonic jerks and immobility necessitating complete nursing care. On August 1, serial DWI showed a continuous spreading of the high-signal area along the cerebral cortex of the entire brain and in the putamina and caudate nuclei [14].

It has been reported that regions with increased signals in magnetic resonance imaging are correlated with a dramatic accumulation of the pathological prion protein, as shown by immunohistochemistry in autopsied brain [2]. A host prion protein for prion disease pathogenesis is abundantly expressed in the central nervous system, where it may be conformationally modified during the course of the disease into a protease-resistant form [10]. Our observations suggest that the infectious prion protein spread from the infected dura mater graft to the adjacent cerebral cortex. The infectious prion protein might have continued to spread to the adjacent cerebral cortex, caudate nucleus, and putamen via nerve pathways. In the advanced stage, the infectious prion protein may spread cell-to-cell via nerve pathways and throughout the perivascular space.

Our previous report suggested that initial manifestations of dura-associated CJD may be related to the grafted sites; in 29 patients with dural grafts in the infratentorium cerebelli,

unsteady gait was found as in the initial manifestation in 48% of the patients, whereas in 14 patients with grafts in the supratentorium, unsteady gait was found in only 7% [12]. A CJD patient with implantation of dura mater in the posterior fossa for Jannetta's operation developed vertigo and nystagmus as the early initial symptom, and neuro-otological examinations suggested an initial lesion occurring adjacent to the grafted site [7]. In our case, the initial manifestation may have developed directly from the site of the dura mater graft, as evidenced by the high signal area on DWI. It has been reported that DWI appears to be much more sensitive than conventional MRI with respect to detecting early abnormalities in sporadic CJD [8], and this is consistent with the MRI and DWI findings in our case with dural CJD in which the initial manifestation correlated well with the abnormalities seen on DWI. Thus, careful observation of the initial symptoms and early DWI should be directed at early diagnosis of dura-related CJD patients.

Atypical dura-Associated CJD with Slow Progression

Almost 85% of dura-related CJD cases show rapid progression in the clinical course and are clinicopathologically similar to sporadic CJD. These cases develop akinetic mutism within 2 to 6 months from the onset of symptoms. On the other hand, about 15% show slowly progressive deterioration and are atypical with respect to the clinical features. These cases are characterized by the development of akinetic mutism over a period longer than 1 year from onset and the lack of occurrence or late occurrence of myoclonus and PSDs in the EEG [13] (Figure 5). The neuropathological findings are also characterized by the presence of many prion protein plaques in the brain. Some of these plaques are the "florid" type surrounded by a zone of spongiform changes, known as a hallmark of variant CJD. The distribution of spongiform changes is different from that in sporadic CJD, and is predominant in the thalamus, basal ganglia, and dentate nucleus of the cerebellum, although mild in the cerebrum. There were no mutations in the prion protein gene of these patients and no difference between rapid and slow progression group at codon 129. Western immunoblot analysis of the brain tissues showed the presence of type 1 prion protein of 3 cases in slow progression [6,15].

Conclusions

We first described signal abnormalities seen on DWI in the region surrounding the grafted dura mater in the cerebral cortex, and these signals were correlated with the focal signs and symptoms. We next discussed atypical cases with dura-associated CJD characterized by a slower progression. The observations presented here are expected to contribute to the early diagnosis of dura-related CJD and strongly indicate the need to prevent iatrogenic CJD.

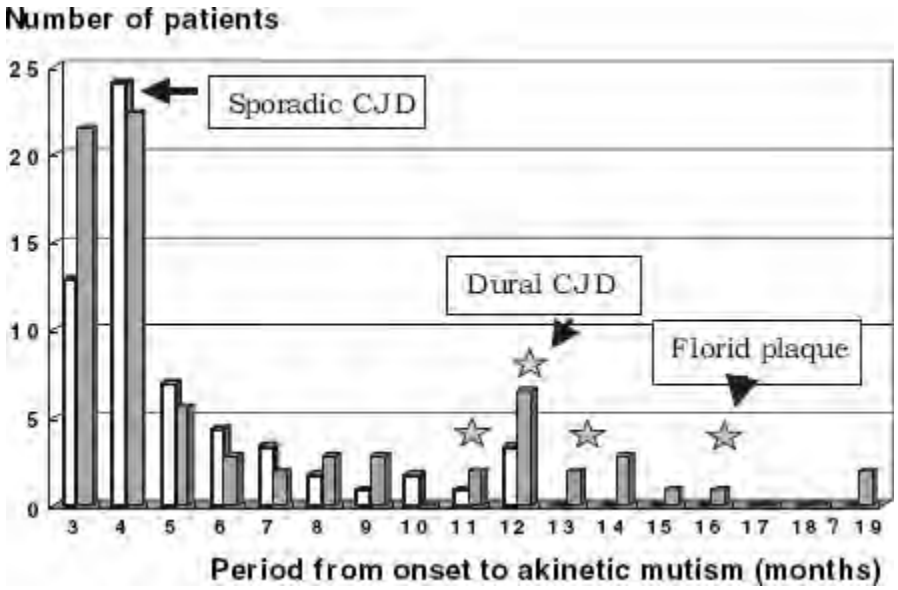

Figure 5. Period from onset of symptoms to akinetic mutism in sporadic (white column) and dural CJD (gray column) patients. Dural patients are characterized by generally slower progression in development of akinetic mutism, sometimes extending 11 months or more after onset of symptoms, and by florid plaque in the brain (star).

Although cadaveric dura mater products have been banned, the danger of unexpected infection from unknown infectious agents will always be present in biological products. Further efforts are required to prevent similar occurrences and to develop better detection methods to improve the safety of biological products for use in humans.

Acknowledgments

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New Bioassay System for Human Prion Disease

Tetsuyuki Kitamoto, M.D.[1] and Shirou Mohri, D.V.M., Ph.D.[2]

[1]Tohoku University Graduate School of Medicine, Japan;
[2]Kyushu University Graduate School of Medicine, Japan

Introduction

Creutzfeldt-Jakob disease (CJD), scrapie, and bovine spongiform encephalopathy (BSE) are transmissible neurodegenerative diseases. Attempts to isolate the agent of scrapie have led to the discovery of a protein, designated prion protein (PrP) [20]. The protease-resistant isoform of PrP (PrP^{Sc}) is implicated in the pathogenesis and transmission of scrapie and CJD [21]. Detection of PrP^{Sc} is useful for a diagnosis of prion disease, and a bioassay method is necessary to check for infectivity. It has proven problematic from the standpoint of sensitivity to use a wild type mouse as a bioassay for human prions. In one study, all mice became sick after 120 days post-inoculation when inoculated with mouse prions, but only 20% of mice became sick after 600 days post-inoculation when inoculated with human prions [25]. Thus, it is important to establish a sensitive bioassay system for evaluations of biosafety against the human prions.

Humanized Mice with Transgenic Technology

To establish an animal model, we used the transgenic technology with the mouse prion protein promoter. In the mouse PrP gene, the intron 2 is more than 20 kbp, and this length was an obstacle when making a transgenic vector. We used the PrP gene of I/Ln mouse which has a deletion of intron 2 (Figure 1), and we constructed a chimeric human/mouse PrP gene (Figure 2), containing 5 octapeptide repeats and either methionine (designated ChM) or valine (designated ChV) at codon 129. This chimeric PrP gene also includes 4 codons between positions 39 and 96, differing from the open reading frame (ORF) of the Tg(MHu2M) construct [26]. We created transgenic mice expressing either ChM or ChV. Transgenic mice with an ablated background ($Prnp^{0/0}$) were produced by repeated backcross with knockout mice [29]. The recombinant PrP (ChM or ChV) was expressed in the neurons of the central nervous system (Figure 3). Immunohistochemistry also revealed the recombinant PrP in the central nervous system of the transgenic mice with a high expression level. Interestingly, the hydrolytic autoclaving method enhanced the immunoreactivity of the recombinant PrP^{C} in the transgenic mice with high expression levels. It was known that this hydrolytic autoclaving method enhanced immunostaining of PrP^{Sc} [9], but it became clear that it enhanced immunostaining both of PrP^{Sc} and PrP^{C}. Therefore, this method might detect PrP^{Sc} not due to a qualitative difference of PrP^{Sc}, but due to a quantitative difference.

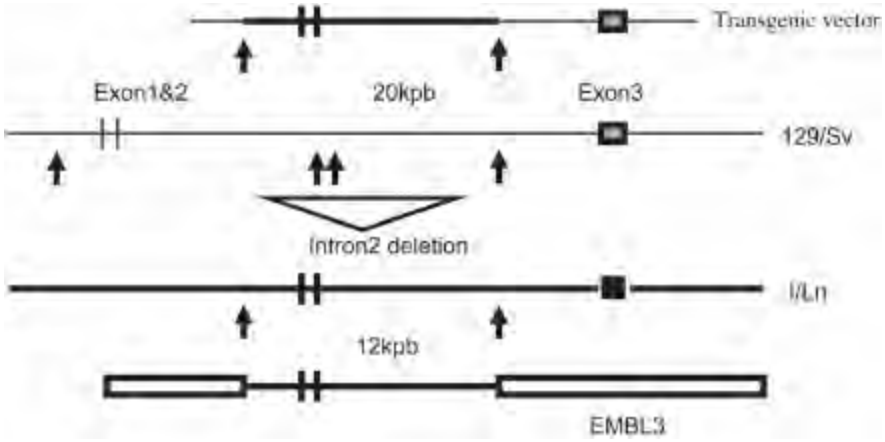

Figure 1. The transgenic vector and the genomic structure of murine PrP gene.

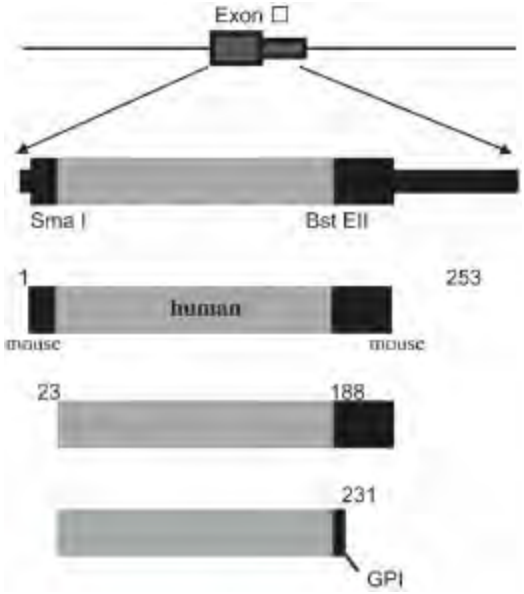

Figure 2. The open reading frame structure of the transgenic mouse.

Figure 3. *In situ* hybridization of the transgenic mouse. The recombinant PrP is expressed in a neuronal pattern.

Susceptibility to Human Prions in Transgenic Mice

To measure the transmission of human prions, brain homogenates were prepared from a sporadic CJD (sCJD) patient (H-3; codon129 Met/Met, type 1; designated MM1). Tg-ChM#30 ($Prnp^{0/0}$) mice possessed short incubation periods (Table 1). Surprisingly, paradoxically long incubation times were found in Tg-ChV ($Prnp^{0/0}$) mice expressing high levels of the transgene. Tg-ChV#21 mice ($Prnp^{0/0}$, expression level; 4x) demonstrated longer incubation periods than Tg-ChV#12 mice ($Prnp^{0/0}$, expression level; 2x) following inoculation with sCJD human prions. In our chimeric PrP construct, overexpression did not shorten the incubation periods seen in transgenic mice expressing wild type PrP [1,22].

Table 1. Transmission of Human Prions to Both Knock-in Mice and Transgenic Mice Via Intracerebral Route and the Resulting Positivity of PrPSc Observed in Lymphoid Organs

Recipient Mouse Line	Expression	Inoculum	Incubation Period*	Spleen	LN	Peyer¶
Tg-ChM#30 (Prnp0/0)	0.7X	sCJD (H-3)	156 ± 14.2 (11/11)	(0/8)	(0/6)	(0/4)
Tg-ChV#12 (Prnp0/0)	2X	sCJD (H-3)	175 ± 15.3 (18/18)	(0/18)	(2/12)	(2/7)
Tg-ChV#21 (Prnp0/0)	4X	sCJD (H-3)	192 ± 4.0 (3/3)	(1/3)	(0/2)	(0/2)
Ki-ChM (PrnpChM/ChM)	1X	sCJD (H-3)	151 ± 6.7 (7/7)	(5/5)	(4/4)	(3/3)

* Incubation Period: Mean ± SD days post-inoculation (transmitted mice/total mice),

¶ Peyer (Peyer's patches): positivity of immunoreactive PrP^{Sc} in the follicular dendritic cells (number of positive mice/number of total mice examined). PrPres typing was done by the method of Parchi *et al.* [18,19].

Key: LN: (lymph nodes); (PrnpChM/ChM): homozygous knock-in mice with chimeric PrP; (Prnp0/0): ablated PrP background mice; sCJD: sporadic Creutzfeld-Jakob disease; SD: standard deviation; H-3: codon 129 Met/Met and type 1 PrP^{Sc} (MM1).

Humanized Mice with Knock-in Technology

In order to get a more natural expression level and distribution of recombinant PrP, we decided to make the knock-in mouse using a homologous recombination technique. The knock-in construct, reported previously [10], contained minor modifications (Figure 4). The open reading frame was replaced with a ChM construct. The ChM contains the same ORF (codon 129 Met) as the transgenic construct. The selection marker for homologous recombination was also altered from MC1 neo-PGK gpt to PGK neo gene. The homologous recombination, germ-line transmission, and Cre-induced recombination to delete the PGK neo gene, have been described previously [10].

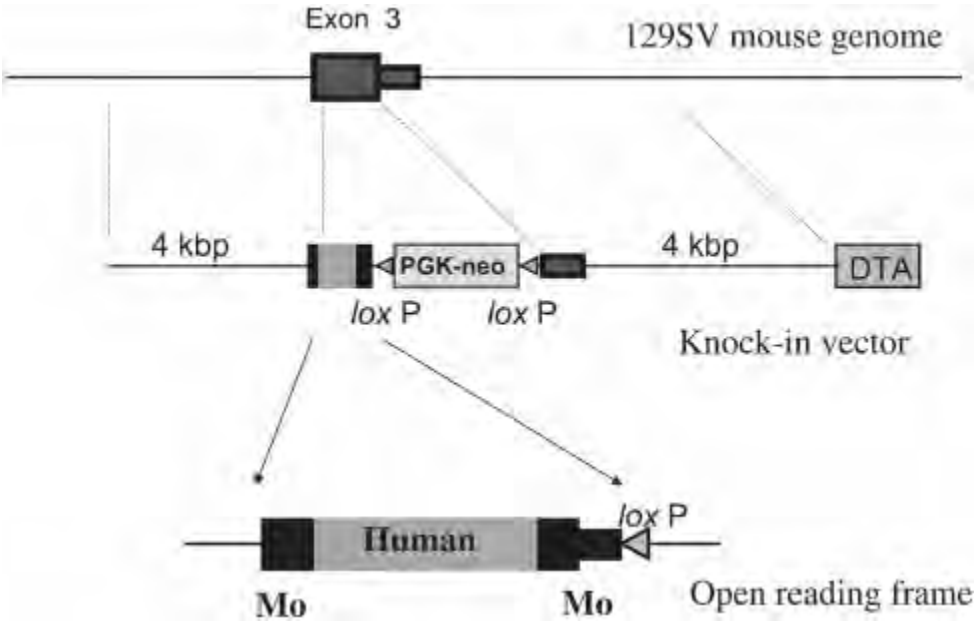

Figure 4. Construction of the knock-in vector. DTA is corresponding to a diphtheria toxin gene.

Susceptibility to Human Prions in Knock-in Mice

The same homogenates were inoculated into knock-in (Ki-ChM) mice (Table 1). Ki-ChM ($Prnp^{ChM/ChM}$) mice were highly susceptible to the same human prions (H-3). ChM PrP contains the human PrP sequence between codons 23 and 188 and the mouse PrP sequence between codons 189 and 231. The differences in these sequences are located in the octapeptide repeat region and the C-terminal chimeric region between codons 189 and 231 (Figure 2). The C-terminal sequence of mouse PrP is thought to be a binding site for protein X [27]. Ki-ChM mice showed almost the same incubation periods after the intracerebral inoculation compared with Tg-ChM#30 mice.

Follicular Dendritic Cells

The infectious agent of CJD or scrapie replicates in the lymphoid organs, especially in the spleen, long before involvement of the central nervous system [4,13]. Practically, PrP^{Sc} could be detected in the lymphoid organs by Western blotting prior to the neuronal phase [3,6,24]. In 1991, we identified follicular dendritic cells (FDC) as the site of PrP^{Sc} accumulations in the lymphoid organs [8]. Severe combined immunodeficiency (SCID) mice were subjected to transmission via the intracerebral route but not via the intraperitoneal route. The SCID mice did not have PrP^{Sc} accumulations in the FDC. Based on these results, FDC may play a major role as reservoir cells in peripheral prion infections. We hypothesized that FDC in the SCID mice may not be well maturated because of B-cell depletion, and therefore FDC of the SCID mice could not play a role in PrP^{Sc} accumulations. Our hypothesis that the maturation of FDC is of importance for the PrP^{Sc} accumulation has been supported by others [11,12,14].

In addition, sequential transmission experiments revealed that PrP^{Sc} accumulated in FDC was detectable in the early stage of the mouse prion infections [15,17]. The preclinical diagnostic value of FDC has been confirmed by the tonsilar tissues of the sheep [23,28] and the appendix tissues from a patient before the onset of variant CJD (vCJD) [5]. Thus, PrP^{Sc} detection in FDC of a mouse model might be a rapid bioassay system for human prions. However, the accumulation of PrP^{Sc} in the FDC did not occur in New Zealand White (NZW) mice inoculated with materials from human CJD (human prions), suggesting that there is the species barrier working in the lymphoreticular system between human and mouse [16]. Therefore, we have a working hypothesis that the FDC of the humanized mouse model could be an excellent tool to detect human PrP^{Sc} in the early stage of the transmission experiment.

Humanized Mice and FDC

Immunohistochemical analysis of lymphoid organs with anti-PrP [7] revealed PrP^{Sc} stainings in the FDC of the spleens, lymph nodes, and intestinal Peyer's patches in Ki-ChM mice at the onset of the disease. In contrast, PrP^{Sc} was not detected in the FDC of Tg-ChM or Tg-ChV mice with prion disease. Exceptionally, only few transgenic mice have PrP^{Sc} stainings in the FDC of lymphoid tissues (Table 1). FDC of the transgenic mouse were negative for PrP^{Sc}, and the knock-in laboratory mouse was a simple result to be positive.

The absence of PrP^{Sc} in the FDC of the transgenic mice was ascribed to the level of recombinant PrP expression. Estimation of recombinant PrP^{C} expression in the membrane fraction of the spleen showed that the Tg-ChV#12 ($Prnp^{0/0}$) mouse had <25% expression compared with Ki-ChM ($Prnp^{ChM/ChM}$) mouse. Immunohistochemistry revealed positive PrP^{C} expression in the splenic FDC of the Ki-ChM mice but not of the Tg-ChV#12 mice. Thus, our transgenic vector led to an attenuated expression of recombinant PrP in the spleen.

Sequential Analysis in the Follicular Dendritic Cells of the Spleen

Ki-ChM ($Prnp^{ChM/ChM}$) mice were inoculated intraperitoneally with 50 mL of 10% homogenate of human sCJD prions (H-3). Ki-ChM mice showing the positive immunoreactions in FDC of the spleen increased in number as the incubation time was prolonged, and 100% of Ki-ChM mice had positive FDC stainings in the spleen at 30 days post-inoculation. Positive FDC stainings in the lymph nodes or the Peyer's patches developed slowly (Figure 5). All of the Ki-ChM mice had positive FDC stainings in the lymph nodes at 60 days post-inoculation, and in the Peyer's patches at 75 days post-inoculation. In our previous results of NZW mice inoculated with Fukuoka-2 mouse prions, mouse PrP^{Sc} in splenic FDC was detected in 80% of mice inoculated intraperitoneally at 14 days post-inoculation, and in 100% of mice at 30 days post-inoculation [16]. As the FDC in NZW mice provided a sensitive bioassay system for mouse prions, the FDC in Ki-ChM mice did so for human prions. Therefore, analyzing PrP^{Sc} in the spleen of Ki-ChM mice could be a preclinial diagnosis and a bioassay marker for human prions.

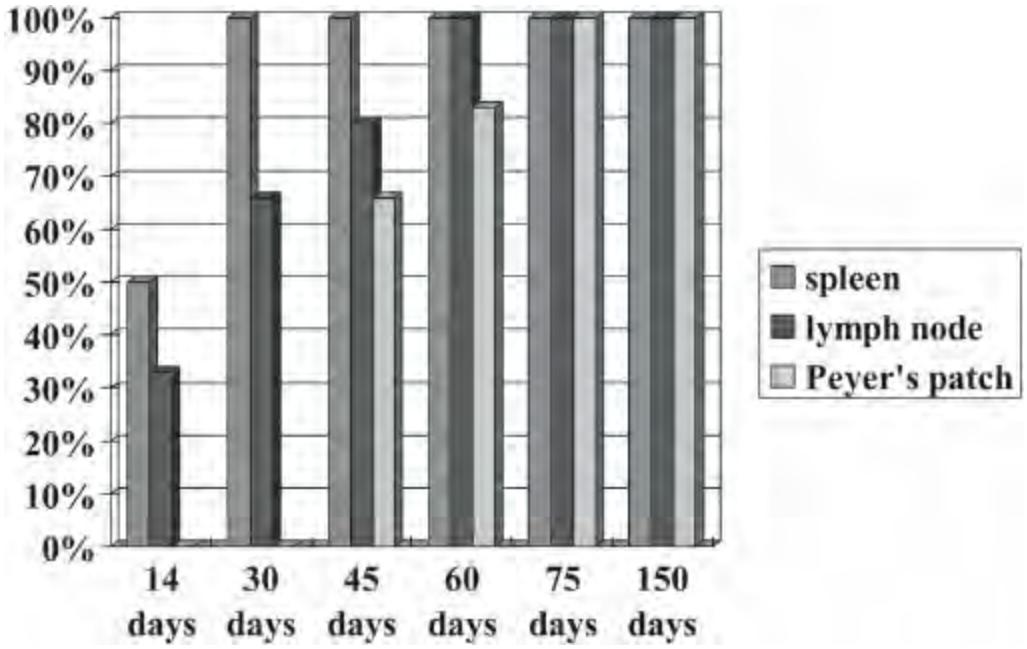

Figure 5. Positive PrPSc immunostainings of FDC.

Bioassay for vCJD

The FDC assay system was used to examine vCJD prion (Table 2). The inoculum consisted of 10% homogenate of the brain tissue prepared from a patient with sCJD or vCJD. As a control, we used 2 patients with Alzheimer's Disease. A 50-mL sample of each homogenate was inoculated intraperitoneally into Ki-ChM ($Prnp^{ChM/ChM}$) mice, and the mice were sacrificed at 75 days post-inoculation. We detected PrP^{Sc} in the splenic FDC of the mice inoculated with the samples from sCJD and vCJD. PrP^{Sc} was also detected in the splenic FDC of all the mice inoculated with vCJD prions. All of the human prions from 3 patients with vCJD led to the positive FDC stainings in each of the inoculated mice. No positive FDC stainings were obtained for the samples from patients with Alzheimer's Disease. Thus, the positivity of PrP^{Sc} in the splenic FDC correlated well with the presence of PrP^{Sc} in the inoculum from human brains.

Table 2. Transmission of Various Human Prions and Alzheimer Brains to Ki-ChM Mice and Positivity of PrP^{Sc} in the Spleen

Inoculum	Spleen*
sCJD(H-3)	(7/7)
sCJD (Sumi)	(5/5)
vCJD (96/02)	(5/5)
vCJD (96/07)	(4/4)
vCJD (96/45)	(6/6)
AD (H3982)	(0/4)
AD (H4023)	(0/7)

* Spleen: positivity in the follicular dendritic cells of the spleen (number of positive mice/number of total mice examined).

Note: Ki-ChM (PrnpChM/ChM) mice received intraperitoneal inoculation of 10% brain homogenates prepared from a patient with sporadic CJD (sCJD), variant CJD (vCJD), or Alzheimer's Disease (AD). The mice were sacrificed and examined at 75 days post-inoculation.

Key: H-3: MM1; Sumi: MV1; 96/02: MM2* [28] or MM4 [29]; 96/07: MM2*/MM4; 96/45: MM2*/MM4; H3982: codon 129M/M and codon 219E/E without PrP^{Sc}; H4023: codon 129M/M and codon 219E/E without PrP^{Sc}.

Bioassay System to Establish Therapy Against Infectious Prion Diseases

Infectious prion diseases occurring in humans are mainly initiated by inoculation via the peripheral routes with prion-contaminated drugs, foods, or organs. Intracranial administration is involved only in dura mater grafting. The recent appearance of vCJD in humans is suspected of being caused by an oral route infection from cattle products infected with BSE prions. It is, therefore, an urgent issue to establish the therapeutic method against the infectious prion diseases, especially vCJD. The humanized Ki-ChM mice could recapitulate the pathogenic process involved in the intraperitoneal inoculation of human prions, and therefore are expected to provide a model to study the process involved in the oral inoculation seen in vCJD patients. For the treatment of prion diseases, it is best to inhibit the onset of prion diseases and/or to find a better way to prolong the incubation period. This model for human infectious prion diseases can be used to assess various therapeutic approaches for this disease, including the inhibitory manipulation of FDC function or the pharmacological modulation of humanized PrP^{Sc}.

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Prions and Processing of Reusable Medical Devices: United States Perspective

William A. Rutala, Ph.D., M.P.H. and David J. Weber, M.D., M.P.H.

University of North Carolina School of Medicine and the Department of Hospital Epidemiology, U.S.A.

Introduction

Human transmissible spongiform encephalopathies (TSEs) are degenerative neurologic disorders transmitted by a proteinaceous infectious agent or “prion” [39,46,88]. They include Creutzfeldt-Jakob Disease (incidence ~1/million), kuru (0 incidence, now eradicated), Gertsmann-Straussler-Sheinker (GSS) syndrome (1/40 million), and fatal insomnia syndrome (FFI) (<1/40 million) [24,39,62]. Prion diseases do not elicit an immune response, result in a noninflammatory pathologic process confined to the central nervous system, have an incubation period of years, and usually are fatal within 1 year of diagnosis [46]. At present, there are no effective vaccines, no completely reliable and validated laboratory tests for detecting infection in presymptomatic persons, and no specific therapy available for prion diseases.

Recently, a new variant form of CJD (vCJD) has been recognized that is acquired from cattle with bovine spongiform encephalopathy (BSE), or “mad-cow” disease [16,23]. As of December 1, 2003, a total of 153 vCJD cases have been reported worldwide, 143 in the United Kingdom (U.K.), 6 in France, and 1 each in Italy, Ireland, Canada, and the United States (U.S.) [19,21]. The U.S. case was reported in a patient who resided in the U.K. during the U.K. outbreak of BSE (E. Belay, June 2003, written communication). Compared with CJD patients, vCJD patients are on average younger (29 vs. 65 years of age), have a longer duration of illness (14 vs. 4.5 months), and present with sensory and psychiatric symptoms that are uncommon with CJD. The association of vCJD with BSE is the first instance of apparent transmission of a TSE across the species barrier to humans. Chronic wasting disease of deer and elk was recognized in the U.S. over 20 years ago. Recently, it has been identified in Northcentral U.S., outside of its original Rocky Mountain area of occurrence. Transmission of chronic wasting disease of elk and deer to humans has not been described [5,22].

CJD and other transmissible spongiform encephalopathies exhibit an unusual resistance to conventional chemical and physical decontamination methods [92]. Since CJD is not readily inactivated by conventional disinfection and sterilization procedures and because of the invariably fatal outcome of CJD, the procedures for disinfection and sterilization of the CJD prion have been both conservative and controversial for many years. The purpose of this article is to critique the literature and develop evidence-based guidelines to prevent cross-transmission of infection from CJD-contaminated medical devices.

Etiology

Prions are a unique class of pathogens, as an agent-specific nucleic acid (DNA or RNA) has not been detected. The infection is associated with the abnormal isoform of a host cellular protein called prion protein (PrP^{c}) [46]. In humans, the PrP gene resides on chromosome 20; mutations in this gene may trigger the transformation of the PrP protein into the pathologic isoform. This conversion of the normal cellular protein into the abnormal disease-causing isoform (PrP^{sc}) involves a conformational change whereby the α-helical content diminishes and the amount of β-pleated sheet increases, resulting in profound changes in properties. For example, the PrP^{c} is susceptible to proteases and the PrP^{sc} is partially resistant. No prion-specific nucleic acid is known to be required for transmission of disease [46,63,87]. The pathogenic prions accumulate in neural cells, disrupting function and leading to vacuolization and cell death.

Epidemiology of CJD

CJD is the most prevalent form of the transmissible spongiform encephalopathies in humans. CJD is manifested clinically as a rapidly progressive dementia (cognitive imbalance) including psychiatric and behavioral abnormalities, coordination deficits, myoclonus, and a distinct tri- and polyphasic electroencephalogram reading. CJD is often misdiagnosed as other neurologic diseases including Alzheimer's disease, Parkinson's disease, and multi-infarct dementia. Eighty percent of sporadic CJD cases are diagnosed between 50 and 70 years of age. Definitive diagnosis of CJD requires a histologic examination of the affected brain tissue [46,63,87]. Increased concentrations of several proteins have been reported in the cerebrospinal fluid (CSF) of patients with sporadic CJD including protein 14-3-3, neuron specific enolase, S 100b, and tau protein [38]. The reported sensitivity and specificity of protein 14-3-3 has ranged from 90 to 97% and 87 to 100%, respectively [38]. Following an evidence-based review, the American Academy of Neurology (AAN) has concluded that CSF 14-3-3 protein assay is useful for confirming the diagnosis of CJD [51]. While promising tests are being investigated (e.g., protease-resistant prion protein [72]), AAN states that no laboratory tests have emerged that are appropriate for routine use in the clinical evaluation of patients with suspected dementia.

CJD occurs as both a sporadic and familial disease. Approximately 10% of CJD cases are inherited and caused by mutations in the PrP gene located on the short arm of chromosome 20. Less than 1% of CJD cases result from person-to-person transmission, primarily as a result of iatrogenic exposure. About 90% of CJD cases are classified as sporadic because there is no family history and no known source of transmission. There is no seasonal distribution, no evidence of changing incidence, and no convincing geographic aggregation of cases [46,63,74,87]. It has been known for some time that genetic influence (i.e., methionine or valine homozygosity at codon 129 of the prion gene) may predispose to CJD or vCJD [62]. Ninety percent of the deaths in the U.S. are among persons older than 55 years of age and both genders are affected equally. Death usually occurs within 6 months (median age at death 68 years) [20].

CJD is not transmitted by direct contact, droplet, airborne, or transplacental routes. Iatrogenic transmission of CJD from person-to-person has resulted from the direct inoculation, implantation, or transplantation of infectious materials either intracerebrally or peripherally. CJD can be transmitted from samples obtained from patients-to-nonhuman primates [46]. Transmission can occur by peripheral routes of inoculation, but larger doses are required than intracerebral inoculation. Oral transmission has been demonstrated with even larger doses [35,46]. The incubation period depends on the dose of prions and the route of exposure. Studies have shown that prions (i.e., scrapie) are not inactivated by 3 years of environmental exposure [9].

variant CJD

BSE was first identified in 1986 in the U.K. and by November 2002 approximately 181,000 cattle had been infected [91]. BSE has been detected in cattle from 21 countries including the U.S. [91]. BSE appears to have resulted from the exposure of cattle to meat and bone meal that was produced by a new rendering process in which the temperature was reduced and the hydrocarbon solvent extraction step was omitted. The protein supplement was made from the remains of sheep and beef contaminated with scrapie and BSE. In 1996, an advisory committee to the U.K. government announced its conclusion that the BSE agent might have spread to humans, based on the recognition of the vCJD in 10 persons during 1994 to 1995. As mentioned, a total of 153 human cases have been diagnosed as of December 2003. The epidemiology, clinical, and pathologic profile differ from sporadic CJD (sCJD). The mean age at onset is 29 years (range, 16 to 48 years) compared with 65 years for sCJD. The duration of illness is 14 months for vCJD and 4.5 months for sCJD. Patients with vCJD frequently present with sensory and psychiatric symptoms that are uncommon with sCJD [87]. All patients with vCJD were potentially exposed to contaminated bovine during the 1980s, before measures to control human exposure were taken.

Both epidemiologic and molecular biologic evidence support a casual link between BSE and vCJD [8,16,26,70]. For example, intracerebral inoculation of cynomolgus macaque monkeys with brain tissue obtained from cattle with BSE resulted in all the monkeys developing a neuropathological phenotype similar to that described with vCJD but which differed from macaques inoculated with sCJD [53]. More recently, Lasmezas and colleagues have demonstrated primate-to-primate transmission of the BSE agent via intracerebral or intravenous challenge of infected brain tissue [54]. One case of BSE was reported in the U.S. in December 2003 but the cow originated in Canada and the one case of vCJD in the U.S. resided in the U.K. during the BSE outbreak.

The sensitivity and specificity of protein 14-3-3 for the diagnosis of vCJD is less than for sCJD [38]. Unlike sCJD [47], abnormal PrP immunostaining has been reported in lymphoid tissues of persons infected with vCJD including the tonsil [41,42], appendix [43], spleen [41], and lymph nodes [41]. Importantly, these studies have reported detecting PrP prior to the onset of clinical vCJD.

Infectivity of Tissue

To date, all known cases of iatrogenic CJD have resulted from exposure to infectious brain, dura mater, pituitary, or eye tissue. This is likely due to the high levels of abnormal prions in the central nervous system. However, from tissue infectivity studies in experimental animals and epidemiological studies in humans, it has been well established that the infectious agent may be present in many body tissues (Table 1) but that prions are present in lower numbers than the brain and transmission less likely. Consistent experimental transmission of infectivity has been possible with homogenates of brain, spinal cord, and eye tissue. The level of prions in corneas of infected humans is several orders of magnitude less than brain (44). Limited data suggest only routine control measures (i.e., no cling film covering over tonometer) are needed to prevent transmission of CJD via instruments that have surface contact with an intact cornea. Transmission occurs in less that half of the attempts with preparation of lung, liver, kidney, spleen, lymph node, placenta, olfactory epithelium, and CSF. Transmission to primates has never been documented with any body fluid other than CSF [7,12]. Using a highly sensitive immunological assay, PrP^{sc} was detected in 10/28 (36%) spleen specimens and 8/32 (25%) musculoskeletal samples (37). Prions have been isolated from the blood of infected guinea pigs, mice, and patients with CJD [56,78]. There are no known cases of CJD attributable to the reuse of devices contaminated with blood or via transfusion of blood products. So while transmission of CJD from human blood to laboratory animals through intracerebral inoculation have been reported (78), attempts to transmit CJD from CJD-infected patients into primates via whole blood or serum have failed (12).

Iatrogenic CJD

Iatrogenic CJD has been described in humans in 3 circumstances: after use of contaminated medical equipment (2 confirmed cases); after the use of extracted pituitary hormones (>130 cases) or gonadotrophin (4 cases); and after implant of contaminated grafts from humans (cornea, 3 cases; dura mater, >110 cases) [13,92]. Transmission via stereotactic electrodes is the only convincing example of transmission via a medical device. The electrodes had been implanted in a patient with known CJD and then cleaned with benzene and "sterilized" with 70% alcohol and formaldehyde vapor [6]. Two years later, these electrodes were retrieved and implanted into a chimpanzee in which the disease developed. The method used to "sterilize" these electrodes would not currently be considered an adequate method for sterilizing medical devices. The infrequent transmission of CJD via contaminated medical devices probably reflects the inefficiency of transmission unless dealing with neural tissue and the effectiveness of conventional cleaning and current disinfection and sterilization procedures. Retrospective studies suggest 4 other episodes may have resulted from use of contaminated instruments in neurosurgical operations [33,60]. In one case, an index source CJD case was identified; in this case the surgical instruments were cleaned with soap and water followed by exposure to dry heat for an unspecified time and temperature [33]. All 6 cases of CJD associated with neurosurgical instruments occurred in Europe between 1953 and 1976 and details of the reprocessing methods for the instruments are incomplete (LM Sehulster, 2000, written communication).

Table 1. Comparative Level of Infectivity in Organs/Tissue/Body Fluids of Humans with Transmissible Spongiform Encephalopathies

Infectious Risks[a]	Tissue
High	Brain (including dura mater), spinal cord, eyes
Low	Cerebrospinal fluid, liver, lymph node, kidney, lung, spleen, placenta, olfactory epithelium
None	Peripheral nerve, intestine, bone marrow, whole blood, leukocytes, serum, thyroid gland, adrenal gland, heart, skeletal muscle, adipose tissue, gingiva, prostate, testis, tears, saliva, sputum, urine, feces, semen, vaginal secretions, milk

[a] Infectious risks: high = transmission to inoculated animals **>** 50%; low = transmission to inoculated animals ≥10-20% (except for lung tissue, for which transmission is 50%); none = transmission to inoculated animals 0% (several tissues in this category had few tested specimens).

Modified from References 34, 68, and 92.

Johnson and Gibbs reviewed the risks associated with blood products [46] and concluded that CJD had not been transmitted by transfusion of human blood products. Evidence supporting this conclusion has included the following: case-control studies have

not linked a history of transfusions to an increased risk of CJD [90], the disease has not been reported in patients with hemophilia [29,55]; intravenous drug use does not increase the risk [46]; investigating recipients of blood components from known CJD donors has not revealed transmission of CJD [64]; and transfusion with full units of blood from CJD patients to chimpanzees failed to induce CJD [36]. Although there have been no proven cases of CJD transmission via blood transfusions these epidemiologic studies could miss very rare events.

There is no evidence of occupational transmission of CJD to healthcare workers. Although cases of CJD have been reported in approximately 24 healthcare workers, this incidence does not exceed what would be expected by chance alone [87]. In the context of occupational exposure, the highest potential risk is from exposure to high infectivity tissue through needle-stick injuries with inoculation [92]. Exposure by splashing of the mucous membranes (notably the conjunctiva) or unintentional ingestion may be considered a hypothetical risk [92]. For these reasons, all healthcare personnel who work with patients with known or suspected prion diseases should use standard precautions.

Control Measures

We believe that infection control measures should be based on epidemiologic evidence linking specific body tissues or fluids to transmission of CJD and/or infectivity assays demonstrating that body tissues or fluids are contaminated with infectious prions. The Centers for Disease Control and Prevention (CDC) [30,71] has used these principles plus inactivation data to develop draft guidelines for reprocessing CJD-contaminated medical devices. Guidelines are also available from the World Health Organization (WHO) [92] and healthcare professionals [34,73]. Other CJD recommendations have been based primarily on inactivation studies [25,67,73]. Our recommendations are also based on epidemiological data, infectivity data, cleaning data using standard biological and protein indicators, inactivation data of prions, the risk of disease transmission associated with the use of the instrument or device, and a review of other recommendations [25,30,34,67,71,73,92].

Standard precautions should be used by healthcare workers when caring for patients with CJD. Added personal protective equipment such as gowns or masks are unnecessary in view of the lack of communicability to healthcare workers.

To minimize the possibility of use of neurosurgical instruments that have been potentially contaminated during procedures performed on patients in whom CJD is later diagnosed, healthcare facilities should consider using the sterilization guidelines outlined below for neurosurgical instruments used during brain biopsy done on patients in whom a specific lesion has not been demonstrated (e.g., by magnetic resonance imaging or computerized tomography scans). Alternatively, neurosurgical instruments used in such patients could be disposable [68] or instruments quarantined until the pathology of the brain biopsy is reviewed and CJD excluded.

Disinfection and Sterilization

Numerous studies have been conducted on the inactivation of prions by germicides and sterilization processes but these studies do not reflect the reprocessing procedures in a clinical setting. First, these studies have not incorporated a cleaning procedure that normally reduces microbial contamination by 4 logs and protein by 2 logs [1,45,59,67]. Second, some prion studies have been done with tissue homogenates and the protective effect of tissue may explain, in part, why the TSE agents are difficult to inactivate [66]. Brain homogenates confer thermal stability to small subpopulations of the scrapie agent and some viruses. This subpopulation may be due to the protective effect of aggregation or population heterogeneity [66]. Third, results of inactivation studies of prions have been inconsistent due to the use of differing methodologies, which may have varied by prion strain (e.g., using thermostable strains of prions such as 22A and 301V), prion concentration, test tissue (e.g., 50-mg versus 350-mg tissue, intentionally smearing and drying or heat-fixing tissue), test animals, duration of follow-up of inoculated animals, exposure container, method of calculating log-reductions in infectivity, concentration of the disinfectant at the beginning and end of an experiment, cycle parameters of the sterilizer, and exposure conditions. Despite these limitations, there is some consistency in the results

[68,73]. In order to provide scientifically-based recommendations, research in which actual medical instruments are contaminated with prions (including vCJD) followed by cleaning and either conventional sterilization or disinfection, or special prion reprocessing should be undertaken.

Favero has explained that the draft CDC guidelines are based on a risk assessment that considers cleaning and prion bioburden from contact with infectious tissues [30]. Another component that must be integrated into the disinfection and sterilization processing is the risk of infection associated with the use of the medical device. The 3 categories of medical devices are critical, semicritical, and noncritical. Items assigned to the critical category present a high risk of infection if contaminated with CJD as the device enters a sterile tissue or the vascular system. This category includes surgical instruments and implants. Semicritical items (e.g., endoscopes, respiratory therapy equipment) are devices that come in contact with mucous membranes or skin that is not intact. In general, these items should be free of all microorganisms with the exception of small numbers of bacterial spores. Transmission of CJD via contact with mucous membranes or non-intact skin has not been described. Noncritical items (e.g., floors, walls, blood pressure cuffs, patient furniture) come in contact with intact skin but not mucous membranes. Intact skin should act as an effective barrier to microorganisms and prions. Thus, a critical or semicritical device that has contact with high-risk tissue (e.g., brain) from a high-risk patient (e.g., suspected or known CJD) must be reprocessed in a manner to ensure prion elimination. The combined contribution of cleaning and an effective physical and/or chemical reprocessing procedure should eliminate the risk of CJD transmission. Critical or semicritical instruments or medical devices that have contact with medium, low, or no risk tissue can be treated using conventional methods, as the devices have not resulted in transmission of CJD (Table 1).

To assess the effectiveness of disinfection or sterilization procedures one must consider the inactivation/removal factor [11,15,49]; that is, the reduction of infectious units during the disinfection or sterilization process. Thus, the probability of an instrument remaining capable of transmitting disease depends on the initial degree of contamination and the effectiveness of the decontamination procedures. An instrument contaminated with 50-mg of CJD brain with a titer of 6.0 LD_{50} intracerebral units/g [12] would have 5×10^4 infectious units. It has been suggested a titer loss of 10^4 prions should be regarded as an indication of appropriate disinfection of CJD [49]. However, the effectiveness of a disinfection or sterilization procedure should be considered in conjunction with the effectiveness of cleaning. Studies with microbial agents demonstrate that cleaning by conventional methods used in healthcare results in a 10^4 reduction of microbes and a 10^2 reduction in proteins [1,45,59]. Thus, cleaning followed by sterilization at 132°C for 18 min (prevacuum) should result in a titer loss of 10^7 (2-log reduction with cleaning plus 5- to 7-log reduction with an effective sterilization process) while tissues with high prion infectivity (e.g., brain) would be contaminated with 5×10^4/gram. Unpublished data suggest that alkaline detergents reduce 5-logs of prions and some enzymes may digest prions at 70°C and reduce 5- to 6-logs of prions [58]. Cleaning with enzymatic or alkaline cleaners followed by a sterilization procedure should destroy infectivity and provide a significant safety margin [52,58]. New

methods for decontamination of instruments (enzymatic breakdown) would offer an attractive possibility for instrument reprocessing either as a single method or in combination with other inactivation treatments.

Disinfection

Results of chemical inactivation studies of prions have been inconsistent due to the use of differing methodologies including: strain of prion (e.g., prions may vary in thermostability but differential susceptibility to disinfectants has not been described), prion concentration in brain tissue, test tissues (intact brain tissue, brain homogenates, partially purified preparations), test animals, duration of follow-up of inoculated test animals, exposure container, log decrease calculated from incubation period assays not endpoint titrations, concentration of disinfectant at the beginning and end of an experiment (e.g., chlorine), exposure conditions, and cycle parameters of the sterilizer [73]. Despite these limitations, there is some consistency in the results. An important limitation of current disinfection research is that prion assays are currently slow, laborious, and costly. Studies evaluating the efficacy of combined cleaning and disinfection have not been published.

It has been established that most disinfectants are inadequate for eliminating prion infectivity. There are 4 chemicals that reduce titer by >4 logs: chlorine, a phenolic (based on ortho-phenylphenol, p-tertiary-amylphenol and ortho-benzyl-para-chlorophenol), guanidine thiocyanate, and sodium hydroxide (Table 2) [11,14,15,27,28,49,57,75,85]. Of these 4 chemical compounds, the disinfectant that is available and provides the most consistent prion inactivation results is chlorine but even chlorine has had unexplainable reduced activity (e.g., reduction of 3.3 logs of CJD in 60 min by 2.5% hypochlorite) [14]. The corrosive nature of chlorine would make it unsuitable for semicritical devices such as endoscopes. Several investigators have found that CJD is incompletely inactivated by 1N NaOH [76,79,85]. Other antimicrobials that have been shown to be ineffective (<3 log reduction in 1 hour) against CJD or other TSEs are listed in Table 3 [11,14,15,27,40,49,80,82,94]. Studies have also shown that aldehydes such as formaldehyde enhance the resistance of prions and pretreatment of scrapie-infected brain with formaldehyde abolished the inactivating affect of autoclaving [10]. A formalin-formic acid procedure is required for inactivating prion infectivity in tissue samples from patients with CJD [17].

Table 2. Disinfectants that Decrease Prions by >3 logs within 1 hour

Disinfectant	Prion Conc. LD_{50}/g brain	Agent (strain)	Animal	Log decrease in LD_{50}	Reference
5,000 ppm chlorine	10.14	Scrapie (263K)	Hamsters	3.1 (15m), 3.5 (30m), 4.2 (1h)	[15]
5,000 ppm chlorine	5.5-6.0	CJD	Guinea pigs	≥3.5 (15m)	[11]
		Scrapie		3.8, 5.6, 5.8 at 30m,	

1,000 ppm chlorine	7.4	(139A)	Mice	1h, and 2h	[49]
10,000 ppm chlorine	7.4	Scrapie (139A)	Mice	≥5.9 at 30m, 1h, 2h	[49]
1,000 ppm chlorine	6.1	Scrapie (22A)	Mice	4.4, ≥4.6, 4.3 at 30m, 1h, and 2 h	[49]
10,000 ppm chlorine	6.1	Scrapie (22A)	Mice	≥4.6 at 30m, 1h, 2h	[49]
8,250 ppm chlorine	3.6	BSE	Mice	>3.6 at 30m	[85]
1N NaOH	5.8	CJD (Kitasato1)	Mice	3.6 at 1h	[75]
2N NaOH	9.3	Scrapie (263K)	Hamsters	5.1 at 2h	[85]
1N NaOH	3.6	BSE	Mice	>3.6 at 30m, 1h, 2h	[85]
NaOH, 0.1 and 1.0N	6.1-7.2	CJD (S.Co.)	Guinea pigs	4.8 (0.1N) at 15 m, 4.5 (1.0N) at 15m	[14]
0.01M sodium metaperiodate	10.11	Scrapie (263K)	Hamsters	3.5 at 30m, 2.3 at 4h	[15]
4M guanidine thiocyanate	8.36	CJD (SY)	Hamster	~5	[57]
Phenolic, LpH, 0.9, 9, 81, and 90%	7.4	Scrapie (263K)	Hamster	>5 at 30m (0.9%), >7.4 at 30m (9, 81, 90%)	[28]

All experiments employed brain homogenates or brain macerates at 20-22°C and no cleaning was employed.

Conc. = concentration; h = hour; LD_{50} = lethal dose intracerebral log units/g; m = minutes; ppm = parts per million

Modified from Reference 68.

Table 3. Chemicals Demonstrated to be Ineffective in Inactivating Prions (≤3-log Reduction in 1 hour)

Alcohol (100%) [27], (50%) [40]
Ammonia (1.0M) [14]
Chlorine dioxide (50 ppm) [11,15]
Formaldehyde (3.7%) [11,15,94]
Glutaraldehyde (5%) [11,27]
Hydrochloric acid (1.0N) [14,80]
Hydrogen peroxide (3%) [15]
Iodine (2%) [15]
Peracetic acid (19%) [27,82]
Phenol/phenolics (10% Lysol) [15], (0.6% phenols) [49]
Potassium permanganate (0.1%-2%) [11,14,15,49]

Sodium deoxycholate (5%) [11]
Sodium dodecyl sulfate (0.5-5%) [11,49,80]
Tego (dodecyl-di(aminoethyl)-glycine) (5%) [49]
Triton X-100 (1%) [11]
Urea (4-8M) [14]

Modified from Reference 68.

Both flexible and rigid endoscopes have been used in neurosurgery [4,61]. If such scopes come into contact with high-risk tissue in a patient with known or suspected CJD, either they should undergo sterilization (if possible, see below) or single-use devices should be used. Endoscopes coming into contact with no-risk tissues (e.g., gastrointestinal tract, joints, abdomen) can be disinfected using conventional methods, while endoscopes having contact with low-risk tissue (e.g., lung) can be disinfected using standard methods.

Sterilization

Prions exhibit an unusual resistance to conventional chemical and physical sterilization methods. These include both gaseous (i.e., ethylene oxide and formaldehyde) and physical processes (e.g., dry heat, glass bead sterilization, microwave irradiation, gamma irradiation, boiling, and autoclaving at conventional exposure conditions [e.g., 121°C for 15 min]) [11,14,27,73]. Rohwer's data suggest that the majority of scrapie infectivity is inactivated by brief exposure to temperatures of 100°C or greater. For example, when scrapie strain 263K was exposed to 121°C, 99.9999% of the infectivity was destroyed during the minute required to bring the sample to temperature. At 100°C, 97% was destroyed within 2 minutes of exposure at temperature. Thus, only a fraction of the infectious activity is extremely resistant [66].

Standard gravity displacement steam sterilization at 121°C has been studied using different strains of CJD, BSE, and scrapie and has been shown to be only partially effective even after exposure times of 120 min. As the temperature and exposure time increases, greater inactivation of the prion agents was achieved (Table 4). While there is some disagreement on the ideal time and temperature cycle [34], the recommendation for 132°C for 60 min (gravity) and 134°C for ≥18 min (prevacuum) are reasonable based on the scientific literature. These methods should result in a decrease of >5 logs and cleaning should result in a 2-log reduction providing a significant margin of safety (hypothetical instrument contamination ~5×10^4) [12]. Other steam sterilization cycles such as 132°C for 15 min (gravity) have been shown to be only partially effective [14].

Some investigators also have found that combining sodium hydroxide (e.g., 0.09N for 2 hr) with steam sterilization for 1 hour at 121°C results in complete loss of infectivity [28,84]. However, the combination of sodium hydroxide and steam sterilization may be deleterious to surgical instruments [73], sterilizers (TK Moore, October 2002, written communication), as well as sterilizer operators who could be breathing vaporized chemicals unless engineering controls or use of PPE prevent exposure. This risk can be minimized by the use of

polypropylene containment pans and lids designed for condensation to collect and drip back into the pan [18]. Hot NaOH is more caustic than NaOH at room temperature so even greater care should be taken to avoid exposure to it when hot (D. Asher, November 2002, written communication).

Recommendations for Processing Reusable Medical Devices

The disinfection and sterilization recommendations for CJD in this paper (Table 5) are based on the belief that infection control measures should be predicated on epidemiologic evidence linking specific body tissues or fluids to transmission of CJD, infectivity assays demonstrating that body tissues or fluids are contaminated with infectious prions, cleaning data using biological indicators and proteins [45,59,69], inactivation data for prions, the risk of disease transmission with the use of the instrument or device, and a review of other recommendations [30,32; LM Sehulster, 2000, written communication). Other CJD recommendations have been based primarily on inactivation studies [25,67,73]. Thus, the 3 parameters integrated into disinfection and sterilization processing are the risk of the patient for having a prion disease, the comparative infectivity of different body tissues, and the intended use of the medical device [30-32; LM Sehulster, 2000, written communication). High-risk patients include: those with known prion disease; rapidly progressive dementia consistent with possible prion disease; familial history of CJD, GSS, FFI; patients known to carry a mutation in the PrP gene involved in familial TSEs; a history of dura mater transplants; or a known history of cadaver-derived pituitary hormone injection. High-risk tissues include brain, spinal cord, and eye. All other tissues are considered low or no risk (Table 1). Critical devices are defined as devices that enter sterile tissue or the vascular system (e.g., implants). Semicritical devices are defined as devices that contact nonintact skin or mucous membranes (e.g., endoscopes). The AORN recommended practices for reprocessing surgical instruments exposed to CJD are consistent with the following recommendations [2,68].

Table 4. Effectiveness of High Temperature Sterilization (With and Without Pretreatment with NaOH) against Prions

Temperature	Prion Conc. LD_{50}/g brain	Agent (strain)	Animal	Log decrease in LD_{50}	Reference
121°C (gravity)	5.8	CJD (Kitasato-1)	Mice	3.1, 3.8, 4.2 at 30m, 1h, 2h	[75]
121°C (gravity)	7.4	Scrapie (263K)	Hamsters	>5 at 1h and 1.5h	[28]
121°C	6.1-7.2	CJD (S.Co.)	Guinea pigs	≥5 logs at 1h	[14]
0.09N or 0.9N NaOH for 2h plus 121°C (gravity) for 1h	7.4	Scrapie (263K)	Hamsters	>7.4	[28]
1N NaOH for 1h plus 121°C for 30m	5.8	CJD (Kitasato-1)	Mice	≥4	[75]

126°C (gravity)	7.4	Scrapie (139A)	Mice	5.9 (15m, 30m), ≥6.9 (1h, 2h)	[49]
126°C (gravity)	6.1	Scrapie (22A)	Mice	1.8, 2.1, 3.6, 2.9 at 15m, 30m, 1h, 2h	[49]
132°C	6.1-7.2	CJD (S.Co.)	Guinea pigs	≥5 at 1h	[14]
132°C (gravity)	5.8	CJD (Kitasato-1)	Mice	>4.8 (30m, 1h)	[75]
132°C (gravity)	7.4	Scrapie (263K)	Hamsters	>6 at 1h, 7.4 at 1.5h	[28]
134°C (porous)	9.3	Scrapie (263K)	Hamsters	7.2 at 18 m	[85]
134°C-138°C, (porous)	3.6	BSE	Mice	2.5 at 18m	[85]
136°C, (porous)	7.4	Scrapie (139A)	Mice	≥6.9 at 4, 8, 16 and 32m	[49]
136°C, (porous)	6.1	Scrapie (22A)	Mice	≥5.6 at 4, 8, 12 and 32m	[49]

All experiments employed brain homogenates or brain macerates and no experiment cleaned the inoculum.

Conc. = concentration; h = hour; LD_{50} = lethal dose intracerebral log units/g; m = minutes

Table 5. Infection Control Precautions for Patients with Known or Suspected Prion Disease such as CJD

General Precautions

1. Precautions are used on all patients with known or suspected prion disease and those at high risk for development of a prion disease, including all patients with:
   a. Rapidly progressive dementia consistent with possible prion disease
   b. Known or possible prion disease-Creutzfeldt-Jakob disease (CJD), Gerstmann-Staussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI), or variant Creutzfeldt-Jakob disease (vCJD)
   c. History of dura mater transplants or cadaver-derived pituitary hormone injection
   d. Familial history of CJD, GSS, or FFI
   e. Patients know to carry a mutation in the PrP gene involved in familial TSEs
2. Standard precautions should be used on all patients with known or suspected CJD. Additional precautions (e.g., contact) are not necessary. Gloves should be worn when handling blood and body fluids (e.g., secretions and excretions). Masks, gowns, and protective eyewear should be worn if exposure to blood or other potentially infectious material to mucous membranes or skin may occur.
3. Since standard decontamination of tissue samples (e.g., formalin) or specimens may

not inactivate CJD, all tissue samples should be handled using standard precautions (i.e., gloves). The tissue and specimens should be labeled as a “biohazard” and “suspected CJD”, prior to being sent to the laboratory.

4. No special precautions are required for disposal of body fluids. Such fluids may be disposed of via a sanitary sewer. Blood or blood-contaminated fluids should be managed per state regulations for medical waste.
5. Regulated medical waste (e.g., bulk blood, pathological waste, microbiologic waste, and sharps) should be managed per state regulations.
6. Laundry should be managed as required by OSHA rule on bloodborne pathogens. No additional precautions are required.
7. No special precautions are required for handling food utensils.
8. When a patient dies, ensure that the morgue and funeral home are notified that the patient has CJD. No excess precautions need to be taken at burial (e.g. no special cemetery).
9. Patients with known or suspected prion disease should not serve as donors for organs, tissues, blood components, or sources of tissue (e.g., dura mater, hormones).
10. Infection control professionals and other involved departments (e.g., Surgical Services, Central Processing, Pathology) should be notified by clinicians when a patient with a known or suspected prion disease is scheduled for any invasive procedure in which there may be exposure of personnel or instruments containing potentially infectious tissues.
11. Infection control professionals should be notified by clinicians of all patients with a known or suspected prion diseases.

Processing Creutzfeldt-Jakob Disease (CJD)-Contaminated Reusable Patient-Care Equipment and Environmental Surfaces (This section does not apply to variant CJD).

1. Keep instruments wet or damp until they are decontaminated. Instruments should be decontaminated as soon as possible after use. Dried films of tissue are more resistant to prion inactivation by steam sterilization than are tissues that have been kept moist. [6,7,11-16,25,27,28, 30-32,40,48, 57,65,67,68,71,73,75,80-83,85,92,94].
2. Use the following recommendations (see a-f, i below) with high-risk tissues (defined as brain [including dura mater], spinal cord, and eyes) from high-risk patients (e.g., those with known or suspected CJD) and to reprocess critical/semicritical items. [6,7,11-16,25,27,28,30-32,40,48, 57,65,67,68,71,73,75, 80,82, 85,92,94].

   a. Clean devices (e.g., surgical instruments) that have been constructed so that cleaning procedures result in effective tissue removal and then sterilize these devices by one of the following methods:

   Option 1 (preferred by the World Health Organization as it combines sodium hydroxide and autoclaving) - immerse in 1N NaOH for 1 hour; remove and rinse in water, then transfer to an open pan and autoclave [121°C gravity displacement or 134°C porous or prevacuum sterilizer] for 1 hour. Alternatively, immerse instruments

in 1N NaOH for 1 hour and heat in a gravity displacement sterilizer at 121°C for 30 min); or

Option 2 - autoclave at 134°C for >18 minutes in a prevacuum sterilizer; or

Option 3 - autoclave at 132°C for 1 hour in a gravity displacement sterilizer. [6,7,11-16, 25,27,28,30-32,40,48,57,65,67,68,71,73,75, 80-85,92,94].

b. Discard devices that are impossible to clean. [45,68].

c. Do not use flash sterilization for reprocessing instruments. [6,7,11-16,25,27,28,30-32,40, 48,57,65,67,68,71,73,75, 80-83, 85,92,94].

d. Discard items that permit only low-temperature sterilization (e.g., ETO, hydrogen peroxide gas plasma). [6,7,11-16,25,27,28,30-32,40,48,57,65,67,68,71,73,75, 80,82,83,85,92,94].

e. Recall contaminated items (e.g., medical devices used for brain biopsy before diagnosis) that have not been processed according to these recommendations and appropriately reprocess. [68].

f. To minimize patient exposure to neurosurgical instruments later determined to have been used on a patient with CJD, use the sterilization guidelines above for neurosurgical instruments used on patients undergoing brain biopsy when a specific lesion has not been demonstrated (via computerized tomography or magnetic resonance imaging). Alternatively, use disposable neurosurgical instruments in such patients. [6,7,11-16,25,27,28,30-32,40,48, 57,65,67,68,71,73,75,80-83,85,92,94].

g. Clean noncritical environmental surfaces contaminated with high-risk tissues (e.g., laboratory surface in contact with brain tissue of a CJD-infected person) with a detergent and then spot decontaminate these surfaces with a 1:5 dilution of sodium hypochlorite (i.e., bleach, 1:5 dilution of 5.25-6.15% sodium hypochlorite provides 10,500-12,300 ppm chlorine). To minimize environmental contamination, use disposable cover sheets on work surfaces. [6,7,11-16,25,27,28,30-32,40,48,57,65, 67,68,71,73,75,80,82,83,85,92,94].

h. Clean and then disinfect noncritical equipment that has been contaminated with high-risk tissue using a 1:5 dilution of sodium hypochlorite or 1N NaOH, depending on material compatibility. Ensure that all contaminated surfaces are exposed to the disinfectant. [6,7,11-16,25,27,28,30-32,40,48,57,65,67,68,71,73,75,80,82,83,85,92,94].

i. Tag equipment that requires special prion reprocessing after use. Train clinicians and reprocessing technicians on how to properly tag the equipment and on the special prion reprocessing protocols. [6,7,11-16,25,27,28,30-32,40,48,57,65,67,68,71,73,75,80,82, 85,92,94].

3. Use only standard disinfection to process environmental surfaces contaminated with low-risk tissues. (Use disinfectants recommended by OSHA for disinfecting blood-contaminated surfaces.) [6,7,11-16,25,27,28,30-32,40,48,57,65,67,68,71,73,75,80,82,85,92,94].

4. Use the following recommendations to reprocess critical/semicritical medical devices

that have been contaminated with low risk (defined as CSF, kidney, liver, spleen, lung, and lymph nodes) or no-risk tissue (defined as peripheral nerve, intestine, bone marrow, blood, leukocytes, serum, thyroid gland, adrenal gland, heart, skeletal muscle, adipose tissue, gingiva, prostate, testis, tears, saliva, sputum, urine, feces, semen, vaginal secretions, milk) from high-risk patients. [6,7,11-16,25,27,28,30-32,40, 48,57,65,67,68, 71,73,75,80,82,85,92,94].

a. Clean and either disinfect or sterilize these devices using conventional protocols of heat or chemical sterilization or high-level disinfection. [6,7,11-16,25,27,28,30-32,40, 48,57,65,67,68, 71,73,75,80,82,85,92,94].

b. Use standard cleaning and high-level disinfection protocols for reprocessing endoscopes (except neurosurgical endoscopes with central nervous system contact) because these devices can become contaminated only with no-risk materials. [6,7,11-16,25,27,28,30-32,40, 48,57,65,67,68,71,73,75,80,82,85,92,94].

5. Use standard disinfection to process noncritical equipment and noncritical environmental surfaces that has been contaminated with (use disinfectants recommended by OSHA for decontaminating blood-contaminated surfaces [e.g., 1:10 to 1:100 dilution of 5.25-6.15% sodium hypochlorite]) low risk or no-risk tissues or fluids. [6,7,11-16,25,27,28,30-32,40,48,57,65,67,68,71,73,75,80,82,85, 92,94].

Recommendations for disinfection and sterilization of prion-contaminated medical devices are as follows. Instruments should be kept wet or damp until they are decontaminated and they should be decontaminated as soon as possible after use. Dried films of tissue are more resistant to prion inactivation by steam sterilization compared to tissues that were kept moist. This may relate to the rapid heating that occurs in the film of dried material compared to the bulk of the sample, and the rapid fixation of the prion protein in the dried film [83]. It also appears that prions in the dried portions of the brain macerates are less efficiently inactivated than undisturbed tissue. For high-risk tissues, high-risk patients, and critical or semicritical medical devices, clean the device and sterilize by using a combination of sodium hydroxide and autoclaving as recommended by WHO [92] (e.g., Option 1 – immerse in 1N NaOH [1N NaOH is a solution of 40 g NaOH in 1 liter of water] for 1 hour; remove and rinse in water, then transfer to an open pan and autoclave [121°C gravity displacement or 134°C porous or prevacuum sterilizer] for 1 hour; or alternatively - immerse instruments in 1N NaOH for 1 hour and heat in a gravity displacement sterilizer at 121°C for 30 minutes; clean; and subject to routine sterilization), or Option 2 – autoclave at 134°C for 18 minutes in a prevacuum sterilizer, or Option 3 – autoclave at 132°C for 1 hour in a gravity displacement sterilizer. The temperature should not exceed 134°C since under certain conditions the effectiveness of autoclaving actually declines as the temperature is increased (e.g., 136°C, 138°C) [81]. Prion-contaminated medical devices that are impossible or difficult to clean should be discarded. Flash sterilization should not be used for reprocessing. To minimize environmental contamination, noncritical environmental surfaces should be covered with plastic-backed paper and when contaminated with high-risk tissues the paper should be properly discarded. Environmental surfaces (noncritical) contaminated with high-risk tissues (e.g., laboratory surfaces) should be cleaned and then spot

decontaminated with a 1:10 dilution of hypochlorite solutions. Environmental surfaces contaminated with low-risk tissues require only standard disinfection [30,32,68]. Since noncritical surfaces are not involved in disease transmission, the normal exposure time (≤10 minutes) is recommended.

Most of the data that form the basis of these recommendations have been generated from studies of the prions responsible for sCJD or animal TSE diseases (e.g., scrapie). Limited data are available on which to base recommendations for the prevention of vCJD. To date, there have been no reports of human-to-human transmission of vCJD by blood or tissue. Unlike sCJD, patients with vCJD have infectivity detectable in the lymphoid tissue. Furthermore, TSE infectivity may be detectable before the onset of clinical illness. This has raised concern about the possible human-to-human transmission of vCJD by medical instruments contaminated with such tissues. On the basis of these concerns, the use of prion disinfection and sterilization guidelines (or single-use instruments) has been proposed in the U.K. for instruments used in dental procedures (3), eye procedures [86], or tonsillar surgery [50] on patients at high risk of sCJD or vCJD. Following complications (death in one patient and increased bleeding) associated with the use of single-use instrument in tonsillar surgery, it is now advised in the U.K. that given the balance of risk, surgeons can return to using reusable surgical equipment. If epidemiological and infectivity data show that these tissues represent a transmission risk, then CJD sterilization precautions (or use of disposable equipment) could be extended to equipment used for these procedures [89].

Conclusion

Prion diseases are rare and hence do not constitute a major infection control risk. Nevertheless, prions represent an exception to conventional disinfection and sterilization practices. These guidelines for CJD disinfection and sterilization are based on consideration of epidemiological data, infectivity data, risk associated with medical/surgical instruments, and cleaning and inactivation studies. Guidelines for management of CJD-infected patients and patient equipment should be modified as scientific information becomes available. A task force involving representation from professional organizations and researchers should develop a consensus guideline for the U.S. that represent the optimal and practical conditions for inactivation of CJD. The CDC draft guideline and other evidence-based recommendations could serve as models for this consensus statement. Lastly, studies consistent with actual clinical practices (e.g., operation in infected animals followed by cleaning with enzymatic detergents and disinfection or sterilization) should be undertaken.

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Discussion

BSE / Prions

Question for Dr. Taylor, Consultant, SEDECON 2000, U.K.: Since BSE is most resistant, why bother using other TSE agents for inactivation studies?

Answer by Dr. Taylor: Coming from the lab where 301V was developed, you might think that I have a vested interest in saying that it's the best model. Certainly as far as thermostability studies are concerned, that would seem the logical agent to use. On the other hand, as far as chemical inactivation is concerned, there are really no convincing data in the literature to support strain differences between agents. So if you are talking about validating chemical processes, I don't see the same necessity to use something like 301V.

Comment by Dr. Brown, National Institutes of Health, U.S.A.: Ideally you'd like to use brain or CNS tissue from cattle or elk. The problem there is that the gold standard for looking at a reduction would be a bioassay. Bioassays using elk or cattle are simply not practical. Thus, you are already compelled to move one step away into a rodent-adapted strain. I suppose the 301V strain, which has been extremely resistant in several experiments to date, would be a desirable strain. The 263K hamster strain which we initially used has one feature that none of the other strains do. It achieves a concentration of infectivity in the brain which is typically higher than in any other strain, including 301V, so that you can get a larger margin of clearance.

Question for Dr. Brown: What is the chemical composition of ash which retains infectivity at 600°C?

Answer by Dr. Brown: I do not know. We have not attempted a biochemical or chemical analysis of that ash, although I think it would be a good thing to do. If any funding remained for basic science in the field, it would certainly be done. Unfortunately, the NIH has opted out of all intramural funding for this category of disease.

Question for Dr. Will, University of Edinburgh, U.K.: Why does Japan have more human dura mater cases than any other country?

Answer by Dr. Will: I think one possibility is the relative usage of dura mater in neurosurgery varies from country to country. Sometimes the practice of neurosurgery varies. So it's a combination of the neurosurgical frequency and the importation of infected dura.

Question for Dr. Rutala, University of North Carolina School of Medicine, U.S.A.: Clearly, there are policies in place for dealing with surgical instruments in individuals identified as being in a particular risk group. One of the problems in the U.K. is that some of

these individuals are not identified until well after the event when instruments may have been re-used on other patients. Do you have any comment on the policy in the United States?

Answer by Dr. Rutala: The surgical instruments that were used on patients at the time of surgery not knowingly infected with CJD are a significant issue. As you know, we and other countries have had CJD exposure via surgical instruments. The one way that we have tried to prevent that is to implement a procedure for special prion re-processing when there is a brain biopsy, or when there are situations where the etiology of disease is not known. If we had, as does the U.K., the situation of variant CJD with contamination of lymphoid tissue, I think the recommendations that I presented would be much different. It would not be as clearcut as it is with only sporadic CJD prevalent.

Comment by Dr. Taylor: Dr. Rutala mentioned the effectiveness of phenolic called LPH. I thought that perhaps a clarification was required. It is true that in 1983 a publication from the Rocky Mountain Lab reported on the high degree of efficacy of this particular proprietary type of compound. Unfortunately for reasons I don't understand, when they were carrying out these studies, the manufacturer changed the formulation of this product. Since that time, the original product which was validated in that study has only been available in Canada. The Rocky Mountain Lab has now tested the revised product that is available elsewhere in the world and has found that it is not effective. Thus, Canada is the only place that has a formulation of LPH that is effective.

Question for Dr. Will: Is it necessary to make a preoperative biopsy of tonsils to prevent contamination of instruments used at the time of surgery?

Answer by Dr. Will: This is a difficult issue in the U.K. because of the distribution of PrP infectivity in the lymphoreticular system. At one stage, in order to respond to the possibility that such instruments could lead to onward transmission, the policy was introduced in the U.K. for the use of disposable instruments. This policy was undertaken but then surveys were done from ear, nose, and throat surgeons to determine the efficacy of these new instruments. What was identified was that the risk of hemorrhage had increased significantly because the quality of the instruments was not as good as the previous instruments. Indeed, there is a possibility that 1 or 2 individuals died of late hemorrhage and this policy was then retracted. This is an interesting issue with regards to this whole field. Sometimes actions are taken to prevent risk and these end up making things worse in some ways. It's a very big problem.

Comment by Dr. Brown: I would like to comment on Dr. Rutala's recommendations and his description of the rationale which in general is very logical and very good. I would prefer, however, that instruments used in any patient with any type of risk who is subjected to neurosurgery, a known high risk tissue, be subject to special precautions. That is, they should be sterilized in such a way that they are optimally inactivated for prions. I say this because a half dozen incidents of which I am personally aware of, are incidents in which a patient has not been suspected of having had CJD but did in fact have CJD. The potential

for cross-contamination could largely be further minimized by using special precautions on all neurosurgical patients.

Comment by Dr. Rutala: This has certainly been discussed in many forums. As you realize, that is the way the U.S. has been practicing the sterilization of neurosurgical instruments for decades. We believe that implementing special prion reprocessing for blind brain biopsies will minimize, if not completely prevent, those episodes from occurring in the future.

Comment by Dr. Will: One of the difficulties with sporadic CJD is that sometimes patients present with cortical blindness. We have a number of individuals in the U.K. who have had eye operations early in the clinical course because they were thought to have the anterior ophthalmic problems. These instruments were then unknowingly used on other patients. We have recently looked at this to see if there is there evidence of transmission through ophthalmic instruments in the U.K. and have found no such evidence despite the fact that there were clearly episodes of cross-contamination. That doesn't mean we shouldn't take precautions, but I think your point that the epidemiological evidence does not suggest any great risk since 1980 is certainly true for sporadic CJD if not for the variant form of the disease.

Session III

Infection Control: Pacific Rim & Emerging Issues

Chairman: **Hiroyoshi Kobayashi, M.D., Ph.D., C.I.C.D.**
Kanto Medical Center NTT EC, Japan

Infection Control in Japan

Hiroyoshi Kobayashi, M.D., Ph.D., C.I.C.D.

Kanto Medical Center NTT EC, Japan

In Japanese hospitals, the Chief Executive has responsibility for hospital infection control and prevention, while on a national level the Ministry of Health, Labor, and Welfare (MHLW) is responsible for these activities. The primary national laws for infection control are the Medical Service Law and its Enforcement Regulations, revised in 2001, and the Law for Infection Control and Treatment of Infected Patients enforced in 1999 and revised and enforced in November 2003. The latter especially concerns infectious diseases. Two guidelines supervised by the MHLW have been published. The first is a guideline for antisepsis, disinfection, and sterilization [3], and the second is a guideline for transportation of patients having infectious diseases [4]. Both are scheduled to be revised soon. National laws also regulate tap water, swimming water, and wastewater and isolation of patients. Recommendations for indoor air quality in hospitals can be found in a guideline from the Hospital Engineering Association of Japan.

Until 1979, the isolation rate for methicillin-resistant *Staphylococcus aureus* (MRSA) among all *S. aureus* isolates was less than 1% at the University of Tokyo Hospital. However, in the early 1980s, the isolation rate had increased rapidly with MRSA isolation rates among all *S. aureus* isolates of 6.2%, 17.8%, 36.0%, and 58.2% in 1984, 1985, 1986, and 1987, respectively. This pattern indicated the need for a national professional organization to deal with hospital infections. Accordingly, the Japanese Society of Environmental Infection was founded in April 1985 and held its first annual meeting in February 1986. This marked the beginning of the recent scientific activity concerning hospital infection control and prevention in Japan. The Japanese Society of Environmental Infection published a “Guideline for Prevention of Hospital Infection” in 1990 that became the first nationwide guideline on this topic in Japan [1].

MRSA is now the resident flora in Japanese hospitals, and the isolation rate of MRSA among *S. aureus* is almost 50 to 60%. Fortunately, the isolation rate of vancomycin-resistant enterococci (VRE), which has an MIC of not less than 32 μg/mL, is very small. Nationwide surveillances on MRSA infections were conducted in 1995 and 1996 through a grant for Scientific Research Expenses for Health and Welfare. The results of these surveillances showed that the prevalence of MRSA infections was approximately 1% in mean per total admissions for both years [5,6]. MRSA prevalence at the Kanto Medical Center was approximately 0.5% for each year from 1998 to 2002, which is almost one-half of national mean values for preceding years.

In 1991, the first Department of Infection Control and Prevention in Japan was founded at the University of Tokyo and this became the scientific center of infection control.

Japanese Nosocomial Infection Surveillance (JNIS) in accord with the National Nosocomial Infection Surveillance (NNIS) of 1999 in the United States was begun as an official task of the Japanese Society of Environmental Infection in November 1998. The early results of JNIS indicated that the incidence of surgical site infections (SSI) was 12.2% for stomach surgeries, 15.2% for colonic surgeries, and 22.6% for rectal surgeries. MHLW began national surveillance on clinical isolates in hospitals in 2000, and national surveillance in the intensive care unit (ICU) was also begun in 2000. The results of surveillance on 2,941 cases in 46 hospitals from October to December 2000 showed an overall hospital infection rate of 5.1% and rates for specific infection types of 1.4% for SSI, 0.4% for urinary tract infections, 3.7% for lower respiratory infections, and 1.0% for primary blood stream infections. Surveillance of SSI and infections in the neonatal ICU was begun in 2002.

In 1993, an educational initiative sponsored by MHLW and aimed at hospital infections was started. Two-day programs for medical doctors and nurses were held annually in 7 districts. In 1995, consultation on MRSA infection via facsimile was begun, and a consultant committee was organized in the office of the Japanese Association for Infectious Disease. Any healthcare worker can send a practical question by facsimile to this committee, which will respond to the question within a few days.

Certification of infection control doctors began in December 1999, with a Joint Commission of 19 medical societies qualifying certification. By October 2003, a total of 3,635 doctors had been certified. The total number of general beds in Japanese hospitals now numbers 1,178,083, so accordingly there are 324 beds for every 1 certified infection control doctor. A training program for certification of infection control nurses by the Japanese Nursing Association began in 2000, and by the National College of Nursing in 2001. In each, the number of trainees is limited to 20 because of the limited teaching staff. This number of trainees, however, is too small to cover the total number of hospitals in Japan.

A guideline for evidence-based precautions with categorization of strength of recommendation and evidence was published in February 2002. The cooperating editor is the MHLW and volumes 2 and 3 were published in 2003 [8-10]. The Japanese Society of Medical Instrumentation (JMSI) officially published its “Guideline for Sterility Assurance in Health-Care Settings 2000” in June 2000 [2]. In the same year, JSMI began a certification program for Sterile Service Technicians. As of October 2003, the number of Certified Sterile Service Technicians was 1,018. The higher grade-certification program was initiated in November 2003 and includes validation of sterilizers in healthcare settings.

Questionnaires addressing the practice of reuse of single-use devices in hospital settings were sent to 500 Japanese hospitals with at least 300 beds in 2000. The response rate was approximately 75%, with responses obtained from 405 departments in 377 institutions [7]. The results of these questionnaires indicated that several high-risk devices, including autosuture appliances, skin staplers, total hip systems, and artificial dura, were being resterilized and reused. In 5 institutions, artificial cardiac valves were reported to have been reused, although these products had only been opened on the instrument table and not used on a patient. Accordingly, official restrictions on the reuse of single-use devices were proclaimed in December 2001 and enforced in January 2002.

In the keynote address by the Society Chairman at the 17th Annual Meeting of the Japanese Society of Environmental Infection held in 2002, the priority projects of the society for the early 21st century were listed as follows: effective organization of infection control and prevention, employment of evidence-based precautions, surveillance on data with good quality, effective intervention, adequate antibiotic use, economic evaluation of infection control, outcome evaluation and feedback, evidence-based disinfection and antisepsis, peer review of infection control strategies in each hospital, and evidence-based research.

In July 2002, the MHLW organized an Expert Panel Committee for Hospital Infection Prevention and Control. This panel is chaired by myself and is comprised of 22 specialists from different fields including journalism, pharmacology, and public health. The committee submitted its report to the Ministry in September 2003. Among the several conclusions in the report, the importance of the nationwide network of district organizations comprised of regional governmental persons and specialists of hospital infection in large hospitals was emphasized. One of the primary purposes of the regional organization network is support and consultation for small hospitals with less than 200 beds. It is strongly expected that the report will be effective in promoting future progress in the area of patient-oriented services concerning hospital infection prevention and control.

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Infection Control in Korea

Moon-Won Kang, M.D., Ph.D.

Catholic University of Korea, Korea

Current data show that the estimated population of Korea is 46 million and the number of hospitals is about 1,061. Forty-eight percent of Korean hospitals are located within the 7 largest cities. The Korean healthcare system is dominated by the private sector, with about 90% of doctors, and the great majority of hospital beds, private. The Korean government has a limited role as provider of curative services and has a laissez-faire policy towards regulating private suppliers. Health financing is a mixture of public and private sources. With the exception of the public healthcare system, almost all infection control (IC), especially nosocomial infection (NI) control, is controlled by non-governmental organizations. IC activity began in the 1970s by several infectious disease specialists and recently, non-governmental organizations were founded such as the Korean Society for Nosocomial Infection Control (KOSNIC, 1995) and the Korean Association of Infection Control Nurses (KAICN, 1996). In 1992, the Ministry of Health and Welfare enacted the Hospital Infection Control Act for hospitals with over 80 beds, but there has been little effect on NI control.

National Infection Control

The National Institute of Health of Korea (NIH) serves as the main organization for national infection control. There are 3 divisions within the Department of Infectious Disease Control of NIH: the Division of Communicable Disease Control, the Division of Epidemiologic Investigation, and the Division of Infectious Disease Information & Surveillance. In the national surveillance system, 56 types of communicable diseases or disease groups were designated as notifiable diseases as stipulated by the Communicable Disease Prevention Act. The act was revised on January 12, 2000 and 29 kinds of communicable diseases or disease groups were added to the existing 27 diseases, and the 3 class-type categorization of these communicable diseases was expanded to 5 class-types (Table 1). This was intended for early detection of newly emerging or reemerging infectious diseases. Class 1 consists of 6 diseases which need to be managed immediately with measures such as isolation to prevent larger-scale epidemics. Class 2 diseases consist of 9 diseases which are preventable by vaccines, while Class 3 consists of 18 diseases including 1 disease group (Sexually Transmitted Infections [STI]). These are important to consistently monitor and to formulate public education programs on their prevention. Class 4 consists of 13 diseases including 1 syndrome of reemerging diseases or imported diseases such as severe acute respiratory syndrome (SARS). There are 8 designated diseases within Class 5 that consist of imported parasitic infectious diseases, viral hepatitis A and C, and vancomycin-resistant *Staphylococcus aureus* (VRSA).

Table 1. List of Notifiable Diseases

Category	Disease	Notify
Class 1	Cholera, Plague, Typhoid fever, Paratyphoid fever, Shigellosis (Bacillary dysentery), Enterohemorrhagic *Escherichia coli*	Immediately
Class 2	Diphtheria, Pertussis, Tetanus, Measles, Mumps, Rubella, Poliomyelitis, Viral hepatitis B, Japanese encephalitis	Immediately
Class 3	Malaria, Tuberculosis, Hansen's disease, Syphilis, Sexually Transmitted Infections (STI)[a], Scarlet fever, Meningococcal meningitis*, Legionellosis, Vibrio vulnificus* sepsis, Epidemic typhus, Murine typhus, Scrub typhus, Leptospirosis, Brucellosis, Anthrax, Rabies, Hemorrhagic fever with renal syndrome, Influenza, AIDS[b]	Within 7 days
Class 4	Yellow fever, Dengue fever, Ebola fever, Marburg fever, Lassa fever, Leishmaniasis, Babesiosis, African trypanosomiasis, Cryptosporidiosis, Schistosomiasis, Yaws, Pinta, New infectious diseases syndrome[c]	Immediately
Class 5	Viral hepatitis A, Viral hepatitis C, VRSA[d], Chaga's Disease, Angiostrongyloidiasis, Gnathostomiasis, Filariasis, Hydatidosis	Within 7 days

[a] STI = sexually transmitted infections including Gonorrhea, Chlamydia, Chancroid, Non-gonococcal urethritis, Genital herpes, Condyloma accuminata.

[b] AIDS = Acquired Immunodeficiency Syndrome,

[c] Including SARS

[d] VRSA = vancomycin-resistant *Staphylococcus aureus*.

The NIH publishes a weekly surveillance report, both on paper and electronically, the Communicable Disease Weekly Report (CDWR), which compromises 23 diseases. The NIH also manages the Expanded Program on Immunization (EPI) for diphtheria, pertussis, tetanus, poliomyelitis, measles, mumps, rubella, viral hepatitis B, tuberculosis, and Japanese encephalitis. Routine immunization with DTaP (diphtheria, tetanus, pertussis), OPV (oral polio vaccine), MMR (measles, mumps, rubella), BCG (for tuberculosis), and HBV (hepatitis B vaccine) is being given to infants and younger children. Thirteen national quarantine stations are in operation which inspect passengers and aircraft for cholera, plague, and yellow fever. They also issue international certificates of vaccination in accordance with International Health Regulations.

According to NIH data [7], typhoid fever, pertussis, measles, mumps, Japanese encephalitis, and malaria were the major communicable diseases in Korea prior to the 1970s (Figure 1). Until the 1990s, there was a remarkable decrease in occurrence of these diseases due to vaccination and improvement of the general hygiene. After 1994, however, outbreaks of measles and mumps, reemerging malaria, and outbreaks of several water-borne infectious diseases were seen.

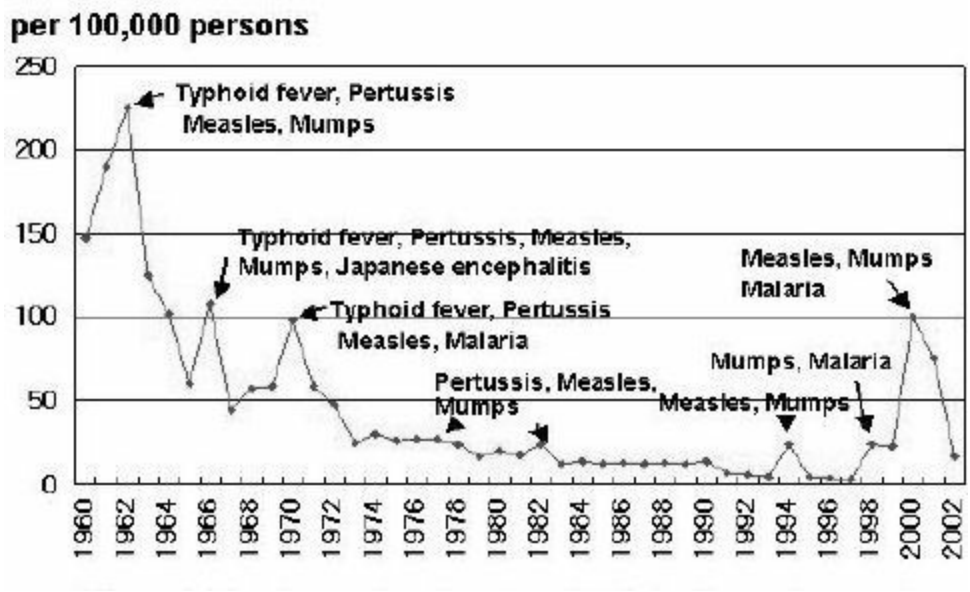

Figure 1. Incidence Rate of Major Notifiable Disease by Year in Korea

There has recently been a major outbreak of measles in Korea. Notification of approximately 55,000 cases of measles was made during 2000-2001. Ninety-two percent of the cases were from students between the ages of 7 and 13 years (Figure 2).

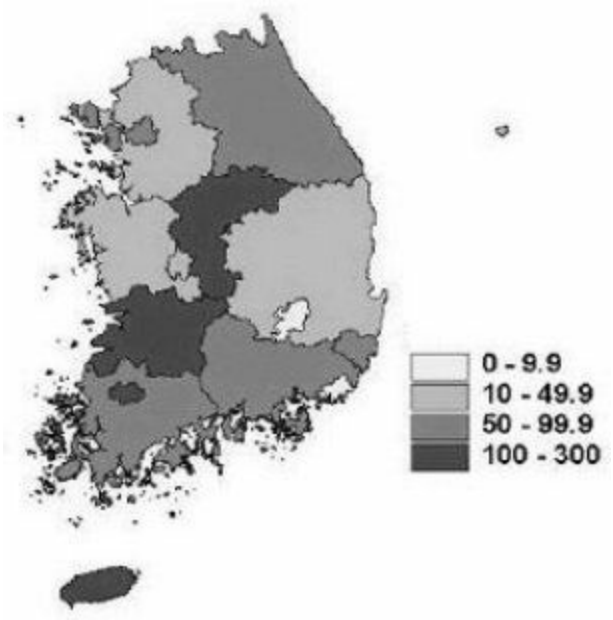

Figure 2. Measles outbreak in Korea. Incidence rates as of December, 2000 (cases/100,000 persons)

Many elementary schools were closed for some time and surveillance was started at the point of the observed initial increase in notification. At this time, the first national meeting to develop a strategy to deal with the resurgence of measles was planned. Subsequently a nationwide seroepidemiologic study, an international meeting with members of World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) was held (Figure 3). As a result, all students who enter school must have certification of vaccination. Following an 8-week campaign, sentinel pediatric surveillance by primary pediatric physicians and strengthening of surveillance were performed. In total, 5.7 million doses of measles vaccination were administered to all students between the ages of 8 and 16 years. The result was dramatic and successful. The outbreak was controlled and few cases were subsequently notified. A 5-year National Measles Campaign is in place for the eradication of this disease.

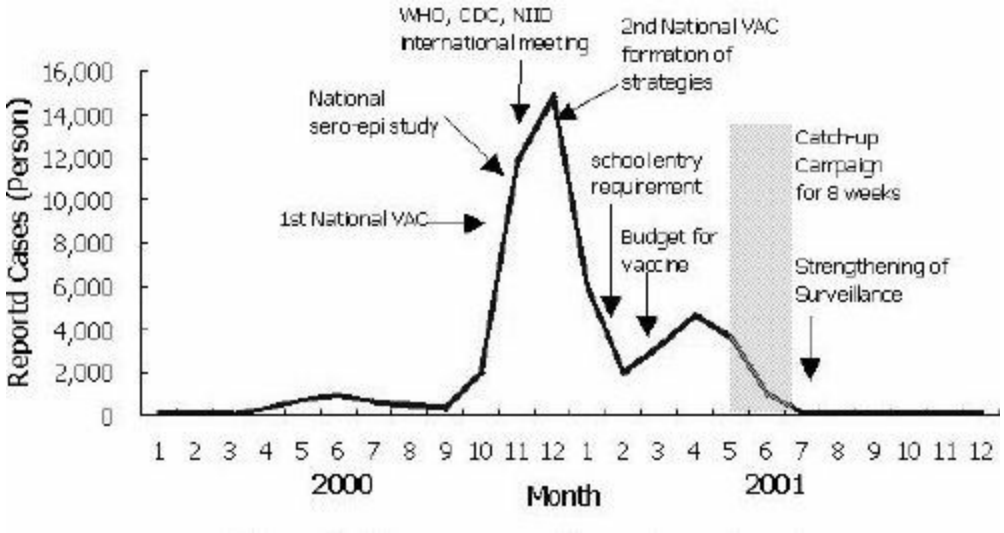

Figure 3. Time Courses of Measles Outbreak

Nosocomial Infection Control

In almost all university hospitals in Korea, there are full-time infection control practitioners (ICPs) who play the main role for hospital infection control. They perform surveillance, education, and research. They also decide the policy of IC practice for their hospitals, such as which hospital disinfectants to use, how to sterilize fiberoptic endoscopes, and the vaccination schedule for healthcare workers. According to their recommendations, in our hospital we use sodium hypochlorite as a universal disinfection, glutaraldehyde for various scopes, isopropyl alcohol for hand washing, and ethylene oxide gas for sterilization of medical equipment. In one example of a successful practice in our hospital, ICP recommended that the intensive care unit (ICU) nurses change the methods for endotracheal suction and hand washing. Repeated training of nurses about hand washing and adherence to single use of a suction catheter and the use of sterile normal saline for endotracheal suctioning was provided for 2 months. Thereafter, hand washing performance increased from 15.6% to 46.8%. The rates of nosocomial pneumonia per patient during the surveillance periods markedly decreased from 10.3% to 3.9%. Patient-days rates of nosocomial pneumonia were decreased from 10.0 cases/1,000 patient-days to 4.1 cases/1,000 patient-days, and ventilator-days rates of pneumonia decreased from 67.6 cases/1,000 ventilator-days to 16.2 cases/1,000 ventilator-days.

There are presently a total of 275 general hospitals in Korea. Nosocomial infection surveillance had previously been performed individually in a few hospitals, but national reference data were not available until 1996. Without defined national data, any improvement, or otherwise, cannot be determined. The first multicenter prospective surveillance study for NI was conducted by KOSNIC [6]. Prospective surveillance was performed at 15 hospitals during a 3-month period from June to August 1996. Non-military hospitals with more than 500 beds and with ICPs actively performing prospective surveillance for NIs were invited to participate in this voluntary study. Of the 15 hospitals that joined the study, 1 had more than 2,000 beds, 6 had more than 1,000 beds, and the remaining 8 had more than 500 beds. Fourteen were university hospitals, of which 12 were located in Seoul.

Two different prospective surveillance methods were used. For general wards, laboratory-based surveillance was used in which culture results were obtained from the microbiology laboratory, followed by review of the medical charts of all patients with positive culture findings. The surveillance was performed by the ICP in charge of NI surveillance at each hospital according to a uniform protocol prepared by the Nosocomial Infection Surveillance Committee of KOSNIC. For ICUs, the ICPs visited all the patients in the ICUs every 2 to 3 days. Patient charts were reviewed for status, vital signs, and laboratory results. When necessary, the ICPs solicited doctors and nurses for any additional information. Criteria for defining NIs were those recommended by the CDC [2,4]. Infection rate was defined as the number of NIs per 100 patients discharged during the period of surveillance.

A total of 3,162 NIs were identified among 85,547 patients discharged from the 15 participating hospitals during the study period, for an overall NI rate of 3.70 per 100

patients discharged (Table 2).

Table 2. Nosocomial Infection Rates and Distribution of Major Sites of Nosocomial Infections by Hospital Size

Hospital size	No. discharged	UTI		PNEU		SSI		BSI		Others		Total	
		R	D (%)	R	D (%)	R	D (%)	R	D (%)	R	D (%)	R*	D (%)
≤1,000 beds	36,334	1.08	29.8	0.59	16.4	0.64	17.6	0.61	16.7	0.71	19.5	3.63	100
>1,000 beds	49,213	1.15	30.7	0.67	17.8	0.54	14.0	0.48	12.8	0.91	24.1	3.75	100
Overall	85,547	1.12	30.3	0.64	17.2	0.57	15.5	0.53	14.5	0.83	22.5	3.70	100

R = Infection rate, number of nosocomial infections per 100 patients discharged

D = Distribution of infection rate

* $P = 0.32$

Nosocomial infection rates in other countries were reported by the National Nosocomial Infection Surveillance (NNIS) system in 1984 (3.35%) [5], the Q-Probes study of the College of American Pathologists in 1989 (3.53%) [9], and a German study in 1994 (3.5%) [3]. Direct comparison of these reported rates cannot be made because of variations in case-finding methods, patient populations, and sample size [1]. Other NI studies that included only a limited area of 1 hospital or 1 ward in Korea showed a 5.3 to 5.5% NI rate. This was higher than the KOSNIC data because the KOSNIC study included only cases with positive culture results. Thus, any case that was not cultured or for whom there was no growth in the culture media despite clinical signs of infection was omitted. Based on the results of the bacterial culture, the sensitivity ranged from 48 to 58%, so the revised rate of NI in Korea may be more in the range of 6.3 to 7.8% (95% confidence interval; 5.3 to 10.2%). Infectious disease experts, however, estimate that the NI rate would be higher because of the misuse of antibiotics and improper quality control of laboratories. Urinary tract infections (UTI) constituted 30.3% of all NIs; pneumonias, 17.2%; surgical site infections (SSI), 15.5%; and primary bloodstream infections (BSI), 14.5%. The distribution of BSI, was not correlated with hospital size ($P = 0.32$).

The NI rates varied significantly by type of service ($P = 0.01$), with the highest infection rate for neurosurgery (14.2%), followed by neurology (8.6%), oncology (6.7%), and chest surgery services (6.0%) (Table 3). Within internal medicine, the distribution of NIs was UTI (27.1%), BSI (23.1%), and pneumonia (18.1%), and for general surgery service, the ranking was SSI (39.1%), BSI (14.1%), and UTI (12.9%). Rates of UTI and pneumonia were the highest for neurosurgery (6.2% and 3.3%), SSI for chest surgery (1.8%), and BSI for oncology (1.7%).

Table 3. Nosocomial Infection Rates by Services

Services	Number discharged	Number of NI	NI Rate (%)*
Neurosurgery	4,180	594	14.2
Neurology	2,703	233	8.6

Oncology	3,119	209	6.3
Chest surgery	2,157	130	5.8
Internal medicine	23,651	1,022	4.3
General surgery	9,735	420	4.1
Orthopedic surgery	7,007	233	3.2
Plastic surgery	2,766	50	1.8
Urology	3,299	44	1.3
Obstetrics/Gynecology	12,957	43	0.3
Others	12,162	184	1.5

NI = nosocomial infection

* $P = 0.001$

The NI rate in ICUs was higher than it was in general wards (10.7% vs 2.6%, $P = 0.001$), although surveillance methods for general wards and ICUs were different. In general wards, the most common NI was UTI (31.2%), followed by SSI (17.5%), BSI (13.9%), and pneumonia (12.6%). In ICUs, the most common NI was pneumonia (30.3%), followed by UTI (27.6%), BSI (16.1%), and SSI (9.5%).

A total of 3,772 organisms were isolated from 3,162 NIs. *Staphylococcus aureus* (17.2%) was the most common pathogen and 83.7% of these isolates were methicillin-resistant. *Pseudomonas aeruginosa* (13.8%), *E. coli* (12.3%), and *Klebsiella pneumoniae* (7.7%) were also commonly isolated pathogens. In general wards, *S. aureus* (18.0%) and *E. coli* (14.6%) were the commonly isolated organisms, while in ICUs, *S. aureus* (20.8%) and *P. aeruginosa* (17.6%) were most frequent. The most common pathogen in UTI cases was *E. coli* (23.8%), while *S. aureus* (23.5%) was the most frequent isolate in pneumonia cases. In addition, *S. aureus* (28.3%) was the most common organism isolated from SSI cases, and coagulase-negative staphylococci (18.2%) was the most common organism isolated from BSI cases.

These data represent the first nationwide surveillance results. However, although participating institutions were tertiary hospitals that had full-time ICPs, there were only 15 institutions and there was not enough investigators to research all the NIs that were associated with negative culture results.

Recently in 2003, KOSNIC performed a nationwide surveillance study of NI in ICUs which surveyed 97 ICUs in 38 hospitals. Results of this study will be published soon. A third study of NI rates in Korean hospitals is currently in progress.

Conclusion

Infection control systems in Korea have been undergoing development in recent years through the effort of infectious disease specialists in concert with the Ministry of Health and Welfare and NIH. KOSNIC plays a major role in research, education, investigation of IC policies, and the execution of hospital IC in the field. KOSNIC provides training courses in IC for allied health professionals; in addition, NIH offers field epidemiology training. The manpower needs have evolved, however, with the improvement of health environment. There is a shortage of trained ICPs in many hospitals. Although there is awareness of this problem, without a viable infrastructure, IC systems cannot be implemented. All practitioners need appropriate resources. Government should reform the system to promote and encourage quality of care, reward good practice through professional associations, and increase financial support to the healthcare system. GDP share of health expenditure (5.9% in 2000) is below the level that could be expected for an OECD (Organization for Economic Cooperation and Development) country (average 8.1%) with Korea's standard of living and real health expenditure [8].

At present, a new organization that would perform the function of the CDC in the United States is under consideration. This organization would play an important role for national efforts in controlling infection, including NI control. In the future, with the effort of the Government and the continuing activities of ICPs, infection control in Korea would achieve noticeable success and would be consistent with other developed nations.

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Prevention and Control of Nosocomial Infections

Hui-Ping Zhou, M.D.

The First Hospital of Beijing University, People's Republic of China

Introduction

In China, there are about 50 million inpatients annually, and nearly 5 million of them develop infections during their stay in the hospital. The extra cost of treating these infections has been estimated to be between 10 and 15 billion RMB (yuan). The management of hospital infections developed on a large scale in the 1980s in China. In recent years, authorities responded to pressure from many sources and have developed several standards and regulations related to prevention and control of nosocomial infections.

General Situation

Hospital Infection Committees

In China, committees in charge of managing hospital infections have been founded. These committees exist at all levels of medical care (province, city, county, and hospital). Within hospitals in which there are more than 500 beds, a department of hospital infections must be present and staffed by at least 3 persons; this number increases to 4 for hospitals having more than 1000 beds. Hospitals with 300 or fewer beds are required to have 1 person in charge of managing nosocomial infections in their facility.

Today in China there are about 33,000 persons engaged in the prevention and control of hospital infections.

Standards and Regulations

Beginning in 1994, many standards and regulations have been made regarding nosocomial infections. These include diagnostic standards for nosocomial infections, management of nosocomial infections, regulation of disinfectant technology, and disposable medical devices management.

Sites of Nosocomial Infections and Primary Pathogens

The overall prevalence rates of nosocomial infections in China are as follows: respiratory tract infections, approximately 40%; urinary tract infection, between 20 and 31%; surgical site infections, about 10%; and gastrointestinal tract infections, approximately 10%.

The main pathogens associated with nosocomial infections in China are gram-negative bacteria (approximately 40%), gram-positive bacteria (28%), and fungi (21%). The primary gram-positive bacteria implicated in these infections are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, β-hemolytic Streptococcus, and *Enterococcus* (*faecalis, faecium*). The main gram-negative bacteria pathogens include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Enterobacter* (*cloacae, agglomerans*), Acinetobacter, and Legionella.

Prevention and Control of Nosocomial Infections Remain an Important Problem

Bacterial Resistance

Antibacterial resistance is the result of complex interactions between the use of antimicrobial agents and microorganisms. The use of antimicrobial agents has become increasingly prevalent, and at the same time, resistance of bacteria to these drugs has become more widespread and severe. In China, antimicrobial agents are administered to about 70 to 80% of hospital inpatients. The use of susceptibility testing results to guide the selection of antimicrobial agents occurs in only about 14% of cases. Thus, understanding the mechanisms underlying bacterial resistance, selection of the appropriate antimicrobial drug, and correct use of an antimicrobial agent are important issues for personnel involved in the management of nosocomial infections.

Penicillin-resistant Streptococcus pneumoniae (PRSP)

Penicillin-resistant *S. pneumoniae* (PRSP) first occurred in 1965 in the United States, in 1967 in Australia, and in 1977 in South Africa, and has now been identified in almost all countries worldwide. In the 1980s the frequency of PRSP was estimated to be about 1%. By the 1990s the rate of PRSP had increased. In China, the rate of PRSP is about 40%, and PRSP has been identified in almost 55% of cases of childhood pneumonia. In addition to penicillin, many *S. pneumoniae* strains also show resistance to erythromycin and sulfamethoxazole/trimethoprim.

Methicillin-resistant Staphylococcus aureus (MRSA)

Methicillin was a highly effective antimicrobial in the 1960s, but by the 1970s, several strains of *S. aureus* had begun to demonstrate resistance to this agent. In the United States (U.S.), only 2.4% of *S. aureus* isolated in hospitals with 200 or more beds demonstrated resistance to methicillin, but by 1999, this rate had risen to 40%. In China, more than 50% of *S. aureus* pathogens isolated were methicillin-resistant, and in some areas this rate was much higher. In the Zhenjiang Province, for example, the rate of MRSA was estimated to be 78.3%.

Vancomycin-resistant Enterococci (VRE) or Staphylococcus aureus (VRSA)

Worldwide, it has been estimated that between 20 to 30% of Enterococci are resistant to vancomycin. In China, the rate of VRE has been estimated to be about 10%. The first case of vancomycin-resistant *S. aureus* was reported in Japan in 1996. Several more cases were identified shortly thereafter, and the minimum inhibitory concentration (MIC) values for vancomycin in these strains were around 8 µg/mL. But in 2002, a strain of VRSA was isolated in the U.S. in which the MIC for vancomycin was 128 µg/mL. VRSA has not yet been identified in China.

Multidrug-resistant Tuberculosis bacterium (MDR-TB)

Today in China, about 6 million patients suffer from tuberculosis, and according to current trends, this number could increase to as many as 20 to 30 million patients within the next decade. Multidrug-resistant tuberculosis bacteria are a problem in the treatment of these patients. In China, the rate of MDR-TB has been reported to be as high as 33% in one local area.

Newer Pathogens and New Technologies

In recent years, several new pathogens responsible for serious and life-threatening illnesses have emerged, including hepatitis C and hepatitis E virus, human immunodeficiency virus, and prions. In 2002-2003, coronavirus was responsible for an outbreak of severe acute respiratory syndrome (SARS) in which 5327 individuals in China were infected as of July 2003, including 968 doctors and nurses.

Potential causes underlying increased risk of nosocomial infection include not only new pathogens and new resistant bacterial strains, but technological advances in the diagnosis and treatment of disease. Some examples of these include organ transplantation and the use of endoscopes and cardiac catheters.

Problems Related to Prevention and Control of Hospital Infection that Need to be Resolved

1. Some emergency measures should be developed to deal with acute communicable diseases such as SARS that may occur in the future.
2. Standardized rules or regulations need to be developed to deal with issues such as disinfection and sterilization by the Central Sterile Service Department within hospitals. This includes a renewed emphasis on regulations concerning hand washing and cleaning on the part of medical workers.
3. Standards defining the role of clinical microbiology in managing nosocomial infections should be developed and should include topics such as the sampling and transport of specimens, reporting results of susceptibility testing, staffing by trained professionals, special laboratory equipment, and quality control.
4. The structure and role of organizations concerned with the prevention and control of nosocomial infections should be strengthened.
5. A new hospital dedicated to the management of infectious disease should be built.
6. Training of hospital staff in the prevention and control of nosocomial infections should continue.

Recently, the Standing Committee of National People's Congress has made a decision regarding a construction plan and research arrangement for a system to deal with public health emergencies. Therefore, in the future, work aimed at preventing and controlling nosocomial infections in China should be improved.

Infection Control in Asia

Ling Moi Lin, M.D.

Singapore General Hospital, Singapore

Introduction

Infection control is safety. We aim to protect the patient, the healthcare worker, and the hospital. Hence, we develop safe patient care and employee health policies and guidelines accordingly. A good test to determine the level of safety in these areas is in the form of a crisis, of which there are currently 2 in the world. One is avoidable as it is man-made, i.e., bioterrorism, while the other occurs naturally – the infectious disease outbreak of SARS (Severe Acute Respiratory Syndrome). The response to these crises is a good measure of a country's level of preparedness and ability to handle an outbreak and control it effectively.

SARS is "a puzzling and difficult new disease" that has affected 8,437 patients resulting in 813 deaths in 32 countries in a relatively short period of 8 months during 2002/2003. While 97% of the cases occurred in China, Canada, Hong Kong, Taiwan, and Singapore, SARS is a global concern as we do not know of any effective treatment or vaccine to combat it. Notably, however, the outbreak was successfully controlled with the same basic infection control measures that the world first knew of – isolation of the affected patients and quarantine of exposed contacts who may potentially harbor the disease. Nosocomial transmission occurred primarily through either direct patient contact or contact with large respiratory droplets in close vicinity to an infected patient. The reported rates of nosocomial transmission were 75% in Singapore, 62% in Hong Kong, and 51% in Canada [5].

Lessons from SARS

A large number of SARS cases were reported in the Asia Pacific region (China, Hong Kong, Vietnam, Taiwan, and Singapore) and Canada. This contrasted greatly with the incidence in the United States (U.S.) where a relatively low number of cases were reported. One possible reason for this discrepancy was the high level of nationwide planning and preparedness in the U.S. following the anthrax scare in October 2001 (see related manuscript, Dorothy Canter).

The “global village” in which we live today has both values and pitfalls with respect to management of a highly infectious disease. Through the internet and e-mail systems, global alerts and updates concerning SARS were successfully delivered via ProMed (the Program for Monitoring Emerging Diseases), World Health Organization (WHO), and Centers for Disease Control (CDC) websites. However, the rapid spread of the virus was facilitated by our advanced transportation systems that allow travel to virtually any destination worldwide in a short period of time.

The level of national response in controlling the outbreak correlated well with the degree of strength in political leadership for a particular country. The early days of the outbreak was essentially a war against a relatively unknown pathogen, and rapid, decisive, and united action was needed for effective control measures. During this outbreak, inadequacies in the healthcare system were uncovered and these were seen in the areas of structure, system, and surge capacity.

Therefore, it was not surprising that the following conclusion was reached at the recent SARS WHO Conference held on 17-18 June, 2003 [5] – that current infection control measures are not effective unless there is proper infrastructure, adequate training, and consistent, appropriate infection control practices. Countries represented at the meeting agreed that they were aware of the problem and recognized the need for a response. It is now the responsibility of each of these countries to review their current infrastructure and act accordingly.

Recommended Responses – The 3 R's

Resources

The 1999 requirements outlined by CDC [2] for an effective infection control program called for the following:

- Management of critical data and information
- Development and recommendation of policies and procedures
- Compliance with regulation, policies, and accreditation requirements
- Institution of an employee health policy
- Education and training of all staff
- Adequate number of trained infection control nurses and a physician trained in hospital epidemiology (infection control)

The establishment of an Infection Control Committee (ICC) is already present in most Asian hospitals. However, the effectiveness of these committees is greatly limited as they meet only as scheduled. The daily happenings at ground level are best handled instead by the Infection Control Team which is comprised of infection control nurses (ICN) and the infection control doctor (ICD) or officer (ICO). In most Asian hospitals, it is far more effective for a physician to talk to another fellow physician to gain his/her acceptance. In larger hospitals, the framework of infection control liaison officers (ICLO) has proven to be very effective. These individuals work closely with the Infection Control Team on a daily basis towards ensuring better compliance with appropriate infection control measures.

Although CDC made the recommendation of the ratio of 1 infection control nurse for every 250 beds, many countries did not/are not able to achieve this as outlined in Table 1.

Table 1. Infection Control Resources

Country	ICN	ICD	ICC
Belgium	1:1000 patients	1:2400 patients	1: hospital
Denmark	---	1: country	1: hospital
Finland	1:400-500 beds	1:800-1000 beds	1: hospital
Germany	1:300 beds	1:450 beds	1: hospital
Great Britain	1:477 beds	1: hospital	1: hospital
Iceland	1:250 beds	1: hospital	1: hospital
The Netherlands	1:250 beds	1:1000 beds	1: hospital
Norway	1:300-400 beds	1:3-5 regions	---
Spain	1:500 beds	---	---
Sweden	60 in country	10-15 in country	1: hospital
Switzerland	1:650 beds	1:4-5 university hospitals	1:4-5 university hospitals
Taiwan	1:350 beds	1: hospital	1: hospital

Data referenced in Reference 6.

Zoutman *et al.* revealed that 12% of the >200 bed hospitals in Canada did not have an infection control nurse in the 1980s [7]. A second survey conducted in 2000 [7] showed some improvement, but with 40% of hospitals having a ratio of less than 1 ICN:250 beds; 80% did not meet Canadian recommendations; and 40% had no physician with infection control training. The infection control doctor/officer is a vital component of the program as he/she is often the best person to garner the support of fellow physicians for compliance with infection control practices.

Both Taiwan and Korea had a boost to the development of infection control in their countries after a major outbreak at one of their hospitals. In Taiwan, it took a shigellosis outbreak at a maternity hospital in 1984 and that of *Legionella pneumophila* at a tertiary hospital in 1985 in Korea to alert the countries of the importance of infection control [1]. Since then, national infection control societies have been set up with active programs ongoing to promote the training and development of trained personnel and programs. In Taiwan, a national recommendation was made to change the ICN: patient ratio of 1:750 in 1985 to 1:300 [3].

In Singapore, the recent national outbreak of SARS helped to alert the nation to the significance of an effective infection control program. Hospitals have seized the opportunity to increase their infection control manpower staffing to cope with the recognized increased need for expansion of infection control work (Table 2).

A recent Delphi study conducted in the U.S. [4] revealed that the ratio of 1 ICN:250 beds (0.4 ICN:100 beds) must be updated since the current job scope for the ICN has changed. Not only does the ICN have to deal with ordinary functions of surveillance, education, teaching, and development of policies, but time also needs to be spent on quality improvement projects. Thus, the study recommended that a more appropriate ratio would be 0.8 – 1.0 ICN per 100 beds.

Table 2. Number of Infection Control Nurses at Hospitals in Singapore

Hospital	Pre-SARS	Post-SARS
Parkway	3	3
MAH	1	1
IMH	0	1
NUH	2	3
TTSH	4	4
CGH	2	2
KKH	1	3
SGH	7	10
NCC	1	2
NHC	1	1
SNEC	1	1

Routine

A system of ongoing surveillance, training, and auditing is essential to achieve the objectives of maintaining safety for the patient, staff, and hospital.

Readiness

The level of preparedness is crucial as we face the question of our level of readiness and ability to deal with the issue of possible outbreak management. The key lies in the state of the surveillance program's ability to promptly detect issues of concern. Effective process management involves gathering the right data in the right format at the right place and time and subsequently delivering these to the right hands i.e., the process owners so that the appropriate action is taken. This data-driven process is a means of achieving the desired outcomes for any system. Data is only useful when it gets transformed into information, and this requires a certain level of training for the ICN.

There may be 3 obstacles to the above – ignorance of the needs or means, resistance to change either due to lack of resources or unwillingness to change, and lack of confidence. The recent SARS outbreak broke the first barrier. Each country throughout Asia will naturally address the second factor now that the need for a good infection control infrastructure and system is apparent. Infection control societies like the Asia Pacific Society of Infection Control (APSIC) hope to address the third factor.

APSIC was formed in 1998 by representatives from 16 countries in the region. The first international congress was held in 1999, with 1,000 international delegates in attendance. The society's mission is to build a network of infection control professionals working towards quality healthcare in the Asia Pacific region. Its objectives are:

a) To facilitate exchange of information and data on infection control principles and practices through workshops, seminars, congresses, conferences, and journal publications
b) To formulate recommendations, guidelines, or standards by consensus working groups
c) To assist in collaborative work of research or investigations of outbreaks

APSIC has held 3 infection control training courses. An increasing number of registrants attended each course, demonstrating the high level of interest in equipping staff for better work capabilities in their own institutions. Each 10-day course aims to provide the student with the necessary knowledge and skills in epidemiology, microbiology, and surveillance as well as to give updates on infection control issues.

Conclusion

It was evident from the recent WHO SARS Conference that the minimum global level of safe practice is that of Standard Precautions. Each country should practice risk-based infection control measures and review its current infection control capacity. In addition, each nation should take a political interest in setting a mandate for a good infection control system within their hospitals and to set requirements for an infrastructure within the country. In addition, resources need to be released to meet these requirements. Finally, the collaborative spirit evident throughout the SARS crisis must continue as each country has much to learn from each other's best practices.

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Hospital Infection Control in Vietnam

Nguyen Viet Hung, M.D., Ph.D.

Bach Mai Hospital, Hanoi, Vietnam

Introduction

Vietnam is a country with 80 million inhabitants. Its system of health facilities is mainly public based, with 847 hospitals at various levels, from central to district ones. Hospital size varies from an average of 50 to 1400 beds. Medical care expenditures are mostly covered by the government and partly contributed by patients through the system of partial hospital fee payment. Private hospitals account for only a small number, approximately 20 in total. With the motto of “Prevention is better than cure”, the local health networks (commune health stations, village health units, etc.) have benefited from important investments of the government in recent years. It should be noted, however, that most hospitals, especially those at the central level, are overloaded and their resources for taking care of patients are very limited.

In recent years, infection control (IC) activities at hospitals have been strengthened. In 1997, the Ministry of Health promulgated regulation on hospital infection control and requested every hospital establish an Infection Control Committee (ICC) and/or an Infection Control Department (ICD). However, IC has not been regarded as an independent medical specialty, and educational programs on IC have not yet been regularly included in the teaching program of medical and nursing schools. IC training activities in the last few years have been mainly carried out under the Project of Hospital Infection Control directed by the Department of Therapy, Ministry of Health, or by a small number of central level hospitals through international cooperation projects. Generally, knowledge and skills of most healthcare workers about IC practices are limited, and IC activities are facing many difficulties.

Structure of Hospital Infection Control

Composition and Functions of the ICC

In most hospitals, members of the ICCs usually include heads of the ICD, General Planning Department, Nursing Service Department, Administrative Department, and one representative from departments of Clinical Microbiology, Infectious Diseases, Surgery, Pediatrics, Intensive Care, and General Internal Medicine. The Committee is chaired by the Director or Vice Director of the hospital. The ICD is led by the Vice Chairman or a standing member of the ICC. This committee is supported by an Infection Control Team (ICT), which belongs to the ICD. Except for the members of ICT, all other members of the ICC, including the chairman, share their tasks in the committee with their other duties within the hospital. The major mandate of the committee is to give technical advice to the hospital director on issues relating to nosocomial infection control such as surveillance of endemic infections, detection and control of communicable outbreaks, development of specific infection control guidelines, and training and education practices of prevention and control of hospital infection. The specific responsibilities of ICCs are as follows:

1. To set up and monitor a surveillance program and reporting system for nosocomial infections in patients and healthcare workers for the prevention of infections in the hospital, especially in the higher risk areas.
2. To develop, review, and revise policies and procedures on IC applicable to the hospital (e.g., disinfection, sterilization, environmental hygiene, management of hospital waste).
3. To monitor and control the use of antibiotics and disinfectants.
4. To provide hospital departments with updated and relevant information on local microorganisms and their resistance to antimicrobial agents.
5. To formulate and provide a continuing education program for all hospital staff and patients.
6. To periodically evaluate the effectiveness of the IC activities and recommend the appropriate and required policies or measures.

Infection Control Department

In order to promote enforcement of regulations on IC, the Ministry of Health requests every hospital at central and provincial levels to establish an ICD. The main task of these departments is to coordinate daily IC activities which are discharged by the respective ICTs. The ICTs usually include full-time IC doctors and nurses. The Ministry of Health recommends that a full-time IC staff be provided for every 200 beds. The responsibilities of the ICTs are shown below:

1. Coordinate the ICCs.
2. Take responsibility before the hospital board for IC activities.
3. Develop and conduct surveillance and investigation of nosocomial infections using

epidemiological methods.
4. Analyze, report, and apply surveillance and investigation findings to clinical practices.
5. Formulate IC policies and procedures.
6. Inspect and monitor implementation of IC policies and procedures in the hospital.
7. Supervise the isolation of infected patients and their management.
8. Develop and implement systems for prevention of infectious diseases in healthcare workers.
9. Provide appropriate consultation and education to patients and healthcare workers.
10. Research and evaluate the effectiveness of IC measures.

In addition to the above responsibilities, the ICD has the following tasks and duties:

1. Centralized management and disinfection of the medical laundry.
2. Centralized management and sterilization of medical instruments and devices.
3. Delivery of sterile medical instruments and devices.

Infection Control Network at Clinical Departments

At hospitals with an ICC/ICD, the network of IC collaborators is mostly established within the clinical departments. Members of this network usually include a head nurse and doctor of each department (link nurses and doctors). Besides providing treatment and care for patients, these staff work closely with the full-time IC staff to implement IC activities within their respective departments. Since most of these individuals have not attended any training courses on IC (even the basic ones), however, the effectiveness of their activities remains limited.

After 6 years of implementing IC regulation, the results are still limited and do not meet the requirements due to several manpower and resource issues.

Reports of 329 hospitals in 2001 showed that the percentage of hospitals in Vietnam with an ICC and ICD was 56% and 41%, respectively. Of these hospitals, however, only 47% had medical doctors and 67% had nurses who work on a full-time basis on IC activities. There is usually 1 infection control doctor at each hospital, although in particular, Bach Mai and Cho Ray hospitals each have 5 such physicians. Infection control activities were mostly focused on disinfection, sterilization, and monitoring of environmental conditions. Only 40% of hospitals had centralized sterilization, and only 30% of hospitals were conducting microbiological monitoring of the environment. Activities of nosocomial surveillance had not been implemented in most hospitals. Similarly, IC training activities had not been strengthened. Reports also showed that only 60% of hospitals had organized IC training courses and only on an irregular basis; most of them held 1 or 2 courses with a limited number of participants.

Activities of Nosocomial Infection Surveillance

To date, the IC Project of the Ministry of Health has conducted 2 nationwide studies on prevalence of nosocomial infections. The latest study was conducted in 2001 and included 5,396 patients from 11 general hospitals at provincial and central levels. A total of 369 (6.8%) nosocomial infections were identified. The most common infection was respiratory tract infection (41.8%), followed by surgical site infection (17.6%), urinary tract infection (16.4%), skin and soft tissue infection (12.2%), intestinal tract infection (5.6%), and bloodstream infection (3.9%). The greatest prevalence rate was found in adult ICUs (22.8%), followed by the wards of general surgery (10%), internal medicine (5.1%), obstetrics (3.4%), and pediatrics (3.6%). The most frequent microorganisms isolated from patients with nosocomial infections were gram-negative bacilli (78%), gram positive bacteria (19%), and *Candida* spp. (3%). During the survey period, 59% of the patients were receiving antibiotics for empirical therapy or medical prophylaxis.

A few hospitals, such as the Bach Mai and Cho Ray hospitals, performed local infection prevalence and incidence studies. Most of these studies focused on surgical site infection (SSI). In 2000, a 1-day point prevalence SSI study was conducted in Cho Ray hospital, and the prevalence of SSI was 14%. In Bach Mai hospital, the first 1-day cross-point surveillance was carried out in November, 2001. The overall prevalence of hospital-acquired infections (HAIs) was found to be 6.75%, with 60% detected from the emergency and surgical patients. The most common HAIs recorded involved lower respiratory infections (45.4%), followed by urinary tract infections (20.6%) and surgical site infections (15.1%). The number of operations, the total number of used devices, and number of invasive procedures were significantly correlated with HAIs. Positive cultures were found in 43.8% of the cases. The most frequently isolated microorganisms were: *Pseudomonas aeruginosa* (28.1%), *Enterococcus* (21.9%), *Acinobacter* (18.8%), and *Candida* spp. (15.6%). The prevalence of antibiotic use was 39.7%.

Two incidence SSI studies were conducted in 1999 and 2001 in Bach Mai hospital; 697 patients were observed in the first study and 911 patients in the second one. The results of these studies showed that the overall rate of SSI had decreased considerably, from 10.9% in 1999 to 4.3% in 2001. These studies also showed that abdominal surgery and wound class IV were significant predictors of SSI. All patients were treated with prolonged courses of perioperative antibiotics. Within the framework of the 2001 study, all patients with clinical signs of SSI were microbiologically tested. Five main pathogens isolated from SSIs are *Enterococcus* (24.2%), *Candida* spp. (15.2%), *Enterobacter* (12.1%), *Escherichia coli* (12.1%), and group D *Streptococci* (12.1%). Most of these bacteria were resistant to frequently used antibiotics (penicillin, cephalosporin, and amynoglycoside).

Some hospitals are implementing programs to report and manage exposure to blood-borne diseases in healthcare workers or the incidence of nosocomial infections aimed at evaluating interventional measures.

Antimicrobial Resistance in Vietnam

In recent years, a national program on surveillance of antibiotic resistance of common pathogens, including nosocomial pathogens, has been underway under the responsibility of Bach Mai hospital. The results of the 3-year surveillance (1999-2001) at 16 central/provincial hospitals showed that 64.9% isolates were gram-negative bacilli, of which *E. coli* accounted for the highest rate (22.4%). Among the gram-positive bacteria, *Staphylococcus aureus* accounted for the highest rate, 20.7%. The rates of antibiotic resistance of the common bacteria were as follows:

- *E. coli*: Over 50% of isolates were resistant to ampicillin (81.7-86.1%), co-trimoxazole (68.6-78.0%), tetracycline (69.5-87.2%), cloramphenicol (51.4-72.2%), and cephalothin (52.3-54.4%).
- *Enterobacter*: Over 50% of isolates were resistant to ampicillin, cefuroxime axetil, cephalothin, gentamicin, co-trimoxazol, tetracyclin, and chloramphenicol.
- *Klebsiella*: Over 50% of isolates were resistant to ampicillin, gentamicin, co-trimoxazole, tetracycline, chloramphenicol, and ceftriaxone.
- *Enterococcus*: Over 50% of isolates were resistant to erythromycin, gentamicin, co-trimoxazole, chloramphenicol, and ceftazidime. The rate of vancomycin-resistant enteroccoci (VRE) ranged from 8.7% to 58.8%. The rate of amikacin- and ampicillin-resistant isolates was under 25%.
- *Salmonella typhi*: Over 50% of isolates were resistant to ampicillin, co-trimoxazole, tetracycline, and chloramphenicol. The rate of bacteria resistant to nalidixic acid was 93.5%.
- *S. aureus*: Over 50% of isolates were resistant to erythromycin, clindamycin, tetracycline, and doxycycline. The percentage of isolates with β-lactamase enzyme was very high (91.9-98.4%). The percentage of MRSA was 33.5-46.5%, and the percentage of bacteria resistant to vancomycin was quite low (0.5-1.0%).
- *P. aeruginosa*: Over 50% of isolates were resistant to tetracycline (>90%), cefotaxime (58.2-64.1%), ceftriaxone (49.8-53.3%), and gentamicin (37.9-54.9%).

Problems and Future Needs

Similar to other developing countries, IC activities in Vietnam have several constraints, such as lack of administrative and financial support, unqualified/untrained personnel, especially the lack of IC nurses, over-crowded wards, and inadequate equipment and supplies. The Ministry of Health needs to more intensively invest in upgrading hospitals, and all hospitals should allocate an adequate budget and appropriate human resources for carrying out IC activities.

Another major constraint is the lack of trained medical doctors and nurses specializing in infection control. In general, the healthcare workers in hospitals are not well aware of the importance of IC activities. The IC project should develop training and educational programs for the full-time IC personnel as well as healthcare workers. Based on these programs, modular training courses for IC doctors, in particular nurses, and healthcare workers, should be organized either by the individual hospitals themselves or by the leading regional hospitals. Currently, scientific evidence-based guidelines on IC do not exist within Vietnam, and the budget for surveillance and research on nosocomial infections is limited. The IC Project of the Ministry of Health requires adequate investment in terms of human and financial resources from the government in order to properly conduct prevalence and incidence studies, to develop the guidelines, and to standardize IC procedures at national level.

Infection Control and Hospital Epidemiology in the United States: Challenges in 2003

Denise M. Cardo, M.D.

National Center for Infectious Diseases, Centers for Disease Control and Prevention, U.S.A.

Introduction

Nosocomial or hospital-associated infections are adverse patient events that affect approximately 2 million persons and contribute to approximately 90,000 deaths annually in the United States (U.S.) [46]. The annual economic burden of these infections in the U.S. is estimated at $6.7 billion per year, in 2002 prices [20]. Nosocomial infections are one of the most common complications affecting hospitalized patients. Based on the Harvard Medical Practice Study II, surgical wound infections constitute the second largest category of adverse events [30].

While many successes have been achieved by infection control programs, several challenges remain for local and national programs, including changes in the delivery of care, difficulties in preventing infections, and new issues such as emerging infections and bioterrorism. This article briefly discusses some of these challenges.

From Hospital-Associated Infections to Healthcare-Associated Infections

Hospital-associated infections typically affect patients who have reduced host defenses because of age, underlying diseases, or medical or surgical treatments. Aging of the population and increasingly aggressive medical and therapeutic interventions have made patients more vulnerable to infections. For many years, the highest rates of infections were observed in intensive care units (ICUs) [5,9,46]. Recent studies, however, have shown that procedures that pose a risk for infection, e.g., use of central venous catheters (CVCs), are also frequent outside ICUs. One study involving 6 medical centers found that 7 to 39% (mean, 28%) of non-ICU patients had CVCs [11].

In the past decade, the delivery of healthcare in the U.S. has shifted from the acute, inpatient hospital to a variety of settings such as outpatient, long-term care and home health. As a result, infection control programs are increasingly challenged to detect and prevent healthcare-associated infections that occur outside inpatient settings.

In 2000, there were 83 million visits to hospital outpatient clinics [31]. There are few data on healthcare-associated infections in outpatient settings; however, several outbreaks have been reported, usually related to breaks in basic infection control. Unsafe injection practices, reuse of syringes and needles, and contamination of multiple-dose medication vials have been associated with transmission of infections in these settings [7,19,23,36].

Approximately 1.6 million Americans reside in the nation's 16,500 nursing homes [10]. Some studies have shown rates of healthcare-associated infections in long-term care facilities range from 1.8 to 13.5 infections per 1000 resident-care days [43]. Approximately 8 million people in the U.S. received medical care at home in 1996 (34), and an estimated 774,113 of these had at least 1 indwelling medical device [13]. Current guidelines for surveillance and prevention of infections in hospitals are difficult to apply in these settings.

Prevention of Infections: Going beyond SENIC

Preventing healthcare-associated infections is an integral part of the national safety agenda developed in response to the Institute of Medicine's (IOM) report "To Err is Human: Building a Safer Health System" [27]. The systematic collection and analysis of data on healthcare-associated infections have yielded critical, evidence-based information that can improve infection prevention and control. The Study on the Efficacy of Nosocomial Infection Control (SENIC) conducted in the 1970s showed that at least 30% of infections could be prevented with systems that combined surveillance, prevention, and control [22]. The IOM report indicated that at least 50% of medical errors could be prevented [30].

Data from the National Nosocomial Infection Surveillance (NNIS) system have shown that during 1990-1999, risk-adjusted infection rates decreased for all 3 body sites monitored in ICUs (i.e., respiratory tract, urinary tract, and bloodstream). As an example, bloodstream infection rates decreased substantially in medical ICUs (44%), coronary ICUs (43%), pediatric ICUs (32%), and surgical ICUs (31%) [5].

Data from the NNIS system have generally served as a national benchmark rate that has been used to motivate institutions with higher than expected infection rates [9]. With this approach, several institutions do not assess the preventability of infections acquired by individual patients unless the institution's rates are high or increasing. The result may be an underestimation of the fraction of preventable infections and missed opportunities to discover new prevention strategies [18].

Several hospitals are adopting the approach of "zero" preventable infections as a goal. The experience of continuous quality improvement focusing on an ongoing cycle of event tracking and process improvement has been applied in some hospitals seeking to reduce serious medication errors [4,18,35].

Several new strategies have been developed and evaluated to improve detection and prevention of healthcare-associated infections. Informatics is widely used in healthcare, and several applications of this science can help facilitate infection surveillance and prevention. These include computerized decision support systems, personal digital assistants for data collection and management of patients, and web-based systems for education/training [17,39]. The value of using electronic databases has been demonstrated in monitoring healthcare-associated infections [39]. In a study conducted to compare computer algorithms to traditional surveillance, rates of bloodstream infections were obtained using pharmacy and laboratory data stored in an electronic database. Such rates were comparable to those obtained by infection control practitioners to detect bloodstream infections, but required fewer resources to obtain [44].

Another prevention strategy involved new approaches to hand hygiene - the single most important practice to reduce the transmission of infectious diseases in healthcare settings. Products such as alcohol-based rubs containing an emollient that do not require the use of water have been developed and are recommended to improve adherence to hand hygiene [6,38].

Other examples of improvements are targeted to the prevention of bloodstream infections. Randomized, controlled trials have demonstrated that strategies such as

preparing the skin with chlorhexidine antiseptic [32], using maximal sterile barriers [40], and using a CVC with anti-infection properties can reduce the risk of catheter-related bloodstream infection to 1% or less [14,33].

However, despite advances in technology and strategies to prevent healthcare-associated infections, it is uncommon to find a healthcare institution in the U.S. with very low rates of such infections. This may be due in part to a lack of adherence to recommended infection control practices. Observed adherence by healthcare personnel to universal precautions has ranged from 43 to 89% [15,25], and adherence to hand hygiene from 5 to 81% (average 40%) [6]. Improving adherence to infection control practices requires a multifaceted approach that incorporates continuous assessment of both the individual and the work environment [29].

Several studies have shown that an education program directed to nurses and physicians may significantly improve adherence to infection control practices and reduce the incidence of infections in specific units [16,42,45,48]. However, more data are needed to assess the sustainability of these improvements.

In addition to behavioral factors, other factors related to the health system have been associated with the occurrence of infections in healthcare settings. The association between nursing staff shortages and increased rates of healthcare-associated infections has been demonstrated in several outbreaks [37]. The qualification and experience of the nursing staff may also affect infection rates. In a recent study, the risk for CVC-associated bloodstream infections increased during the time that a patient was cared for by a temporary nurse [1].

Many experts are trying to use industry-based principles to improve the delivery of care. For example, in the Pittsburgh Regional Healthcare Initiative, the principles of the Toyota Production System (TPS) are being applied. TPS is a systems engineering strategy that can be applied to a healthcare setting to improve patient safety. In the Pittsburgh initiative, frontline healthcare personnel were trained to use specific work principles that exposed problems in a non-threatening manner and encouraged problem solving in the course of daily work. TPS has been used to overcome barriers to adherence with infection control practices and identification of methicillin-resistant *Staphylococcus aureus* (MRSA)-colonized patients. Preliminary results have shown a decrease in the rate of MRSA infection in the intervention unit [35].

New Challenges: Antimicrobial Resistance, Bioterrorism, Emerging Infections

Antimicrobial Resistance

Antimicrobial resistance is a growing public health threat in healthcare settings. Many of the bacterial pathogens that cause healthcare-associated infections are becoming increasingly resistant to the antimicrobials most commonly used to treat these multi-drug resistant organisms (MDROs) and include MRSA, vancomycin-resistant enterococci (VRE), and certain gram-negative bacteria.

During the last several decades, the prevalence of MDROs in U.S. hospitals and medical centers has increased steadily [9]. MRSA, first recognized in the 1960s, became endemic in many hospitals during the 1990s. Until recently, vancomycin has been the only uniformly effective treatment for Staphylococcal infections. In 1996, the first clinical isolate of *S. aureus* with reduced susceptibility to vancomycin was reported, and as of March 2003, 11 such infections have been confirmed in patients in the U.S. [21, CDC personal communication]. In June 2002, the first clinical isolate of vancomycin-resistant *S. aureus* (VRSA) was identified, and in September of the same year the second case was reported [12,47].

In ICUs at hospitals participating in the NNIS system, the percentage of *S. aureus* isolates that were MRSA increased from 2.4% in 1975 to 26% in 1992 and to 62% in 2002 [26]. Methicillin resistance has also been frequently reported in *S. aureus* isolates from non-ICU inpatient and outpatient areas [9]. Once considered exclusively a hospital-acquired pathogen, MRSA has recently emerged in the community. Several reports have documented single episodes or sporadic clusters of MRSA infection among populations with little or no documented contact with healthcare facilities. An outbreak resulting from transmission of community-associated MRSA in a hospital has been recently described [41].

Most MDROs are transmitted from one person to another via the hands of healthcare personnel which can be easily contaminated during the process of care giving or having contact with environmental surfaces in close proximity to the patient. Without appropriate hand hygiene and/or glove use, contact with an infected or colonized patient may result in transmission of MDROs to other patients. While recommended measures to control the spread of MDROs, including MRSA and VRE, in hospitals have been promulgated for several years, surveillance data suggest that the existence of these recommendations has not significantly slowed the increase in infections or colonization with either organism in the U.S. [9]. The reasons for this lack of impact are unclear. For some institutions, the recommended measures may be ineffective, or poorly implemented or adhered to. The first 2 VRSA cases in the U.S., which occurred in non-hospitalized patients, illustrate the need to extend and adapt the current recommendations for preventing MDROs to the continuum of healthcare delivery.

Because of the growing public health threat of MDROs, in 2002 the CDC launched a campaign to prevent antimicrobial resistance in healthcare settings. This campaign targets

clinicians and focuses on 4 strategies: 1) prevention of infections, 2) effective diagnosis and treatment of infection, 3) use of antimicrobials wisely, and 4) prevention of transmission. The campaign consists of multiple, evidence-based, 12-step programs targeted to clinicians who treat specific patient populations (i.e., hospitalized adults, hospitalized children, surgical patients, patients in dialysis, residents in long-term care). Additional information can be found at http://www.cdc.gov/drugresistance/healthcare/.

Bioterrorism and Emerging Infections

Infection control programs have been important in the control of emerging threats. Following the terrorist attacks on September 11, 2001 and the subsequent outbreaks of anthrax, many healthcare facilities developed plans to address preparedness and response. Infection control issues addressed by these plans include preventing transmission among patients, healthcare personnel, and visitors; identifying persons who may be infected or exposed; providing treatment and prophylaxis; protecting the environment; and providing appropriate staffing [3].

Healthcare facilities were at the center of the 2003 outbreak of severe acute respiratory syndrome (SARS). SARS is a newly discovered respiratory disease caused by a novel coronavirus (SARS-CoV) that emerged in China at the end of 2002 and spread globally [2,8,24,28]. SARS posed a major challenge to healthcare facilities from both the impact of healthcare-associated transmission and the resource burden needed/required to control its spread. Healthcare personnel accounted for most of the cases, and hospital transmission accounted for 55% of all cases in Taiwan and 72% of cases in Toronto [2,8].

Data from a survey conducted in the U.S. to assess preparedness for emerging threats revealed that 1) infection control programs are limited in smaller and rural hospitals, and 2) the number of negative pressure rooms and other equipment may not be adequate in some hospitals, especially those in rural areas. These results show the importance of coordinating preparedness plans with other hospitals and municipalities to strengthen the capacity for meeting emerging infections and bioterrorism challenges. It is also very important to have communication among professionals in a healthcare facility and between healthcare facilities and local/state health departments.

Conclusion

In conclusion, despite many successes in the last 30 years, infection control and healthcare epidemiology programs face several challenges. The engagement of healthcare personnel and administrators is crucial since infection control programs should be supported by the administrative level, but infections are prevented at the patient level by healthcare personnel. We have to make infection control a fundamental part of everyone's job.

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Reuse of Single-Use Medical Devices

Susan Alpert, Ph.D., M.D.

Medtronic, Inc., U.S.A.

Introduction

Medical devices have been evolving over many decades, and during that time, demands from users/purchasers have varied widely. The device developer and manufacturer have attempted to meet the needs of its customers even as those needs evolved. Today, in the environment of cost control, facilities are looking at the reuse of disposable devices as one avenue to savings. Such potential savings must be weighed against the risk to patients and to the facility itself from liability since reuse of single-use devices is a risky practice that is neither mature nor sufficiently evaluated to date.

Background

In the 1960s and 1970s when labor was relatively plentiful and not expensive, durability and reusability was a focus of medical device design and manufacture. Devices were designed to withstand many cycles of use, cleaning and, predominately, steam sterilization. As materials evolved and the costs associated with cleaning and sterilization increased – especially the costs of personnel required to perform these activities – hospitals sought other alternatives. They turned to the device industry and asked for products that were disposable and made from materials that provided greater flexibility and utility. The medical device industry responded.

Beginning in the 1980s, biomaterials developers and the device industry worked hard to design materials and products that met these evolving customer needs and wants. The results of this effort are devices that are easy to use, low in cost, and require little to no maintenance. Changes in materials have allowed manufacturers to provide highly flexible, drug- or lubricant-coated devices that meet user needs and are then thrown away.

Products made for disposal after a single use can be made more simply, the coatings require less additives since they don't need to withstand cleaning, and the user can be sure that the product carries no risk of infectious disease transfer from patient to patient. Since it is not necessary to disassemble such products for cleaning, or to create channels that support sterilization, these products can be machine molded, have fewer parts, and can be economically manufactured. Because no reassembly is required, directions for use are simple and focus on patient care not on device care.

Nevertheless, to assure reliable performance of disposable products, these devices are designed to function in excess of their actual intended use. This assures that they will not fail during the initial use and will safely and reliably perform their assigned task. For example, a disposable biopsy device can cut tissue many times, not just once, since during the same procedure in a single patient it may be necessary to obtain multiple tissue samples.

Today, the pendulum has swung once more. Many sophisticated devices today are designed as disposables, made of new materials and coatings, and possess unique functional aspects. Given their sophistication, the cost of such products often seems high to the user. With pressure on containment of healthcare costs around the world, hospitals are looking for sources of potential savings and have focused on disposable medical products as one area where savings might be realized. Since these products function as well upon completion of their initial use as at the start, the question of savings from reuse of these devices was raised. Conceptually, it makes sense that if something works this well once it could continue to do so over repeated uses. Practically, however, many questions and problems arise with the reuse of single-use medical devices, few of which have yet been answered despite the widespread nature of this practice.

Single-Use Disposable Devices

From their conception, single-use medical devices are not like those designed for use on multiple patients. The goal of a single-use product is finite, short-duration functionality and disposal. There is no need to design for durability during multiple cycles of physical and chemical abuse as there would be for a truly reusable product. For the single-use disposable device, cost-effective materials with limited tolerance for harsh chemical cleaning agents are just right. Simple, solid state, often molded designs work well for disposables, while products intended for multiple cycles of cleaning and sterilization require designs with cleanable, accessible channels and compartments, or no channels. Nooks and crannies in general are not a problem for the disposable product, but if the product is intended to be reprocessed, these can lead to failure and contamination. Coatings sufficient for single use need not be applied in ways to prevent erosion from chemical cleaning and sterilization. The goal is to expose patients to just the right amount of coating or drug – the same for each use – with no need to focus on loss or additional chemicals to provide for durability beyond the initial use.

Economic issues are often sighted as the reason that facilities reprocess single-use medical devices. While this paper is not focused on this aspect of the issue, a few questions must be raised in this regard. Who actually is saving money as a result of reprocessing – patients, hospitals, the government? Are there actual, reproducible savings? In the United States (U.S.), reprocessors are charging approximately half of the cost of a new product for a reprocessed disposable device. The original cost of the disposable device reflects expenses incurred during research and development, manufacturing, and distribution. The costs associated with reprocessing reflect those of collection, cleaning, and sterilization. Do these activities really amount to one-half of the original costs of development and manufacturing/distribution? Reprocessors often argue that original equipment manufacturers (OEMs) fight the practice of reuse because they are losing revenue from additional sales of new products. Who actually is making the larger profit?

Patient Health and Safety Issues

There are a number of health and safety issues that must be addressed when considering reprocessing single-use disposable devices. Included in these issues are the potential contamination of the device with blood, tissue, or organisms from the preceding procedure(s); the spread of infection from a previous patient due to difficult and often inadequate cleaning and sterilization; and the risk of emboli, both infectious and sterile, due to material not adequately removed after previous patient exposure. In addition, the loss of device performance along with breakage and failure of the product to perform with repeated use are also significant potential problems.

Why are these significant risks greater for reprocessed single-use devices than for standard reusable products? To address this question requires an understanding of typical reprocessing procedures.

The important first step in reprocessing is a thorough cleaning of all device surfaces that potentially came in contact with the initial patient on whom it was used. Single-use disposable products are not designed for such cleaning, and their design features often make it difficult to thoroughly remove all debris from the preceding procedure. Oftentimes, the channels and compartments of single-use devices are not accessible or may require special tools to adequately clean due to their size or shape. As a result, residual contamination by tissue and/or blood from a previous patient after cleaning is a real possibility, as is the continued presence of bacterial, fungal, and/or viral organisms.

Sterilization is the next step in reprocessing. The standard sterilization procedures used by reprocessors have been developed and tested for effectiveness on clean device surfaces. Since single-use medical devices are not designed to be cleaned and re-sterilized, there is a real potential for foreign materials to collect in places inaccessible to these processes. Microorganisms present in debris located in difficult-to-access areas may not be killed by traditional sterilization procedures. Time, temperature and concentration of a sterilant are just a few examples of parameters in standard sterilization processes designed for relatively clean surfaces that are likely to be inadequate for assuring that any residual contaminating organisms have been killed. In fact, the variability and variation of contamination within or on previously used, disposable devices precludes the use of acceptable standards for cleaning and sterilization designed for reusable products as a means to assure sterility of the disposable device.

Testing of Reprocessed Single-Use Disposable Devices

Although there are few published studies on this issue, several independent laboratories have conducted tests of reprocessed disposable devices collected from hospital shelves (personal communications). These tests revealed that 20 to 40% of reprocessed products contained contaminating materials, and up to 70% in some series were non-sterile. Other series have demonstrated that some reprocessed single-use devices were missing components or had the wrong parts or components packaged with them. Directions for use contained in the packaging of reprocessed disposable devices were also found to be inaccurate. Errors of these types can lead to inappropriate use and patient injury.

Of great importance is the fact that the performance of a reprocessed disposable device may be significantly impacted by cleaning and sterilization processes – processes these products were not designed to tolerate. Edges that need to be sharp to perform appropriately may be dulled by such treatments. Dull edges on biopsy forceps, for example, can lead to ragged and difficult to remove biopsy tissue. In addition, the jaws of such reprocessed devices may not close adequately after reprocessing since durability with repeated use was not a necessary feature of its original design. Some disposable medical devices are coated to assure ease of use and patient comfort. Loss of the coating after cleaning and re-sterilization can lead to the need for increased force on insertion and patient discomfort. Electrical single-use products may not perform well after reprocessing due to loss of electrical integrity, prolonging a procedure when a non-functional product must be replaced, or leading to risk of sparks and fire in the procedure area.

It has been reported that the electrodes of some disposable cardiac mapping catheters have become displaced after reprocessing, leading to exposed wires, damage to heart tissue and valves, and lack of performance. Device mechanisms clogged with residual tissue and/or blood can lead to malfunctions, delaying procedures while failed products are replaced, and put patients at risk. For example, such failures have been reported with reprocessed disposable stapling devices. Single-use devices with unique characteristics such as harmonic blades may be rendered actually unsafe after being subjected to treatments to clean and sharpen them. Balloons used to dilate arteries and stents have been reported to rupture at high rates during normal use after reprocessing cycles, requiring additional procedures to retrieve materials and debris.

Difficulties in Reprocessing Single-Use Disposable Devices

Whether the reprocessor is an independent commercial entity or the healthcare facility itself, there are significant hurdles to accomplishing the goal of a clean and sterile device that is safe for use on the next patient. A recent publication in *Biomedical Instrumentation and Technology* outlined one process that the Cleveland Clinic has found to be useful in evaluating reprocessing of single-use disposables [1]. This facility looked at the issue from cost as well as effectiveness perspectives, and the lessons they highlight are well worth repeating here.

One point worth emphasizing is that not all single-use medical devices pose the same risks to the patient when reprocessed. An example cited of lower risk reprocessed device was blood pressure cuffs. Even here, however, the authors note that some patients such as those with non-intact skin may be at risk for complications following use of a reprocessed blood pressure cuff.

Another important point is the need to determine the number of times a single-use product may be reprocessed without detriment and still function properly. The types of testing for performance will differ from product to product, but such testing is critical before one commits to reprocessing. Sterilization testing of reprocessed disposable devices poses significant challenges, and each device or model must be evaluated to assure proper sterilization parameters.

These same authors note that studies published to date regarding reprocessing have not taken into account the costs of doing the preparative validations and testing to assure the cleaning and sterilization procedures are adequate and that products will continue to perform properly. Such evaluations must take into account the time and personnel required to perform the background work. In addition the care and difficulty required to implement appropriate reprocessing should also be considered in these costs.

Regulatory Issues

Questions have been raised around the world regarding the regulatory status of reprocessed single-use devices. Although the device itself has been allowed into the market legally for single use, the fact of the reprocessing creates a multiple use product. Is this now legally in the market? Who is the manufacturer? The original equipment manufacturer clearly has designated the product for single use only and does not have the objective intent that it be used on more than one individual. Who then takes responsibility for this product? Should the product now be considered legally in the market and under what conditions?

In some jurisdictions, the practice of reprocessing has been considered the purview of the original buyer. If this entity chooses to reprocess the product for another use, then in some areas of the world that is considered legal. If, alternatively, the ownership of the device is transferred to another entity, then it may not be legal for the reprocessed device to be used. In many cases, reprocessing is actually below the level of visibility of the regulatory agency or considered of such low risk as to raise no concern.

In contrast, other jurisdictions have proposed to ban the reprocessing of single-use disposable devices. In these locations concerns about the safety and performance of reprocessed products have raised sufficient question to lead to discussions focused on not allowing the practice to continue with government support. Injuries and reports of contamination have led to reconsideration of the effectiveness of reprocessing in these arenas.

Still other governments, like that in the U.S., have developed programs intended to legally allow reprocessed single-use devices to be introduced into use. In these areas, oversight has been extended from the original manufacturer and the single-use product, to address the reprocessing entity as the manufacturer of a “new” product. In these areas, reprocessors are subject to manufacturing controls as well as some form of pre-market evaluation. Such constructs are intended to provide a level of assurance that the reprocessed product is reasonably safe and effective for use. The adequacy of such programs is, however, yet to be established. No systematic evaluation of the impact of these programs has been performed.

Remaining Questions

How Safe is Reprocessing of Single-Use Devices?

Although the real and potential risks of reprocessing are becoming better defined, there is no body of literature that addresses this process. Reprocessors in the U.S. have identified a series of papers that they claim support the safe use of reprocessing. Examination of the publications, however, raises questions about their scientific validity. This body of publications was reviewed for an article prepared for the Association of Disposable Device Manufacturers [2]. The authors, who were independent experts, reviewed selected articles claiming to demonstrate safe use of single-use devices and found that most were not actually reports of experiments or case series that could be seriously considered to support the practice.

Are there Injuries Associated with this Practice?

As noted above, no body of literature or reports can determine if reprocessing leads to increased injuries. Given that the reprocessed single-use product almost always looks exactly like the product prior to its first use, visual inspection alone cannot determine if a product was in fact used and reprocessed. To make such a determination, detailed examinations of the device including microbiological, physical, and chemical testing would be required. Since such testing is not routinely performed, even on devices that have failed or have caused injury, it is not possible to determine the occurrence rate of injuries due to reprocessing of single-use devices.

How Should the Actual Impact of Reprocessing be Determined?

In order to accurately determine the impact of reprocessing on patient care, valid focused unbiased studies should be performed. Such studies could address the safety and effectiveness of the products and potential for injury, as well as address the issue of cost effectiveness. A major reason why this has yet to be done is that the reprocessor has not been required to actually demonstrate the clinical impact of the practice. The original equipment manufacturer would have no credibility in the conduct of such a study, and the practitioner as yet has had no reason to perform such a study. How such studies will be funded remains an impediment to their conduct.

Should Patients Know about this Practice?

In today's environment, patients play a much greater role in decision-making regarding their healthcare. They want to be involved not only in the selection of their healthcare provider but also in the selection of the type of intervention and specific procedures that will be used to treat many of their diseases. Given this background, there are many who

believe that patients have a right to know that products are being used in these procedures in a manner that is contrary to the directions provided by the original equipment manufacturer. This parallels their being informed when a pharmaceutical is being used off label in their treatment, a practice that has been evolving in medical care over the past decade.

How, when, and by whom patients should be informed about the reuse of single-use disposable products has begun to be discussed in a number of venues. Patient advocates, particularly those representing vulnerable populations and others, including the Center for Patient Advocacy in the U.S., believe that such information should be included in what a patient is told and agrees to during the informed consent processes for any procedure. To not do so they believe is unethical. Although not standard language in consent today, advocates advise patients who are concerned about reuse to add language about this practice to any consent form they sign and to raise this question with each of their healthcare providers. Whether this will be a standard part of patient education around the world is not at all clear.

What Should the Future Bring?

As in all questions of science, only when the questions raised in this paper are addressed by scientific study and where valid data are developed to support a conclusion of safety and product performance should the reuse of single-use disposable devices be accepted by patients and their healthcare providers.

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New Directions in Aseptic Processing

Rainer F. Newman, M.S.

Johnson & Johnson, U.S.A.

Definition

Aseptic processing in the pharmaceutical, medical device, and diagnostic industries is the practice of bringing together technologies and processes that ensure that manufacturing and packaging steps prevent sterilized components from being contaminated with microorganisms.

Some of the key elements of this practice consist of appropriate facilities, utilities, equipment, and procedures all designed to maintain product sterility. Facilities and utilities include clean rooms or critical zones that are supplied with air via High Efficiency Particulate Air (HEPA) filters, Water for Injection (WFI), sterile process gases, and other systems required for manufacturing. Equipment, including laminar flow hoods (LFH), liquid or powder fillers, tanks, lyophilizers and more, as well as the activities required to process the sterile components, are all designed to handle, transport, fill, assemble, or package sterile articles while preventing their contamination.

By regulatory mandate, terminal sterilization must be the first approach to sterilizing those drugs and devices required to be sterile. Sterile products must be evaluated for their ability to undergo terminal sterilization before aseptic processing can be considered. Many of today's drugs and some devices are not able to undergo terminal sterilization without undesirable consequences. Terminal sterilization by chemical, heat, or radiation exposure will render many drugs and some devices unusable. Products can degrade forming undesirable by-products, they can lose potency and efficacy or otherwise change their pharmacological or functional characteristics through exposure to terminal sterilization processes. In that event, aseptic processing is the only alternative.

It is important to keep in mind that aseptic processing is not a sterilization method but a means of accomplishing all the manufacturing steps while preventing contamination.

Background and Current Practices

Aseptic processing has seen numerous improvements in the past 30 years. These improvements have come through changes in the regulatory environment, in equipment design, and in the practices and procedures employed in aseptic processing. The continual improvement efforts have resulted in today's state-of-the-art aseptic processing capability that delivers products with very high reliability of sterility and safety.

In the United States (U.S.), the Food and Drug Administration (FDA) promulgated the current Good Manufacturing Practices (GMPs) during the 1970s [6]. The Aseptic Processing Guideline was issued in 1987 and is currently undergoing revision by the FDA [3,4]. While it may appear that little regulatory activity impacting aseptic processing has occurred in recent decades, that is hardly the case. Regulatory approaches and best aseptic practices have been discussed and written about at length, and perhaps most effectively, became a key focus of inspections. Further, the industry and its equipment suppliers have, for sound economic and safety reasons, consistently raised the aseptic processing technology bar ever higher. Advances in equipment and practices quickly spread throughout the industry by 3 main mechanisms.

- Industry and regulatory employees are highly mobile and move freely and frequently within the aseptic processing arena allowing new ideas and best practices to spread rapidly from one organization to another.
- Industrial and professional organizations such as the International Society for Pharmaceutical Engineering (ISPE) and the Parenteral Drug Association (now just PDA) have had significant impact. Their activities, include publishing, teaching, and serving as technical experts, are widely recognized.
- Regulatory activities, in particular inspections, observation reporting, and recall actions have similarly been effective means of spreading the technology.

"Harmonization" is a term applied to the international effort to align medicinal regulations across borders. Many medical device and drug regulations continue to be subjected to harmonization efforts. Differences remain among the various regulatory agency rules and guidelines, but the differences tend to be in details rather than in basic aseptic processing concepts.

The various pieces of equipment used in the conduct of aseptic processing have significantly changed over the years. In the early days of drug manufacturing, equipment such as liquid fillers were essentially borrowed from related industries, for example the dairy and brewing industries. In early recognition of their critical nature, these devices were usually sanitized, and some components were steam sterilized.

Modern aseptic processing equipment has been specifically designed with attributes that simplify cleaning and sterilization and allow the process to be conducted with minimal potential for contamination. Every aspect of the aseptic manufacturing process has been redesigned and purpose-built. Virtually, every nut and bolt, as well as entire systems were improved.

Some of the key equipment improvements included designs to:

- **Minimize the number of parts** – Fewer parts make it easier to clean and sterilize them. It also reduces the likelihood of incorrectly reassembling key components.
- **Minimize or entirely eliminate the need for changeover** – While minimizing the number of steps can help speed changeover, specific designs to eliminate changeovers altogether vastly improves the process, and further minimizes the potential for contamination.
- **Relocate moving parts and other components from the critical (product exposure) zones** – Moving the so called “dirty” parts such as motors, other moving parts, and service components outside the critical zones, thus exposing only the necessary interface connections or functions within those zones, allows maintenance and instrument calibration operations to occur without potentially affecting the product or the aseptic process.
- **Implement sterilizable parts and surfaces** – The ability to sterilize all product contact surfaces of tanks, valves, lines, tubing, and filling equipment greatly improves the chances for a successful aseptic process.
- **Automate** – Eliminating manual intervention is key. An example of a common intervention is the weight checking of vials. This is a process that is routinely performed during sterile drug filling and requires an operator to intervene in the process to obtain samples. With automated weight-checking, operator intervention is eliminated as is the associated contamination risk.

Adjuncts to aseptic processing equipment that have gained wide acceptance in recent years include clean-in-place (CIP) and sterilize-in-place (SIP) systems. CIP and SIP equipment and control systems are designed to clean and sterilize product contact surfaces in a highly reproducible (validatable) manner without the potential for contamination due to operator intervention. Facilities and equipment are designed with surfaces that are easily cleaned, disinfected, or sterilized. This is accomplished through the use of hard, non-porous surfaces that minimize the potential for bacterial build-up and resist degradation from the various agents used to clean, sanitize, or sterilize.

In addition to the direct processing equipment improvements, equipment for transporting and transferring materials in an aseptic manner have been implemented and improved. Among these are the laminar flow carts and rapid transfer ports (RTPs). These devices allow the movement of sterile components from one system and location to another through means of a protective environment such as HEPA-filtered laminar flow air showers or by providing a contiguous aseptic pathway as in the case of RTPs.

Improvements in operator equipment, particularly gowning designs and materials, have resulted in greater operator comfort and ease of movement. It is difficult to maintain good aseptic technique in a hot and uncomfortable environment with goggles that fog and where movement is hampered. Protection from operator contamination has significantly improved through the use of modern, full-body gowns and ancillary components such as face masks designed to cover 100% of operator-exposed areas. The “bunny suit” has been around for many years, and while full coverage from head to foot has been the norm, it is only recently that full face covering has been employed. This was enabled by the introduction of materials

and designs for goggles that prevent fogging and permit better visibility.

While improvements to gowning have continued, a simultaneous effort has been to preclude the human operator from the critical zones altogether. The concept of a separation barrier began with flexible plastic curtains that surrounded the sides of product exposure zones. However, normal processing still required periodic intervention by operators for adjustments and samples and to reload glassware and stoppers. These interventions often occurred while the process (e.g., filling) continued. It took newer equipment designs such as limited access barriers and isolators to minimize and eliminate these interventions. Limited access barriers were introduced and are typically constructed of glass or rigid clear plastic-hinged doors that completely surround the process sides. Most barrier systems have the additional feature of being interlocked such that when a door is opened the operation stops and alarms are raised.

Currently, the most evolved protection scheme is the isolator system. Borrowing from technologies that use “glove boxes” to protect the operator from dangers such as radioactive or toxic materials, aseptic equipment designers have reversed the concept and began to develop isolators that protect the sterile products from contamination by the operator. This technology has gained widespread application in the sterility testing laboratory. The use of isolators in production settings such as for vial filling has been adopted at a slower rate. It is difficult to retrofit an existing traditional facility to isolation technology and as a result adoption of this technology is currently largely limited to new facilities.

Component Sterilization

Since aseptic processing is not a sterilization method, the product and the primary container components of a sterile article will have had to be sterilized at a prior step in the manufacturing process. The sterilization of the components is typically achieved through fairly standard approaches. Some common product and component types include:

- **Fluids** – Gases, liquids, and even some creams and ointments are sterile filtered. This is accomplished through the use of filter systems with a nominal pore size of 0.22 µm, usually called sterilizing-grade filters. A sterilizing-grade filter is so designated because it produces a sterile effluent when challenged with at least 10^7 cells/cm^2 of filter surface area using a suspension of the microorganism, *Brevundimonas diminuta.*
- **Glass** – Container components such as glass vials, ampoules, and syringes are typically sterilized and depyrogenated through the use of dry heat at temperatures above 170°C. Today's equipment uses significantly higher temperatures, near 300°C. The process employs ovens or in the more current technology, containers continuously moving within a depyrogenation tunnel.
- **Elastomers** – Other containers components such as stoppers and syringe plungers are usually steam sterilized.
- **Process Equipment** – Vessels such as reactors, tanks, pressure containers, and the associated piping and other equipment is also typically sterilized by steam. SIP is sometimes called steam in place and is becoming the current practice in many applications
- **Plastics** – Plastic components including filters and tubing are sterilized by a variety of methods depending on the polymers and the intended use. Steam and ethylene oxide, as well as gamma and e-beam irradiation, can all be the method of choice.

Safety & Sterility

Since it is both desirable and required to use terminal sterilization wherever possible, we must ask the question, “Does the alternative, aseptic processing, provide a sterile product?” Terminal sterilization is preferred because it is considered the process with higher reproducibility and better control. Aseptic processing consists of many steps, with the resultant potential for variability. For many years, both regulators and the industry have recognized the need for process control and reproducibility. In modern pharmaceutical and medical device manufacturing operations, aseptic processing is highly managed, controlled, and validated to insure reproducibility and efficacy.

Using FDA data related to aseptic processing we can evaluate some aspects of the process’ capability as a method of delivery for sterile product. It is certainly true that in the U.S. and other countries, aseptic processing of medical products is a highly scrutinized and much considered subject. With the advent of modern concepts of validation and GMPs, indirect indicators of sterility concerns have been applied. Among these indicators is the so called “Lack of Assurance of Sterility” (LAS) findings that are used to identify conditions and processes that may not meet the highest standards of validation and control, and may, as a consequence, have an impact on sterility.

For a number of years, the FDA has reported and ranked the reasons for product recalls [2]. LAS has consistently been among the top 10 reasons and in 2000, the FDA reported that LAS was the No. 1 reason for recalls during that fiscal year. It must be remembered, however, that this ranking and classification is often related to deviations in documentation, to insufficient validation data, or to other issues not necessarily directly related to sterility. Some prefer to call these “paper issues”. LAS is not the same as, nor does it directly imply, “contaminated” or “non-sterile”.

The FDA recall data referred to above indicates that 63 LAS drug recalls occurred between 1996 and 2002. These data represent sterile drug manufacturers only, and do not include devices, certain biologics such as blood products, or recalls incurred by third party processing. Of these 63 recalls, 3 were drugs suspected of being contaminated, 1 of which was a contaminated terminally-sterilized product. It is not entirely clear from the available documentation, but the likely scenario for the remaining 2 drug recalls was that they failed the initial sterility test, were retested and passed, and then subsequently were released by the manufacturer. In the FDA’s view, the retest was unjustified, thus the recall.

With the millions and millions of doses of drugs produced aseptically every year and the low incidence of actual contamination, we can conclude that aseptic processing has become a highly reliable and safe method of delivering a sterile product.

New Directions

It is fortunate that aseptic processing has been so successful. Had it not, many new drugs and devices might never have become available, since so many need to be sterile but can't be terminally sterilized. There are currently many new directions that impact aseptic processing and growth in volume, new products, and new technology have all had a direct impact.

Through an informal assessment of the volumes involved we have estimated the annual worldwide output of sterile drugs to be close to a billion (10^9) units. If we only consider therapeutic sterile pharmaceuticals and biopharmaceuticals, the number would still be several hundred million units.

One estimate suggests that about 85% of all sterile pharmaceuticals are produced aseptically [1]. Medical devices are most frequently terminally sterilized, although aseptic processing is playing an ever increasing role as medical devices are increasingly incorporating a biological component.

The newer drug forms are almost exclusively produced by aseptic processing and this class is estimated to grow at an annual rate that could exceed 15% through the year 2010 [5]. Growth will come both in the form of additional units of existing products and through the development and introduction of new classes of products that require the technology. Drugs and devices that require aseptic processing will include:

- Large molecule drugs
- Novel drug delivery systems
- New medical devices
- Drug / device combinations
- Engineered biological tissue

In order to sustain the growth, and indeed to ensure that these medical articles become a reality as sterile products, aseptic processing will need to be applied in new and different ways, perhaps in ways not yet defined. Some of the advances that will enable new applications will be:

- Improved, more efficient, more cost effective, and smaller isolator systems
- Effective use of automation to fully eliminate variability and human intervention
- Highly integrated systems to ensure process control and reliability
- Better alignment of technology and regulation
- Possible adjunct processes to reduce pre-processing bioburden

Current and Emerging Applications

New platforms that will require aseptic processing are being developed, seemingly daily. Some key examples include:

- **Liposomes** – These are lipid-complexed formulations used to deliver drugs, both old

and new, in a novel way. They may provide sustained release, target a specific organ or tumor site, or reduce toxicity. Liposomal formulations are typically not terminally sterilizable since the lipid envelope would denature or disassociate under such conditions. In many instances the lipid complex is also not sterilizable through filtration, which means that the sterilization step must be conducted even earlier in the process, before the lipid envelope is formed. The challenge to aseptic processing under these conditions can be significant.

- **Biopharmaceuticals** – Like liposomes, protein or monoclonal antibody-sourced drugs are typically not terminally sterilized for similar reasons. Additionally, many of these formulations are not stable in the liquid solution form and require lyophilization to remove water as a means of providing stability.
- **Drug / Device Combinations** – Combination products fall into 1 of 2 general categories, those where both the drug and the device have a medical function, and those where the device is simply a drug-delivery system. Examples of the former category include drug-coated stents and drug-loaded tissue structures such as collagen fleeces, while examples for the latter category include implantable osmotic pumps to deliver extended release dosing of drugs and the intra-dermal patches where the drug is loaded onto micro needles.
- **Engineered Tissue Devices** – Many new medical devices include any of a number of biological tissues, both from human and animal sources. Human bone as well as eye, connective, cardiovascular, and skin tissues are all being used in medical device technology. Even nonhuman, allograft tissue such as bovine bone, porcine valves, and skin are common. It is interesting to note that amphibian, reptilian, and avian tissues were used as artificial skin since ancient times. The new tissue-based devices are processed aseptically.

Summary

There is an ever growing list of exciting and unique, and in many cases life-altering, even life-saving, biopharmaceuticals and medical devices that will continue to challenge aseptic processing and will continuously drive it in new directions. Most of these new medical products will require sterility as one characteristic, but most will not be able to be terminally sterilized, making aseptic processing a key element to the success of bringing these products to patients around the world.

It is fortunate that modern aseptic processing technology is a safe and effective means of producing sterile products. Were it not so, many highly effective and critical products might never be available.

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Climate Instability and Public Health

Paul R. Epstein, M.D., M.P.H.

Center for Health and the Global Environment, Harvard Medical School, U.S.A.

Introduction

Today, the overuse of resources and generation of wastes, widening social inequities, and changes in ecologic systems and climate are conspiring to unleash a barrage of emerging diseases that afflict humans, livestock, wildlife, marine organisms, and the very habitat that we depend upon. According to the World Health Organization, previously unknown diseases have surfaced over the last three decades at a pace without precedence in the annals of medicine, and have included HIV, Ebola, Legionnaires', and Lyme disease [24]. Older diseases such as malaria, cholera, tuberculosis, and dengue fever are showing a resurgence, while others such as West Nile virus have undergone redistribution.

As the climate becomes more unstable, its role in the spread of infectious diseases increases [3]. Weather and climate can influence host defenses, vectors, pathogens, and habitats. Climate influences the range of infectious diseases, while weather affects the timing and intensity of outbreaks. Extreme weather events such as flooding and droughts are becoming more intense and are likely to become more frequent as the world climate changes [1,11]. Thus, it is important to understand the impact of these trends on infectious disease patterns with the ultimate goal of predicting those weather and climate conditions that contribute to the spread of specific diseases, which will enable the development of policies for prevention.

Climate Change and Stability

For the past 420,000 years, as measured by the Vostock ice core in Antarctica, atmospheric CO_2 levels (which parallel average global temperatures) have remained steady within the range of 180 and 280 parts per million (ppm) [19]. Today, however, CO_2 levels in the troposphere are close to 370 ppm and rising [1,11]. The accumulation of CO_2 and other heat-trapping greenhouse gases in the lower atmosphere has altered the atmospheric heat budget, world ocean and land surface temperatures, and the cyrosphere (ice cover), and is associated with the observed global warming trend observed for this century [17].

Warming this century has been rapid but is not occurring uniformly. While the overall rate of maximum temperature increase since 1950 is approximately 1°C per century, minimum (nighttime and winter) temperatures have increased at a rate of approximately 2°C per century [7], and winter warming is occurring faster near the poles than at the tropics [1]. Temperatures within the Artic Circle have warmed 5.5°C over the 30 years, and since 1950, seasonal fluctuations in the northern hemisphere are such that spring arrives earlier and fall appears later. These changes are already affecting species distribution worldwide [5].

A warmer atmosphere holds more water vapor (6% for each 1°C), which in turn insulates escaping heat and enhances greenhouse warming. As heat builds up in the deep ocean, more water evaporates and sea ice melts [1,11]. More evaporation also fuels intense, tropical-like downpours, while warming and parching of the Earth's surface intensifies the pressure gradients that draw in winds and large weather systems [22]. Over the last 100 years, droughts have lasted longer and heavy rainfall of more than 5 cm/day (>2 in/day) have become more frequent [13], and these weather patterns have been especially punishing for developing nations. No nation, however, is immune and in the late 1990s and early 21st century extreme weather events have also increased significantly in the Northern Hemisphere, especially in the United States (U.S.) and Europe (unpublished data).

As seen with global temperature fluctuations, there is considerable variability in the observed changes in temperature gradients of the ocean. Although a warming trend has been seen for the ocean as a whole, the northern Atlantic Ocean has cooled in the past several decades. Possible explanations for this observation include melting and thinning of Arctic and Greenland ice and increased rain falling at high latitudes. The cold, freshened waters of the northern Atlantic accelerate transatlantic winds, and this may be one factor driving the frigid fronts down the eastern U.S. seaboard and across to Europe in recent winters. Growing temperature contrasts between cold poles and warm tropics also generate windstorms, such as the winds that raced across the Atlantic in late 1999 and destroyed much of the forests in France.

The El Niño/Southern Oscillation (ENSO) is one of the Earth's ocean-atmospheric systems that has helped to stabilize our climate by undulating between states every 4 to 5 years. ENSO events are associated with weather anomalies especially droughts and floods [15]. Between 1976 and 1998, the pace, intensity, and duration of ENSO events quickened, and extremes are becoming more pronounced [3]. But extremes, in general, have become

more intense, independent of the ENSO cycle. Recent extreme weather events have included multi-billion dollar disasters such as Hurricane Mitch in 1998; Mozambique floods in 2000; the summer 2003 heat waves (~35,000 attributable deaths), crop failures, and wildfires in Europe; and the summer 2003 drought, bark beetle infestation, and wildfires in the U.S. and Canada.

Climate Destabilization and Infectious Disease Patterns

Warming nights and winters, along with intensification of extreme weather events, have begun to alter – among other things – the ecological connections (e.g., relations among competitors, predators, and prey) that help regulate the populations of opportunistic vectors and animal reservoirs of infectious diseases.

Warming, itself, affects the survival and distribution of pests and pathogens. Northward movement of ticks in Sweden as winters warm have been reported and is associated with an increased incidence of tick-borne encephalitis [16].

Mosquitoes are also appearing in mountainous regions where plant communities and freezing levels have shifted upwards and glaciers are retreating [9]. Once limited by temperature to about 1,000 meters in elevation, *Aedes aegypti*, which transmits both yellow and dengue fever, have recently been found at considerably higher elevations in Mexico and the Colombian Andes [21]. Malaria, carried by *Anopheline* mosquitoes, is circulating in highland areas in Kenya [20] and Papua, New Guinea [18]. While excessive heat is fatal to mosquitoes, within their survivable range, warmer temperatures increase reproduction and biting activity as well as the rate at which pathogens mature within them. Small outbreaks of *locally-transmitted* malaria have occurred during hot spells in several U.S. states including Texas, Georgia, Florida, Michigan, and New York [25], dengue fever has spread into far northern regions of Australia [10].

Extreme weather events also provide favorable conditions for clusters of vector-, rodent-, and water-borne diseases [7]. Flash floods leave behind mosquito breeding grounds, drive rodents from burrows, and seed waterways with microorganisms such as Cryptosporidium and *Vibrio cholerae*. Woodruff *et al.* [23] reported a strong association between heavy rainfall and outbreaks of Ross River virus disease in Australia, which is spread by the mosquito *Culex australicus*. In 1998, Hurricane Mitch – nourished by a warm Caribbean – stalled over Central America where it dumped 11 inches of precipitation over 3 days. In its aftermath, 30,000 cases of cholera, 30,000 cases of malaria, and 1,000 cases of dengue fever were reported in Honduras.

Droughts are also conducive for the spread of infectious diseases. In 1998, bats bearing Nipah virus swept onto Malaysian pig farms after fleeing forest fires fueled by the intense drought associated with the largest El Niño event of the century. (The fires affected fruit-bearing trees on which the fruit bats feed.) As a result, Nipah virus killed more than 100 people and crippled the swine industry [12]. The alterations in predation pressure, food supply, and habitat provoked by sequential weather extremes of drought followed by intense rain worked together synergistically to release rodent vectors reservoir hosts and can amplify viral transmission, and such a sequence resulted in a localized outbreak of hantavirus pulmonary syndrome in the southwestern U.S. in the 1990s [6].

There is considerable evidence that mild winters, coupled with prolonged droughts and heat waves, favor the amplification of West Nile virus. This disease made its explosive debut in New York City during the prolonged spring drought and heat wave of 1999 [8], and spread across the nation during the hot, dry summer of 2002. The experience with West Nile virus is reminiscent of that of St. Louis encephalitis (SLE), both of which share the

common carrier, *Culex pipiens*, and have common life cycles. From 1933 to the mid-1970s, 10 of the 12 urban SLE outbreaks in the mid-west U.S. were associated with 2 months of drought; after the mid-1970s outbreaks were associated with anomalous weather conditions that included both drought and heavy rain [8]. In addition, all of the major outbreaks of West Nile virus in Europe were associated with droughts and heatwaves.

Crops and habitat are also subjected to the dual threats of climate change and emerging infectious diseases. Cassava mosaic virus, one of the family of gem-iniviruses transmitted by white flies [2], has caused enormous losses of cassava (manioc, yucca, or tapioca) in sub-Saharan Africa where it is a staple in the diet of millions. In Alaska, spruce bark beetles have denuded 4 million acres of conifers on the Kenai Peninsula as warming allows the beetles time for an extra generation each year [14]. Oaks in New Orleans are bristling with termites as killing frosts have become less frequent [4], and New England hemlock trees are under assault from the woolly adelgid, an aphid-like insect that has migrated northward with warmer winters (D. Foster, Harvard Forest, personal communication, 2002).

No aspect of our environment is immune to the adverse impact of changing climate conditions. High sea surface temperatures have caused widespread bleaching of corals, threatening the integrity and longevity of these coastal buffers and ancient nursery habitats for numerous marine species and birds.

Forecast

Our capacity for long-term weather forecasting has greatly improved in recent years with monitoring of Pacific Sea surface temperatures and the state of the North Atlantic Ocean and the North Atlantic Oscillation or NAO. Integrating health surveillance into long-term terrestrial and marine monitoring programs can help to anticipate conditions conducive to future infectious disease epidemics. For example, anticipating the health risks posed by the extreme weather conditions facing the U.S. East Coast in the summer of 1999 could have resulted in enhanced mosquito surveillance, heightened sensitivity to avian mortality, and selective treatment of mosquito breeding sites. Each of these actions would likely have reduced the incidence of West Nile virus infection and ensuing disease. Greater collaboration among wildlife, insect, human health, and climate specialists can help generate early warning systems designed to limit the spread of infectious diseases occurring in response to changing climatic patterns.

But epidemiologic monitoring of infectious diseases may have even more fundamental utility. Just as we have underestimated the rate at which climate would change [11], so too we have underestimated the sensitivity of biological systems to this change [14,23]. Volatility of infectious diseases may be one of the earliest biological expressions of climate instability, and close monitoring of the spread of infectious diseases may prove helpful in catalyzing timely, environmental-friendly policies designed to stabilize the climate system, and generate jobs and enterprises that can drive a healthier, cleaner, more equitable and sustainable development.

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Discussion

Infection Control: Pacific Rim & Emerging Issues

Question for Dr. Alpert, Medtronic Inc., U.S.A.: The reuse of single-use hemodialyzers has been established in the United States. How prevalent is the practice of hemodialysis instrument reuse?

Answer by Dr. Alpert: Hemodialyzer reuse is a little bit different. I was at the FDA when that issue came up. In hemodialysis, the hemodialyzer is used within a single patient. What was learned is that those products can be properly managed and tested to assure that they remain efficient use after use. But they do have to be handled properly, managed properly, and monitored to assure that the patient will continue to get the benefit of dialysis. Actually, these are the kinds of things that are needed for the reuse of single-use devices – validation of the methodologies and good studies of the impact.

Question for Dr. Alpert: Shouldn't the practice of reprocessing be one of the activities reviewed in the accreditation process?

Answer by Dr. Alpert: There are many issues that are problematic in the reuse of single-use devices. The one that is most troublesome is that work in this area has not been done. There are probably many single-use devices that can be effectively reprocessed, but the procedures have not been well established. Furthermore, there is no good accreditation of the re-processors around the world, so I think we still have some work to do to establish this as a safe process. As I mentioned previously, we are currently reusing single-use devices at great risk.

Question for Dr. Kang, Catholic University of Korea, Korea: Regarding the high incidence of measles in Korea during 2000, did a particular resistance to the vaccine contribute to the outbreak?

Answer by Dr. Kang: I think that the measles outbreak was due to vaccine failure because most people were only vaccinated once in early childhood.

Question for Dr. Epstein, Harvard Medical School, U.S.A.: Will the next couple of decades see great change in fresh water availability that will affect human health?

Answer by Dr. Epstein: This is certainly a major question. There are many issues driving the problems of availability, accessibility, quantity, and quality of water, including overuse of ground water and surface water. We see changes in the water cycle that are affecting the timing, intensity, and geographic distribution of precipitation, both in winter and summer, and these can exacerbate fresh water shortages. I always like to end with good news, because I do believe that this problem of water can be a driver and one of the key

levers towards moving us to clean energy. Solar panels are being used today in many countries to purify water, pump water, and irrigate lands, as well as to light up clinics and homes and cook food.

Question for Dr. Cardo, Centers for Disease Control and Prevention, U.S.A.: In the outbreak of SARS in Toronto, several healthcare workers who took precautions became infected. Was the source of the infection identified?

Answer by Dr. Cardo: They are still looking at the data to better understand why healthcare workers who were exposed became infected, especially when the exposure was during the intubation procedure and they were wearing personal protective equipment. There are several theories to explain why they became infected. One is that their respiratory protection was not good enough. The other potential reason is that it was possible that there was a major contamination of the environment around where the procedure was done and people became infected through touching surfaces. We know there are special procedures that may be associated with a higher risk of transmission of SARS and for those procedures we need to be even more careful in the way we wear respiratory protection and also the way we clean the environment.

Session IV

Viral Hepatitis in Asia

Chairman: **Shunji Mishiro, M.D., Ph.D.**
Toshiba General Hospital, Japan

Viral Hepatitis in the 21st Century

Stanley M. Lemon, M.D.

The University of Texas Medical Branch at Galveston, U.S.A.

Overview of the Hepatitis Viruses A to E

Five very different viruses comprise the etiologic agents most often responsible for acute or chronic viral hepatitis in humans. Each belongs to a different taxonomic virus family with a distinctive genome organization and unique molecular replication strategy. The viruses share only a common tropism for the liver, with the hepatocyte representing the dominant site of viral replication, and either acute hepatitis or the consequences of chronic hepatic infection (fibrosis, cirrhosis, and hepatocellular carcinoma) representing the major clinical manifestations of infection. With one notable exception, the shared hepatitis B surface antigen (HBsAg) specificities of hepatitis B virus (HBV) and hepatitis D virus (HDV), these viruses are antigenically distinct and infection with one does not confer protection against another. In general, infections with the hepatitis viruses are limited to humans and perhaps a few higher primates. There may be a zoonotic component to hepatitis E virus (HEV) infections, however, as viruses very similar to those found in man are prevalent in domestic swine populations.

Hepatitis viruses possess distinct but overlapping epidemiologic characteristics that allow them to be categorized into 2 major groups that correlate well with the presence or absence of a viral envelope, a fundamental aspect of viral structure (Figure 1). Thus, those viruses that lack a lipid-containing outer envelope, namely hepatitis A virus (HAV) and HEV, share a number of clinical and epidemiologic features that distinguish them from the enveloped viruses, HBV, HDV, and hepatitis C virus (HCV). The absence of a lipid envelope in HAV and HEV confers stability on these viruses when they are secreted from infected hepatocytes into the bile, allowing rapid egress of virus to the intestinal tract and efficient spread via feces into the environment. This virus excretion pattern explains why these viruses are particular problems in developing regions of the world with inadequate public health sanitation. It also explains why common source outbreaks of hepatitis A and E occur.

In contrast to HAV and HEV, the other 3 hepatitis viruses, HBV, HCV, and HDV, possess lipid envelopes and therefore are likely to be rapidly inactivated by bile salts if they were secreted into the biliary canaliculi from infected hepatocytes. These viruses are not found in feces as infectious particles in biologically significant quantities, and their transmission is dependent upon several other routes, most often involving virus shed from a mucosal surface or by direct percutaneous exposure to blood. Common source outbreaks of these viruses are exceptionally uncommon, and typically related to contamination of blood products. In contrast to HAV and HEV, each of these 3 enveloped viruses is capable of causing long-term, persistent infection, and each has been shown to be an important cause of chronic viral hepatitis and cirrhosis. Both HBV and HCV have evolved mechanisms that promote their persistence in infected hosts. Virus persistence over decades or more increases the probability of transmission to a new host, and may be critical to survival of these particular viruses within populations. HCV appears most sophisticated in this respect, having evolved several distinct mechanisms that appear to either block the induction or mitigate the effects of innate cellular antiviral defenses.

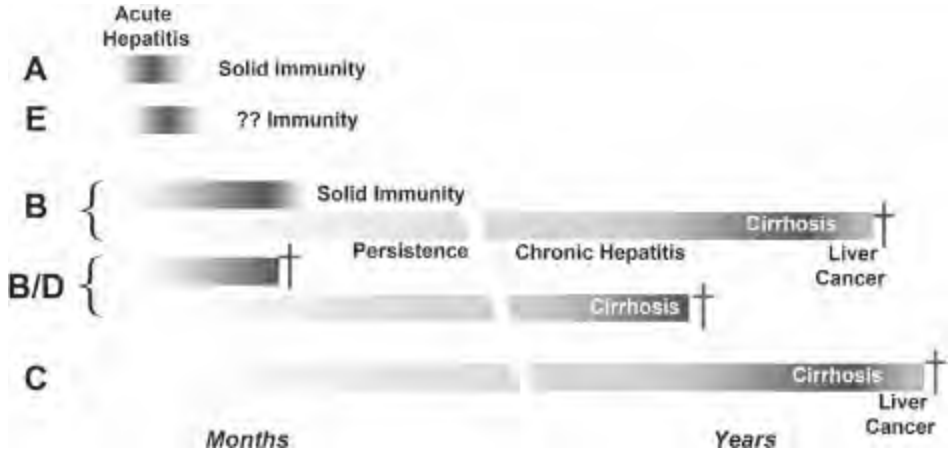

Figure 1. The spectrum of viral hepatitis. Hepatitis A and E infections do not become persistent and are associated only with acute hepatitis, while hepatitis B and C infections may become persistent and are associated also with chronic hepatitis, cirrhosis and hepatocellular carcinoma. Hepatitis D virus can only infect persons who are already infected with HBV, and is generally associated with more severe liver disease.

Disease Burden Associated with Hepatitis Virus Infections

Viral hepatitis accounts for a considerable burden of disease worldwide. Within the United States (U.S.) alone, about 16,000 cases of acute hepatitis due to HAV, HBV, and HCV were reported to the Centers for Disease Control and Prevention during 2003. This represents a substantial reduction from the numbers of cases (of all types) reported a decade ago. These numbers represent only a fraction of all cases, however, because less than 1 out of every 5 to 10 cases appears likely to be reported to public health authorities. Fulminant hepatic failure and death occur in a small proportion of patients with acute hepatitis A or B (the latter much more likely with coincident HDV infection), but are rarely associated with acute HCV infection in the U.S. [22].

Although acute viral hepatitis is often a dramatic illness, the major disease burden associated with hepatitis virus infections stems from the development of cirrhosis, liver failure, and hepatocellular carcinoma in individuals with long-term persistence of HBV (with or without superinfection with HDV) or HCV. Between 50 to 85% of persons who are initially infected with HCV fail to clear the virus and resolve the infection. Most eventually develop at least chemical and histologic evidence of chronic liver injury [5], even though the large majority of these individuals remain free of symptoms for years. HBV has an intermediate tendency to establish persistence. Unlike HCV, which has clearly evolved efficient mechanisms allowing it to persist in most healthy individuals despite a relatively robust cellular immune response, persistent HBV infection is largely confined to those who are immunologically impaired, such as the neonate, the cancer patient, or the individual infected with human immunodeficiency virus (HIV).

In most persons, chronic infections with either of these viruses are well tolerated, with relatively little evidence of disease save elevated serum activities of liver-derived alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes. However, like the tip of an iceberg that floats above water, a small proportion of those who are infected go on to develop life-threatening liver failure or malignancy. There is little understanding of what selects these unfortunate individuals for either the insidiously progressive fibrotic reaction that characterizes the development of hepatic cirrhosis, or the malignant transformation of infected hepatocytes that leads to hepatocellular carcinoma (Figure 2). Nonetheless, within the U.S. today, it is estimated that approximately 10,000 of the ~3,000,000 persons who are currently infected with HCV succumb each year to liver disease, usually after decades of infection. Most die of liver failure and cirrhosis, while a much smaller fraction die as a result of liver cancer. Interestingly, for reasons that remain obscure, these proportions are reversed in Japan, where most HCV-related deaths appear to be due to liver cancer and a much smaller fraction to cirrhosis and liver failure.

Viruses with Disease Burden Related Primarily to Acute Hepatitis

Hepatitis A

Clinical Virology

HAV is a positive-sense, single-stranded RNA virus classified within the genus *Hepatovirus* of the family *Picornaviridae* [35]. The virus shows relatively little genetic variation, and little antigenic diversity. Unlike other hepatitis viruses, HAV can be propagated with relative ease in conventional cell cultures, although wild-type virus typically replicates poorly. Several cell culture-adapted HAV variants have been shown to be highly attenuated in their ability to cause disease in otherwise susceptible primates, and have been used for production of both killed (formalin-inactivated) and candidate live (attenuated) HAV vaccines. These vaccines are very safe, and highly effective, providing protection that appears to be based primarily on the presence of serum neutralizing antibody.

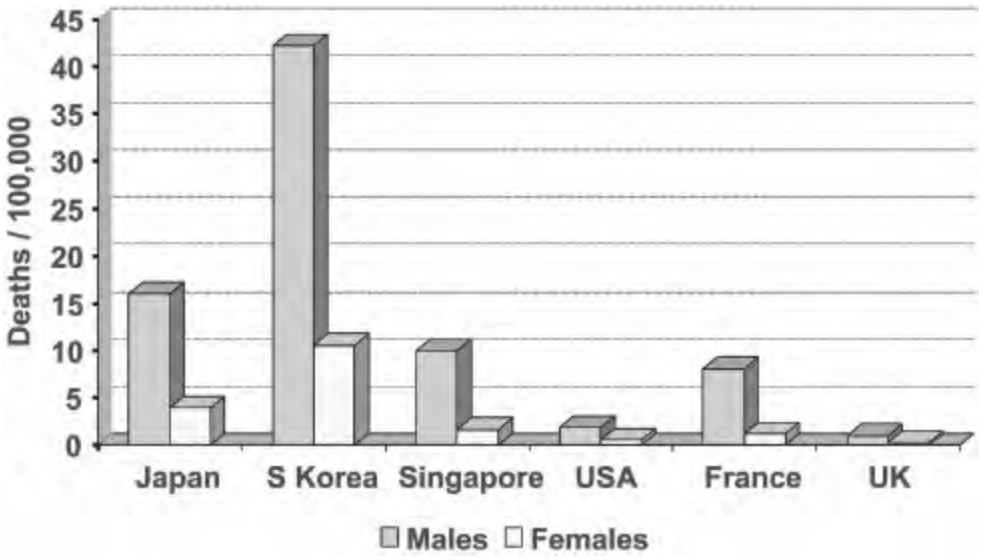

Figure 2. Mortality rates from primary liver cancer, 1994. Chronic infections with HBV and HCV contribute to the increased risk of death due to hepatocellular carcinoma in many countries, particularly in Asia. Data from the World Health Organization Databank.

Epidemiology and Disease Emergence

Infection usually occurs by the fecal-oral route of transmission, and is associated with extensive shedding of the virus in feces during the 3- to 6-week incubation period and extending into the early days of the illness [31]. Consistent with this mode of transmission, the prevalence of anti-HAV is clearly related to age as well as socioeconomic factors. The age-related nature of antibody prevalence in many Western countries appears to be due largely to a cohort effect created by an overall decline in the incidence of HAV infection [24].

Infection occurs during early childhood in many developing countries, conferring protection against symptomatic reinfection that probably persists for life [12]. Infections occurring in individuals below 2 years of age are rarely associated with specific symptoms of hepatitis A, but nonetheless can be associated with efficient spread of the infection. Paradoxically, hepatitis A may become more of a problem for developing countries as improvements occur in public health sanitation, as decreased circulation of the virus leads to increased susceptibility to the virus among young adults. The potential impact of this effect is typified by an epidemic of hepatitis A in Shanghai which reportedly involved over 300,000 persons in early 1988 [27]. This emerging nature of hepatitis A, however, is perhaps better typified by an increase in sporadic adult and school-centered disease outbreaks in countries such as China, in which it appears that most children were previously asymptomatically-infected at an early age. While the relatively high cost and lack of availability of inactivated hepatitis A vaccines has precluded a significant impact on hepatitis A rates in most regions, use of an attenuated vaccine has been explored in China.

Large outbreaks of hepatitis A have occurred among urban homosexual men in recent years, reflecting a different aspect of the emergence of hepatitis A. In one study from Seattle [17], an annual infection rate of 22% was observed when seronegative gay men were followed prospectively. Epidemics of hepatitis A have been described among urban gay men in Copenhagen, Amsterdam, Melbourne, London, and other large, cosmopolitan Western cities in recent years. When studied, risk factors for the acquisition of hepatitis A in these disease outbreaks have generally been related to sexual behaviors that promote fecal-oral transmission of the virus [28].

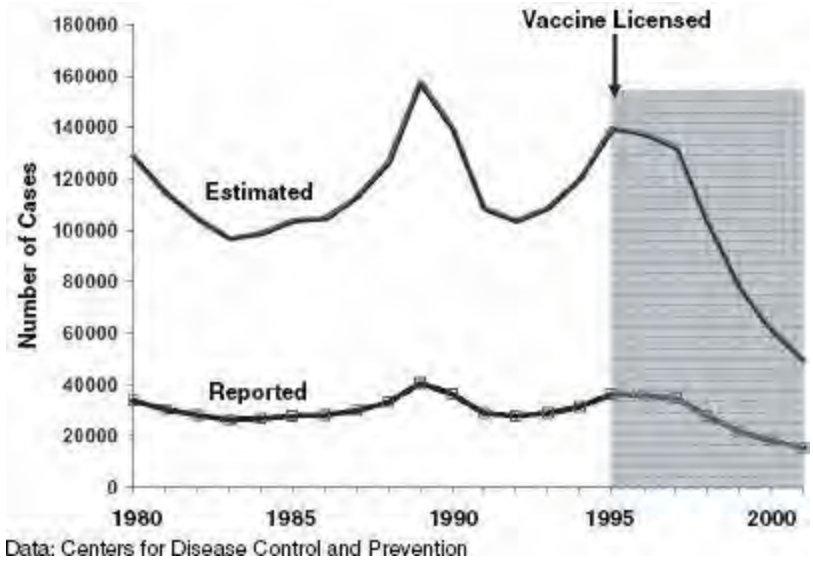

Figure 3. Reported and estimated cases of acute hepatitis due to HAV within the U.S. Data from the Centers for Disease Control and Prevention.

Blood-borne hepatitis A is a rare but potential problem when units of blood or plasma are collected early in the infection, prior to the onset of symptoms. Viremia is present throughout most of the incubation period, and correlates temporally with the fecal shedding of the virus. If persons incubating the disease donate blood or plasma, or share needles with others for the injection of illicit drugs, the virus appears to be readily transmitted.

Although transfusion-transmitted cases of hepatitis A remain relatively rare despite the absence of specific screening, numerous outbreaks of hepatitis A have been reported among users of illicit injected drugs. Interestingly, the incidence of hepatitis A cases in illicit drug users peaked in the late 1980s within the U.S., and has since declined in parallel with decreases in the incidence of HBV and HCV, 2 viruses which are well documented to be transmitted by exposure to contaminated blood (Figure 3). This argues that HAV may be similarly spread by parenteral means, rather than by fecal-oral transmission among drug users as previously suspected. Several outbreaks of hepatitis A have also been reported among hemophilic patients receiving high purity, solvent-detergent inactivated factor VIII preparations. Solvent-detergent methods that were introduced for the inactivation of lipid-enveloped viruses (HIV, HBV, and HCV) contaminating such blood products have no effect on the infectivity of HAV [34]. Other factors contributing to the spread of HAV by such products included the very large plasma pools used in their manufacture, as well as the high purity of the product, which effectively excluded potentially protective immunoglobulins from the final product.

Prevention

HAV remains a common cause of acute hepatitis in many countries (for example, it is the most common cause of acute hepatitis in the U.S.). Compared to other hepatitis viruses, HAV causes relatively little mortality, but nonetheless probably results in approximately 50 to 75 deaths annually in the U.S. due to fulminant hepatitis. Although inactivated hepatitis A vaccine is very safe and highly effective, its relatively high cost prevents universal use. The vaccine is recommended for those at increased risk of acquiring the infection, and those who are at increased risk for severe disease if they become infected [40]. Pooled immune human globulin also provides protection against symptomatic hepatitis A, but is used today only in post-exposure settings.

Hepatitis E

Virology

Like HAV, HEV is a single-stranded, positive-sense RNA virus [21]. Its phlyogenetic relationship to other viruses is uncertain. Although the organization of the genome of HEV closely resembles that of the caliciviruses, its proteins show a much closer relatedness to those of the alphaviruses. Reliable propagation of HEV in cell culture has not been achieved, and much less is known about the biology of HEV than HAV. Unlike HAV, infection with HEV does not appear to give rise to a long-term antibody response, and much remains to be learned about the immune response to this virus. Nonetheless, in many ways the course of HEV infection mimics the course of HAV infection, with extensive fecal shedding of the virus during the latter part of the 4- to 6-week long incubation period [37]. Fulminant hepatitis is a more common complication than with HAV, however, particularly in pregnant women in whom the overall mortality rate may be as high as 20% during the third trimester.

Epidemiology and Disease Emergence

HEV has been identified in developing nations of both hemispheres, where transmission generally occurs via the fecal-oral route and is frequently associated with contaminated drinking water [37]. Outbreaks at times have been very large, such as that which occurred in Delhi in 1956 and involved over 50,000 cases. Surprisingly, in many countries where HAV is highly endemic and HAV infection occurs early in life, hepatitis E is largely an infection of adults. Thus, although the transmission of both viruses is generally dependent upon spread from contaminated feces, there appear to be significant yet still unexplained differences in the epidemiology of these hepatitis viruses.

Strains of HEV recovered in Asia and in Mexico differ significantly with respect to their nucleotide sequence, but these differences are not sufficiently large to suggest the existence of different serotypes. Given the sporadic nature of hepatitis E, even in regions with very poor public health sanitation, a nonhuman reservoir for this infection has long been suspected. Much attention has been focused in recent years on closely related viruses that commonly infect domestic swine [37], but the contribution of this epizootic agent to human disease remains uncertain. In fact, recent studies have shown that closely related viruses are prevalent in many animal species, including not only swine, but also rats, deer, sheep, cattle, and even chickens. Evidence suggests that humans have become infected with HEV by ingesting uncooked deer meat as well as wild boar in Japan [48]. How these zoonotic viruses relate to HEV bears much further study, as commercial swine stocks are commonly infected in the United States despite the virtual absence of indigenously acquired hepatitis E in humans in this country.

Vaccine

An investigational vaccine containing a recombinant capsid protein antigen appears promising, and is in the late stages of clinical evaluation for efficacy [40]. Like hepatitis A vaccines, the sporadic nature of hepatitis E and the likely high cost of this vaccine (should it prove successful and be licensed for use in the future) are likely to make it difficult for health authorities to justify immunization programs in the poorest regions, where competing health needs are extensive but in which this disease historically imposes its greatest burden.

Viruses with Disease Burden Related Primarily to Chronic Infection

Hepatitis B

Clinical Virology

HBV, an hepadnavirus, is the only hepatitis virus with a DNA genome. The organization of its small, circular genome is complex with extremely parsimonious use of the protein-coding capacity [45]. The HBV replication cycle is unique and involves an RNA intermediate from which new progeny DNA genomes are produced by reverse-transcription. The HBV particle (sometimes called the Dane particle) is a spherical double-shelled structure approximately 47 nm in diameter. The outer shell is a lipid envelope containing varying proportions of 3 distinct viral surface proteins (large, middle, and small "S" proteins). The envelope can be removed by mild detergent treatment to reveal a stable nucleocapsid which has a distinct antigenic specificity (core or "C" antigen). A soluble form of the core protein (HBeAg) is also expressed during virus infection. The viral nucleocapsid contains the genomic DNA, which in the mature virus particle consists of 2 complementary linear strands of DNA that are base-paired with each other to form a partially double-stranded, circular molecule of about 3.2 kb [45]. The virally encoded reverse transcriptase/DNA polymerase is also associated with the virion. In addition to the mature virion, serum from infected persons typically contains a large excess of subviral particles (22 nm sphere and tubules) containing the small S coat protein.

Infection with HBV usually entails a lengthy incubation period (2 to 6 months) between exposure and the onset of disease. Viremia can be present throughout much of this incubation period; virus is also shed from mucous membranes resulting in relatively efficient sexual transmission. Symptomatic hepatitis occurs in the majority of immunocompetent infected adults, almost all of whom (probably over 99%) successfully clear the infection and develop permanent immunity. Protective immunity is largely dependent upon the S antibody response, but resolution of infection appears to be largely mediated by virus-specific cytotoxic T cells, perhaps via a primarily noncytolytic, cytokine-dependent mechanism of virus clearance [26]. Newborn infants and immunocompromised adults who are infected with HBV are very likely to become persistently infected. Such individuals have chronic viremia (associated with circulating S protein, HBsAg) and usually substantial anti-HBc (IgG) but not anti-HBs antibody responses. Persistent carriers of HBV may or may not have associated liver disease. Carriers of the virus have a significantly increased risk of developing hepatocellular carcinoma.

In recent years, much interest has focused on so-called "precore mutants" of HBV that are unable to express HbeAg, generally due to the presence of a stop codon within the precore region of the genome [16]. These viruses retain the ability to replicate and express the core protein (HBcAg), and have appeared in the course of persistent HBV infection, generally in association with an HBeAg to anti-HBe seroconversion despite the continuing presence of circulating HBV DNA. Alternatively, they may be the cause of new infections. In

some situations, these precore mutant HBVs have been associated with more clinically aggressive forms of liver disease.

Epidemiology

The prevalence of HBV infection varies widely in different geographic regions, with adult HBV carrier rates being high in China and many other Asian countries, and intermediate in Africa and the Middle East. The prevalence of the infection is dynamic, however, and has been reduced in some of these countries by intensive childhood immunization programs [15]. Infection prevalence has always been low in the well developed countries of North America and Western Europe, where the impact of the disease is greatest within special high risk populations. The virus is readily transmitted at birth from carrier mothers to infants in the absence of immunization. However, most transmission of virus in the developing world is among young children (so called “horizontal” transmission) [19], the details of which have always been obscure. It is not clear whether saliva or blood is the source of virus for such transmission, but both may contain high titers of virus. In developed countries, with effective screening of blood transfusions for hepatitis B, the major means of transmission among adults is sexual [7,46].

Prevention

Immunization is the mainstay of hepatitis B control and is recommended universally for newborn infants in many countries [3]. Vaccination is generally accomplished with recombinant vaccines that contain the small S protein expressed in yeast. Immunization programs have been aggressively promoted by the World Health Organization and other agencies, with impressive decreases resulting in the incidence and prevalence of hepatitis B infection and its attendant complications. No significant serotypic differences have been described among HBV strains from various regions of the world, although minor antigenic variation is common (antigenic subtypes). Although mutations in the S coding region have been suggested to be involved in rare cases of apparent vaccine failure in infected infants, such S mutants do not appear to be emerging as a major threat under the pressure of these successful immunization programs.

Recombinant hepatitis B vaccines are safe and generally effective, but many at risk are not immunized [3]. HBsAg prevalence remains high in many countries, and HBV infection remains the leading cause of liver cancer in many Asian nations (Japan excluded), requiring early detection for successful intervention.

Treatment

The treatment of chronic HBV infection is difficult. As important, access to therapy is limited and only a relatively small proportion of HBV carriers are offered therapy (almost all of whom reside within economically-developed countries with a low infection prevalence, or more recently in the emerging urban economic centers of Asia). Earlier use of parenteral interferon therapy has increasingly given way to oral lamivudine, a nucleoside analog

inhibitor of the viral reverse-transcriptase originally developed for treatment of HIV [51]. Lamivudine is efficacious, typically leading to impressive reductions in viremia and occasional conversions of HBeAg to anti-HBe status which is thought to be associated with an improved clinical prognosis [32]. However, lamivudine therapy is only rarely associated with complete resolution of the infection. In addition, drug-resistant variants emerge in as many as 50% of patients with chronic hepatitis B who are treated with the antiviral for periods as long as 24 months [30]. Consistent with *in vitro* assays showing the resistance of such variants to lamivudine, patients in whom these variants appear usually have a dramatic return in the magnitude of their HBV viremia to near pretherapy levels. The mutations in these virus variants may compromise their replication capacity, however, and these variants are often replaced by virus with wild-type polymerase sequence upon cessation of drug therapy. There is as yet no evidence that the emergence of these drug resistant variants carries any adverse prognostic implications.

A second nucleoside analog, adefovir dipivoxil, has recently been licensed in the U.S. for the treatment of chronic hepatitis B and appears to carry a significantly lower risk of generating drug-resistant variants [39]. Newer and more potent antiviral agents are also in advanced stages of clinical development. It is interesting to note that the commercial markets for these antiviral drugs are likely to be primarily in Asia, given the distribution of the virus worldwide.

Hepatitis D

Clinical Virology

HDV (or “hepatitis delta virus”) is a defective subviral satellite of HBV that may cause severe and often fatal liver disease in persons infected with HBV [14,47]. The HDV particle is approximately 35 nm in diameter, with an outer lipid envelope containing predominantly the small S protein of HBV. The envelope contains a poorly organized ribonucleoprotein structure, comprised of a 1.7 kb single-stranded, minus-sense, circular RNA genome in association with an HDV-encoded protein, the hepatitis delta antigen (HDAg). The RNA is catalytically active, and ribozyme activities in both minus and plus strands result in self-cleavage during replication of the RNA. HDV is dependent upon a coinfecting hepadnavirus for provision of its envelope, and thus cannot replicate in the absence of HBV infection. HDAg is the only protein expressed from the HDV genome, but is expressed in 2 molecular forms with different functions in the viral life cycle.

Infection with HDV can either occur simultaneously with HBV (acute coinfection) or in previously infected chronic carriers of HBV (HDV superinfection) [20]. HDV infection is diagnosed by demonstration of antibodies to HDAg, with superinfections distinguished from acute coinfections by the absence of IgM antibodies to HBcAg. Severe and even fulminant hepatitis is common in both coinfections and superinfections, with appreciable mortality rates in both settings. Individuals who survive acute HDV/HBV coinfection, however, appear no more likely than other patients with acute HBV infection to develop a persistent HBV carrier state. HDV superinfection often leads to transient (sometimes permanent)

suppression of HBV replication, but more typically these individuals develop persistent HDV infection and chronic hepatitis with progression to decompensated cirrhosis over a relatively small number of years.

Epidemiology

HDV coinfection of the HBV carrier state is found worldwide but varies in its prevalence. Clusters of cases of severe liver disease associated with HDV infection have been reported in South America, including both Venezuela and Peru [13]. HDV coinfection of HBV carriers has been relatively common in the Mediterranean region, where it was first recognized. Its impact on the course of chronic hepatitis B in this region seems to have waned in recent years, however. While recognized in Asia, HDV coinfection is relatively uncommon despite relatively high rates of HBV infection prevalence. Within the U.S., multiply-transfused individuals (e.g., hemophilic patients) and users of illicit parenteral drugs have historically been at highest risk of acquiring HDV infection. Clotting factor concentrates now carry little risk of HDV infection, due to the application of solvent-detergent and other virus inactivation procedures.

Prevention and Treatment

Immunization against hepatitis B provides nearly absolute protection against HDV infection, but for HBV carriers, there are no specific preventive measures available. In small scale trials, interferon therapy has suppressed viremia as well as ALT elevations in HDV carriers, but there are no well proven therapies. The large form of the delta antigen is prenylated, however, and it has been shown recently that prenylation inhibitors inhibit the replication of HDV in a murine model [10].

Hepatitis C

Clinical Virology

HCV is a single-stranded, positive-sense RNA virus with a genome length of approximately 9.6 kb. It is currently classified within a separate genus of the flavivirus family. The genome contains a single large open reading frame (ORF) which follows a relatively lengthy 5' nontranslated region containing an internal ribosome entry segment (IRES) directing cap-independent initiation of viral translation [33]. The large ORF encodes a polyprotein which undergoes post-translational cleavage, under control of cellular and viral proteases. This yields a series of structural proteins which include a nucleocapsid protein (C), 2 envelope glycoproteins, E1 and E2, and nonstructural proteins including NS3/4A (a serine protease/NTPase/RNA helicase), and NS5B (RNA-dependent RNA polymerase). In any individual patient with persistent infection, considerable sequence diversity is found among virion RNA molecules circulating in the blood. These related but distinct RNA sequences represent HCV "quasispecies", and reflect a remarkable tendency of this virus to undergo mutation with the continuous selection of more fit variants during persistent

infection, some of which may be capable of escaping previously formed antibodies or cytolytic T cells.

The natural history of this infection has been controversial [6,44]. Even fully immunocompetent persons who are infected with HCV usually fail to clear the infection, and at least 50% of these individuals will become persistently infected with the virus. As many as two-thirds of chronically infected persons will develop elevations of serum ALT, and have chronic hepatitis evident on biopsy of the liver. Cirrhosis may develop in as many as 20 to 30%, but typically only after 2 to 3 decades of chronic infection [43]. Once cirrhosis is present, the risk of hepatocellular carcinoma is dramatically increased, occurring in a few percent of patients each year. Despite this, in cohort-based studies carried out in the U.S., there has been no increased mortality over a 20-year period following infection with HCV that was acquired by blood transfusion [42]. Nonetheless, although the majority of individuals with chronic hepatitis C appear likely to die of unrelated causes, it is estimated that approximately 10,000 persons die each year of liver disease related to HCV infection within the U.S. (out of a population of approximately 4 million infected persons). Alcohol ingestion clearly plays a role in promoting HCV-related liver injury, and may play a role in many of these deaths. The explanation for the synergy that is apparent between HCV infection and alcohol in promoting liver injury may reside in a common tendency to adversely affect mitochondrial function within hepatocytes.

The basis for viral persistence remains a topic of intense investigation. Both innate and adaptive cellular immune responses to the virus have been identified within the liver of chronically infected persons [50]. Persistence may be associated with less vigorous CD8+ cytotoxic T cell responses [49], but a variety of other mechanisms of specific immune evasion have been proposed to explain the high frequency of persistence in normal individuals. These include the ability of the NS3/4A serine protease to block the induction of endogenous cellular antiviral defenses as well as the ability of the viral NS5A protein to inhibit the antiviral effects of the ds-RNA activated protein kinase R [23,25]. In addition, the core protein has been shown to bind to the cytoplasmic domain of some members of the TNF receptor family, potentially interfering with signal transduction through these receptors [53].

Epidemiology

Transmission occurs by both parenteral and nonparenteral routes [2]. Sexual transmission of HCV occurs, but appears to be very inefficient, as is maternal-infant transmission. HIV infection is associated with increased levels of HCV viremia, however, and thus may increase the risk of both sexual and neonatal transmission. The majority of cases of hepatitis C within the U.S. can be related to prior blood transfusion or illicit use of injection drugs. Transfusion no longer caries an appreciable risk of infection, because all blood donations are screened for infection by sensitive immunoassays. Acute infection with HCV is often difficult to diagnose, but the incidence of acute hepatitis C appears to have decreased significantly over the past 15 years within the U.S. [4]. HCV accounts for only about 10 to 15% of acute cases of hepatitis in the U.S. at present. Nonetheless, it remains

the leading cause of chronic viral hepatitis in the U.S. because of its unique ability to cause persistent infection in immunologically normal persons.

HCV infection is found worldwide in 1 to 2% of the population, but in certain geographically restricted areas it has been found to be present at much higher rates. For example, local regions in Japan and Taiwan have been shown to have very high prevalences of infection in older individuals, presumably because of traditional medical practices that previously promoted exposure to blood from infected persons (Figure 4).

In the U.S., the age-related prevalence shows a peak prevalence in the 4th and 5th decades of life, consistent with extensive spread of HCV within the U.S. in association with illicit injection drug use in the 1970s and 1980s [4]. In contrast, in Japan, the age-related prevalence increases with advancing age, peaking in the very oldest age groups [38]. This is likely to be due to spread of HCV within Japan by illicit injection drug (amphetamines) use as well as transfusions in the 1950s. Japan has noted a greater than 2-fold increase in the rate of liver cancer over the past 25 years (Figure 5), almost all of which can be attributed to HCV infection and which probably stems from the earlier increase in the population prevalence of the infection [52]. Unlike most other Asian nations, HCV infection now exceeds HBV infection in its contribution to the etiology of hepatocellular cancer in Japan (Figure 6).

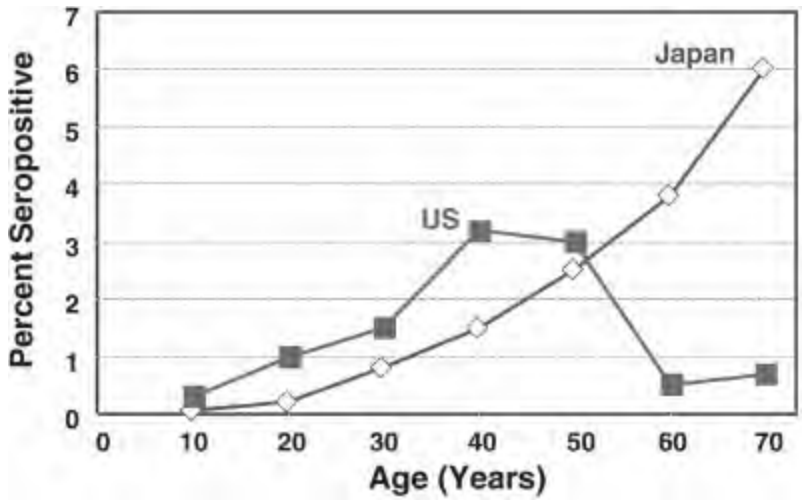

Figure 4. Estimated prevalence of hepatitis C antibodies in the general populations of the United States and Japan. Data obtained from References 4 and 38.

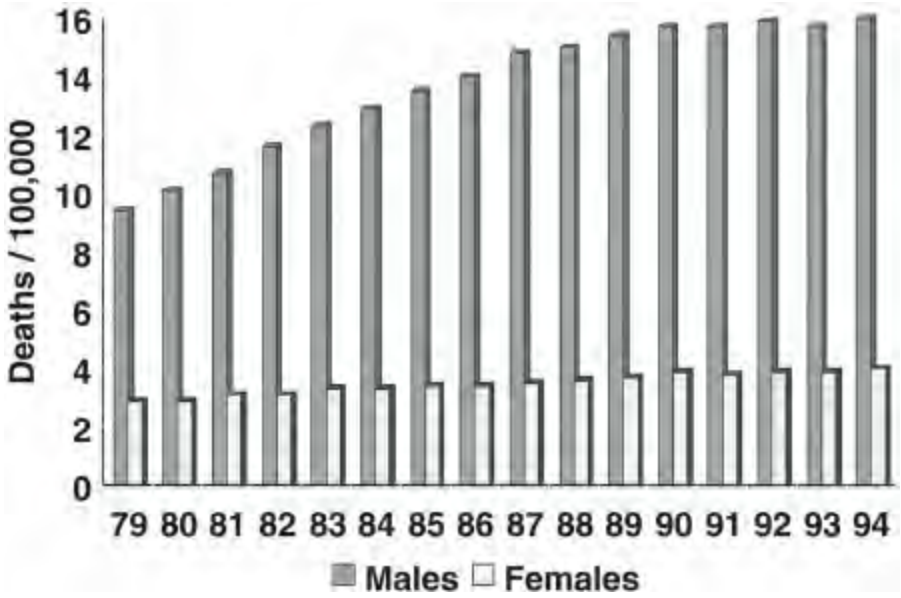

Figure 5. Age-standardized death rates for primary liver cancer in Japan, 1979-1994. Data obtained from the World Health Organization Databank.

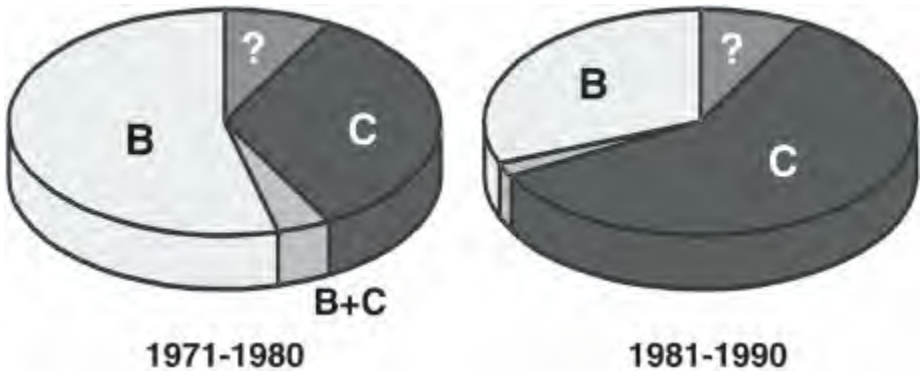

Figure 6. Proportion of cases of primary liver cancer associated with hepatitis virus infections in Japan 1971-1980, and 1981-1990. Data obtained from Reference 29.

Prevention

Effective hepatitis C vaccines appear to be far off in the future, although progress continues to be made [1]. Infection with HCV leads to limited protection against reinfection, even with the virus responsible for the original infection. This has been clearly demonstrated in chimpanzees, the only animal model for hepatitis C, which have been challenged twice or more with the same strain of HCV. However, in chimpanzees, second infections appear to be milder, briefer in duration, and to be associated with rapid viral clearance and a reduced probability of going on to long-term persistence [9]. In recent studies, similar protection against persistent infection may have been achieved with an experimental envelope protein vaccine. The existence of substantial genetic heterogeneity among different stains of HCV poses a particular problem for vaccine development. These differences have led to the classification of HCV strains into a series of genetically distinct “genotypes” [11]. While the

genetic distance between some of these genotypes is large enough to suggest that there may be biologically significant serotypic differences, some studies in chimpanzees suggest that there may be cross-protection between genotypes. Additional research is needed to confirm this. Such studies may be helped by the recent development of pseudotyped lentiviruses allowing a surrogate measure of virus neutralization in the absence of any cell culture system that is fully permissive for HCV [8].

Treatment

Treatment with interferon represents the most effective approach to control of hepatitis C. The efficacy of interferon alfa monotherapy in chronic hepatitis C is about 40% in terms of normalization of ALT by the end of therapy, but relapses are common. Sustained virologic responses (i.e., complete and permanent eradication of the infection) can be achieved in about 50% of persons with genotype 1 infections with 48 weeks of therapy with a pegylated interferon alfa given in combination with ribavirin [36]. Success rates are better with infections due to other genotypes of virus, and may require only 24 weeks of treatment. Pegylation extends the half-life of interferon following its administration, and significantly increases the proportion of end-of-therapy responders who do not relapse after discontinuation of therapy. The mechanism of action of ribavirin in this setting is controversial, but may relate to its action as a mutagen for replicating RNAs and its ability to induce viral RNA “error catastrophe” [18]. Small molecule inhibitors of the NS3/4A protease and NS5B polymerase are under development, and have shown great promise in preliminary clinical trials.

While associated with a significant rate of adverse effects ranging from depression to depression of leukocyte counts, interferon therapy may improve liver histology as well as lower ALT levels. Some studies also suggest that successful therapy may delay or prevent the development of hepatocellular carcinoma, although this is somewhat more controversial. Ongoing studies are examining whether continued interferon maintenance therapy in partial responders or people who fail to clear the virus might also be helpful in reducing the liver injury associated with viral persistence. Some anecdotal evidence suggests that liver fibrosis, and even cirrhosis, may be reversible with treatment. The major issue again is access, as current therapies are offered to only a small proportion of infected patients at risk for cirrhosis or liver cancer. Newer, high technologies for treatment of hepatitis C are being actively explored, such as the use of RNA silencing to reduce the intracellular abundance of viral RNA. However, the hope is that simple, relatively inexpensive, and potent treatments will emerge from current drug development efforts, and that such therapy may enjoy more widespread use in the future.

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Hepatitis B Virus Genotypes in Asia

Masashi Mizokami M.D., Ph.D.

Nagoya City University Graduate School of Medical Sciences, Japan

Introduction

Hepatitis B virus (HBV) was identified by Blumberg in 1965. Today, there are still 350 million infected people worldwide, and an estimated 1 million people die of HBV-related diseases yearly. The majority of the infected population are from Asia.

Recent advances in molecular biology have provided us tools to study the virus at the molecular evolutionary level. Accordingly, a number of HBV genotypes have been described. This review summarizes our current knowledge of the epidemiologic and clinical implications of HBV genotypes in Asia.

Epidemiologic and Clinical Implications of HBV Serotypes in Asia

In his investigations of racial differences in reactivity to human allotypes, Blumberg found a substance reactive with sera from hemophiliac patients in sera from native Australians. He named the substance Australia antigen (AuAg). Later, AuAg was found to be widely present not only in native Australians but also prevalent among Asian and African populations [3]. Ohkochi later discovered that AuAg was associated with post-transfusion hepatitis. Dane described the structural aspect of HBV (Dane particle) in 1972. AuAg was subsequently renamed HBsAg since it was shown to be one of the surface glycoproteins constituting the HBV envelope [5,16].

Further serologic characterization reclassified HBsAg into 4 major serotypes: adr, adw, ayr, and ayw, through combination of 2 pairs of mutually exclusive serotypes, d/y and w/r. Since HBV is considered to transmit from generation to generation through mother-to-child infection, these serotypes were employed in the study of human racial migration [12].

Serotyping studies demonstrated that for China, adr is prevalent in the north, adw is prevalent in the south, and ayw is prevalent in the western part spreading to Mongolia along the region of the Silk Road. In Taiwan, liver cancer is prevalent and most patients have the adw serotype. Liver cancer is also more prevalent in southern China, an area where adw is prevalent. This suggests that the HBV serotype adw may be linked to liver cancer from an epidemiologic perspective. Certainly, a detailed epidemiologic study that takes into consideration all possible confounding variables is required before a firm conclusion can be drawn.

In Japan, the HBV adr serotype accounts for more than 90% of the HBV infection in the Kyushu, Chugoku, Shikoku, Kinki, and Chubu areas. However, HBV adw serotype is more prevalent in the Okinawa and Tohoku areas. It is important to note that while Okinawa is geographically close to Kyushu, HBV adw serotype is more prevalent in the Tohoku area (Figure 1). From an anthropologic perspective, Nishioka proposed an explanation of this HBV serotype distribution, taking into consideration the 2 ethnic groups of people, Jomon and Yayoi, in Japan. According to his proposed model, approximately 20,000 years ago the Jomon-originated population, comprised of HBV adw serotype carriers, migrated from southern regions and settled in Japan as the first group of Japanese natives. Several thousand years ago, the Yayoi-originated population, who are HBV adr serotype carriers, migrated to Japan via the Korean Peninsula, and drove the native Jomon-originated population south to Okinawa and north to the Tohoku area [12]. Nishioka's hypothesis is consistent with the observation that people in the Korean Peninsula are mostly HBV adr serotype, while Taiwanese are mostly HBV adw serotype.

It is interesting to note that while the prevalence of HBV in Okinawa is the highest in Japan, the incidence of hepatocellular carcinoma is relatively low. This suggests that the association between HBV adw serotype and hepatocellular carcinoma is not as strong for this area as in other geographic regions, southern China and Taiwan. In Japan, epidemiologic studies showed that the incidence of hepatocellular carcinoma is high in

patients with HBV adr serotype, while there is a lower incidence in patients with HBV adw serotype. This is in direct contrast to China where the incidence of hepatocellular carcinoma is higher in patients with HBV adw serotype compared with patients with HBV adr serotype. These observations suggested that either there are different HBV genotypes within the adw serotype that account for the difference in etiologic and epidemiologic associations, or that other significant non-viral factors are also critical in determining the etiology of hepatocellular carcinoma and its epidemiologic pattern.

Establishment of HBV Genotypes

Mutually exclusive amino acid substitutions specific for each serotype within the S region of HBV DNA introduced through point mutations have been identified [14]. In 1988, Okamoto compared all the HBV genomic sequences known at that time. He concluded that when 8% or more of the nucleotides of the entire base sequences between isolates are different, they should be considered as different types. Based on this approach, he classified HBV into 4 types, A, B, C, and D [15].

Since molecular evolutionary classification by comparison of the entire base sequences is labor intensive and not robust, comparison of a sequence longer than a specified length of an HBV gene with an important function is a more rational approach. Although only a specified genomic sequence is used, evolutionary analysis based on this approach should still be more reliable than determination of the HBV genotypes based on the substitutions of a few specific amino acids.

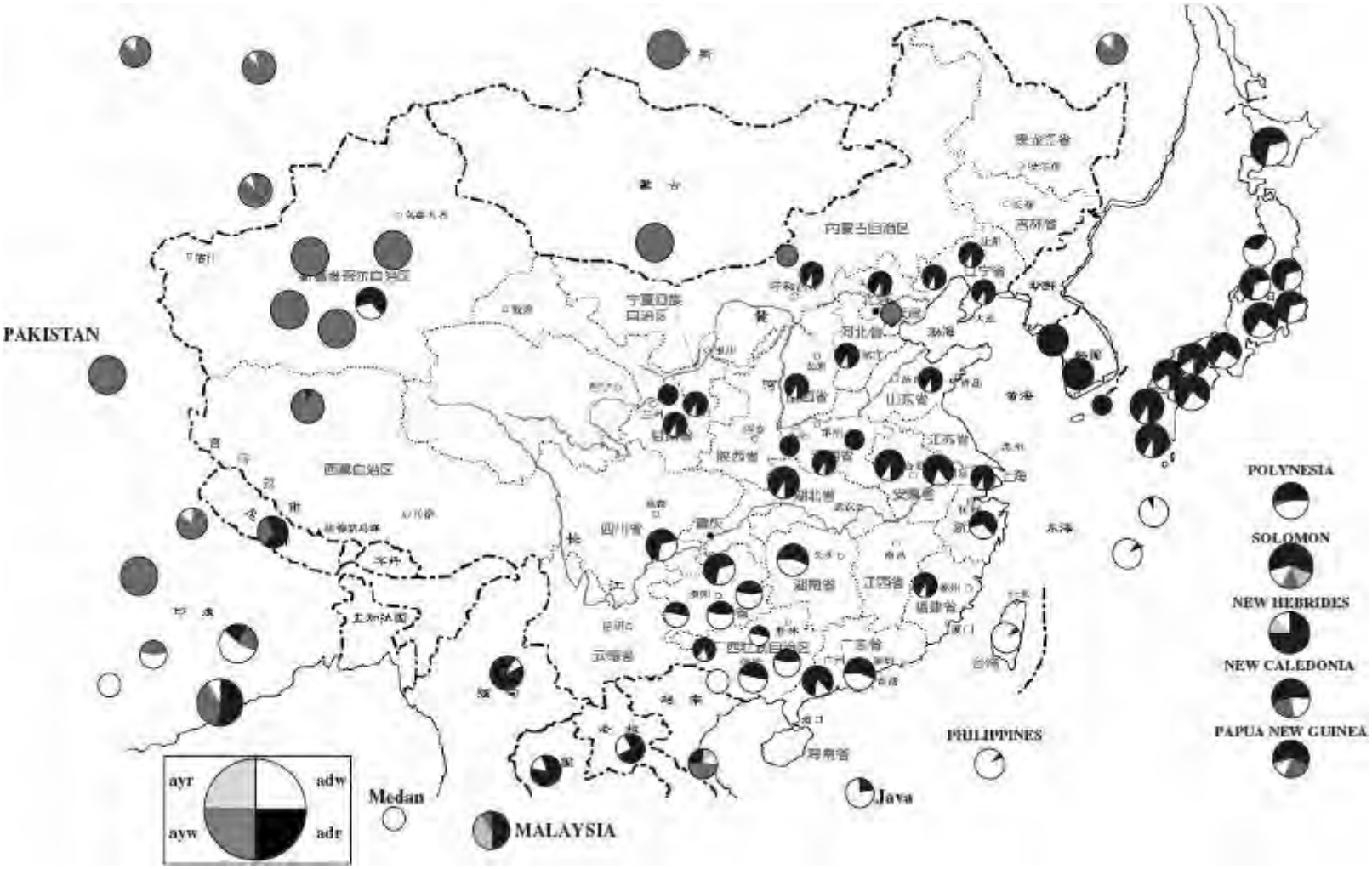

Figure 1. Distribution of HBV serotypes in Asian countries.

To establish an objective assessment of the impact of genomic variation in the overall classification, molecular evolutionary analysis was established through the work of many scientists over the last few decades. In 1968, Kimura proposed the neutral theory, in which DNA and proteins are regarded as molecules carrying genetic information [9]. These molecules were selected because they can be extracted from most biologic systems, are

relatively easy to handle, and provide easily recordable information. In all biologic systems that replicate, replication errors occur at a constant probability and cause mutations. Mutations accumulate over time, and this phenomenon is regarded as molecular evolution. The speed of mutation varies depending on the importance of the protein encoded by the gene. It has been demonstrated that the mutation rate is constant if averaged over a long period of time as long as the function of the protein does not change [9]. Application of this constancy allows us to be able to estimate the divergence time of the protein.

Molecular evolution is a fascinating analytical method. Its application is limited, however, if mutations are uncommon, e.g., for a human gene in which mutations in base sequences occur only every several million years. For viruses, the story is very different. The viral replication machinery does not contain an error correction component. This is critical for virus survival since it allows a more flexible genome capable of adapting to selective pressure through mutations. Mutations (or more appropriately called nucleotide substitutions) frequently occur in HBV since this virus employs reverse transcription as part of its replication strategy. In addition, the replication machinery does not have a correction/repair system, thus allowing mutations to accumulate. As a result, the number of nucleotide substitutions/mutations that occur in humans over a period of several million years will occur in HBV over a few decades. This biologic feature allows the application of molecular evolutionary analysis on hepatitis viral sequences and permits robust genotyping of HBV to determine its clinical implications [10].

Several laboratories, including ours, have embarked on investigations of HBV genotypes based on molecular evolutionary analysis of the phylogenetic relationships comparing the base sequences of Hepadna viruses including all known HBV sequences, woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus (GSHV), and duck hepatitis B virus (DHBV). Based on these studies, it was concluded that HBV genotypes do not necessarily correspond to HBV serotypes [17].

In 1992, Norder *et al.* reported HBV genotype E from Africa and HBV genotype F from South America, and classified HBV into 6 genotypes from A to F based on molecular evolutionary techniques [13]. In 2000, HBV genotype G was reported from patients in America and France, while genotype H was reported in a patient from Central America in 2002. However, reanalysis of the data showed that the difference between the entire base sequences of genotypes H and F is less than 8%, indicating that HBV genotype H should be classified as a subtype of HBV genotype F. At present, most investigators agree that there are 7 HBV genotypes, named HBV genotype A to G [2,20].

Clinical Implications of HBV Genotypes

We have developed a convenient assay method of HBV genotyping based on restriction fragment length polymorphism [11]. This simple assay was employed to evaluate the geographic distribution of HBV genotypes in Japan. A total of 800 blood samples from HBV-infected patients at 13 facilities from Hokkaido University to Ryukyu University were studied. HBV genotype C was the major genotype in Kyushu, Shikoku, and the western mainland of Japan, accounting for more than 90% of the isolates. However, HBV genotype B, which accounted for only 2% of the isolates in Kyushu, was the major HBV genotype in Okinawa, accounting for approximately 60% of the isolates. Interestingly, many patients in the Tohoku area had HBV genotype B infection (Figure 2) [18].

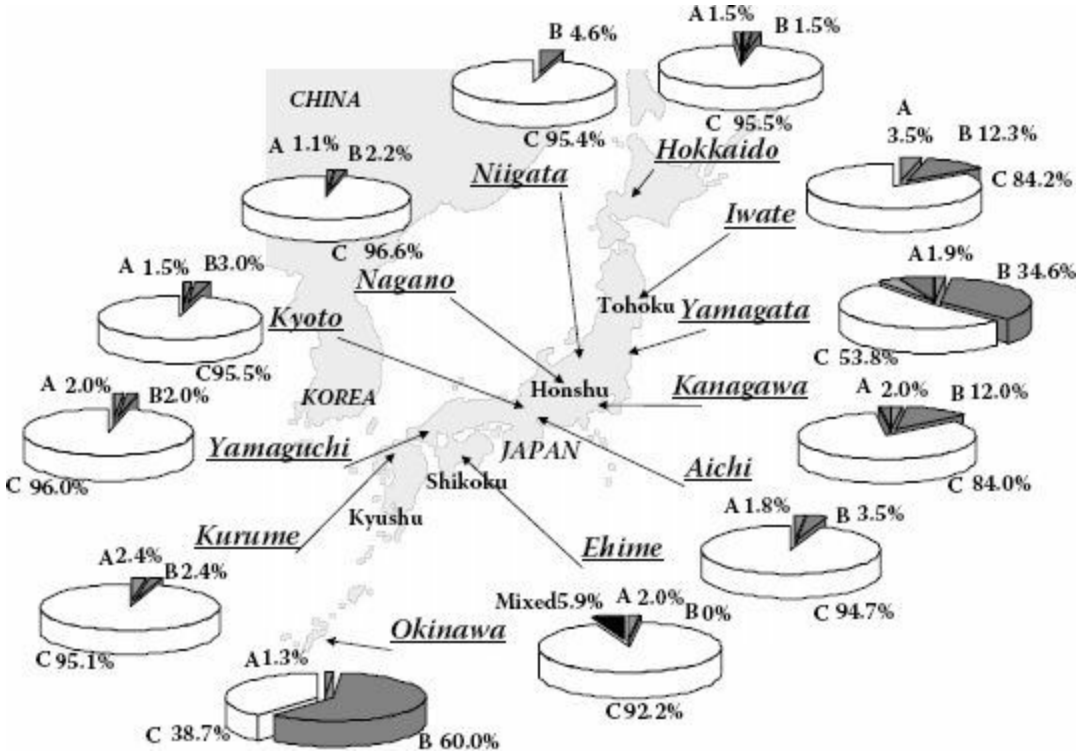

Figure 2. Distribution of HBV genotypes in Japan.

These findings indicate that HBV serotypes adw and adr, corresponding to HBV genotypes B and C, respectively, are more prevalent in Japan. According to the origin of the Japanese people, the Jomon population is infected mainly by HBV serotype adw and genotype B. When the Yayoi population migrated to Japan, they brought with them HBV serotype adr, corresponding to HBV genotype C. When the prevalence of HBV genotype was correlated with hepatocellular carcinoma, liver cancer was more prevalent in HBV genotype C compared to HBV genotype B infection in Japan. It is also interesting to note that the onset of liver cancer occurs much earlier in patients with HBV genotype C infection (on average, in the 50's), compared to patients with HBV genotype B infection (on average, in the 70's). The earlier onset of hepatocellular carcinoma with HBV genotype B was also mirrored by a better prognosis in patients with HBV genotype B infection [19].

As described above, HBV genotypes B and C are also the major HBV genotypes in Taiwan and the Korean Peninsula, respectively. As hepatocellular carcinoma is also common

in these areas with similar HBV genotypes, the better prognosis of HBV serotype/HBV genotypes in Japan cannot be explained by HBV genotypes alone. A report in 2000 showed that HBV genotype B is the major HBV genotype in Taiwan. However, the incidence of hepatocellular carcinoma is higher with HBV genotype C infection in Taiwan compared to HBV genotype B, which is similar to the epidemiologic profile in Japan. In young patients with hepatocellular carcinoma in Japan, HBV genotype B infection was more prevalent, in contrast to the older population in which HBV genotype C was more prevalent [6].

Because of these observations, we have conducted a study to determine the entire viral genome sequence of 38 HBV isolates derived from patients from Vietnam, Thailand, Taiwan, Indonesia, Hong Kong, China, and Japan. In conjunction with the 32 sequences already registered with the hepatitis virus database, a total of 70 isolates of genotype B were analyzed. As seen in Figure 3, the isolates can be divided into 2 subtypes based on molecular evolutionary analysis [21] – one subtype in Japan and the other subtype in other Asian countries. The subtype that occurs mainly in Japan is designated as subtype Bj, while the subtype that is prevalent in other Asian countries as subtype Ba. When virologic differences between these 2 subtypes were further analyzed, we found that the core region of HBV subtype Ba derived from other Asian areas had a similar sequence to HBV genotype C, suggesting that viral recombination had occurred in that region between the HBV genotypes [21].

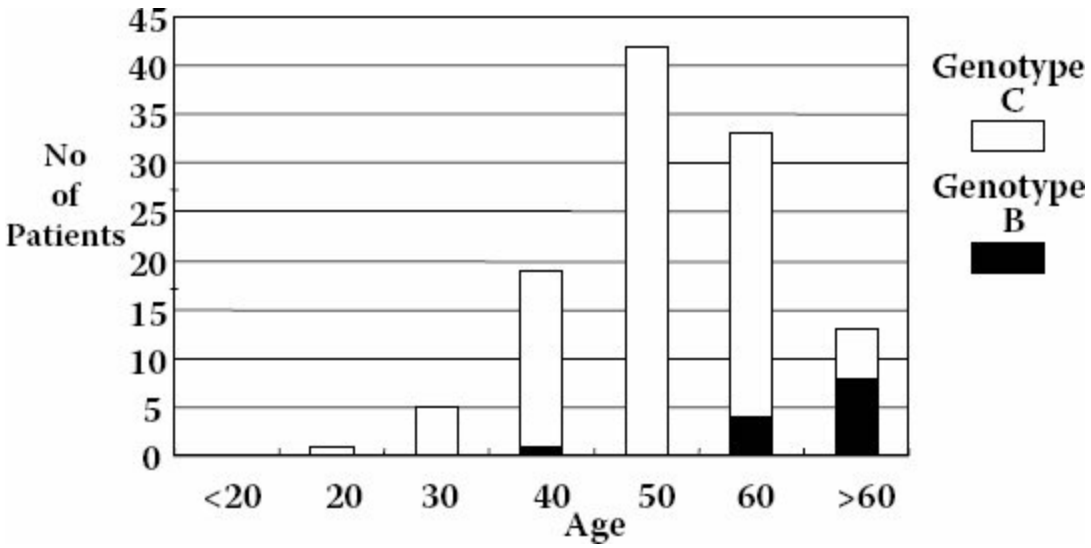

Figure 3. Distribution on hepatocellular carcinoma stratified by age and HBV genotype B and C.

To further elucidate the epidemiologic and clinical implications of these subtypes, an assay system that readily determines HBV subtypes Ba and Bj was established. HBV subtypes Bj and Ba account for 85.8% and 14.2%, respectively, of the HBV genotype B infection in Japan. In contrast, HBV subtype Ba account for 100% of the HBV genotype B infection in Taiwan, China, Hong Kong, Vietnam, Philippines, Thailand, Indonesia, and Chinese in North American, and subtype Bj has not been detected in these populations thus far [23] (Figure 4).

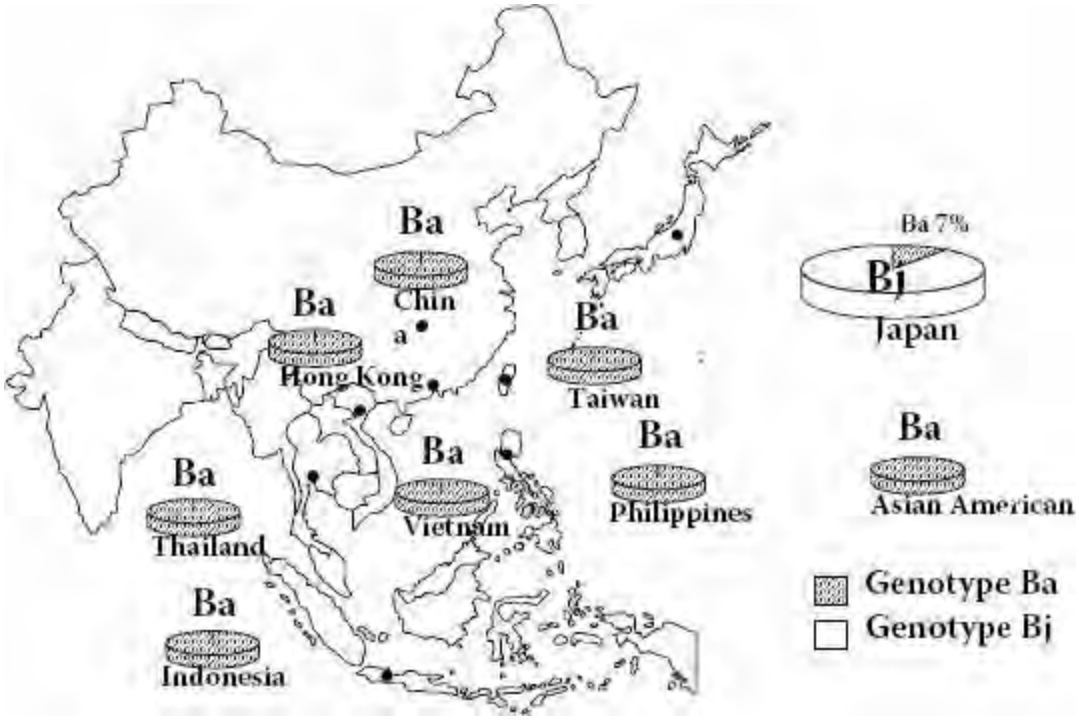

Figure 4. Geographic distribution of HBV genotypes Ba and Bj in Asian countries.

When the clinical profile of these patients were analyzed in light of the HBV genotypes, we found that despite the fact that HBV genotype Ba is prevalent in Taiwan, most Taiwanese patients with HBV-induced hepatocellular carcinoma had HBV genotype C infection. However, we also noted that most young Taiwanese patients with hepatocellular carcinoma were infected with HBV genotype Ba. Hence, the association of hepatocellular carcinoma with HBV genotype C infection may have a number of other confounding variables involved [6]. In Japan, HBV-induced hepatocellular carcinoma is mainly linked to HBV genotype C infection. Although fewer Japanese patients with HBV-induced hepatocellular carcinoma were linked to HBV genotype Bj infection, those patients with HBV genotype Bj-related hepatocellular carcinoma were mainly from an older age population [19] (Figure 5). As there are a number of other possible confounding variables in the etiologic relationship, additional studies on different populations with different confounding variables are critical for elucidating the actual implications of HBV genotypes in the etiology of hepatocellular carcinoma.

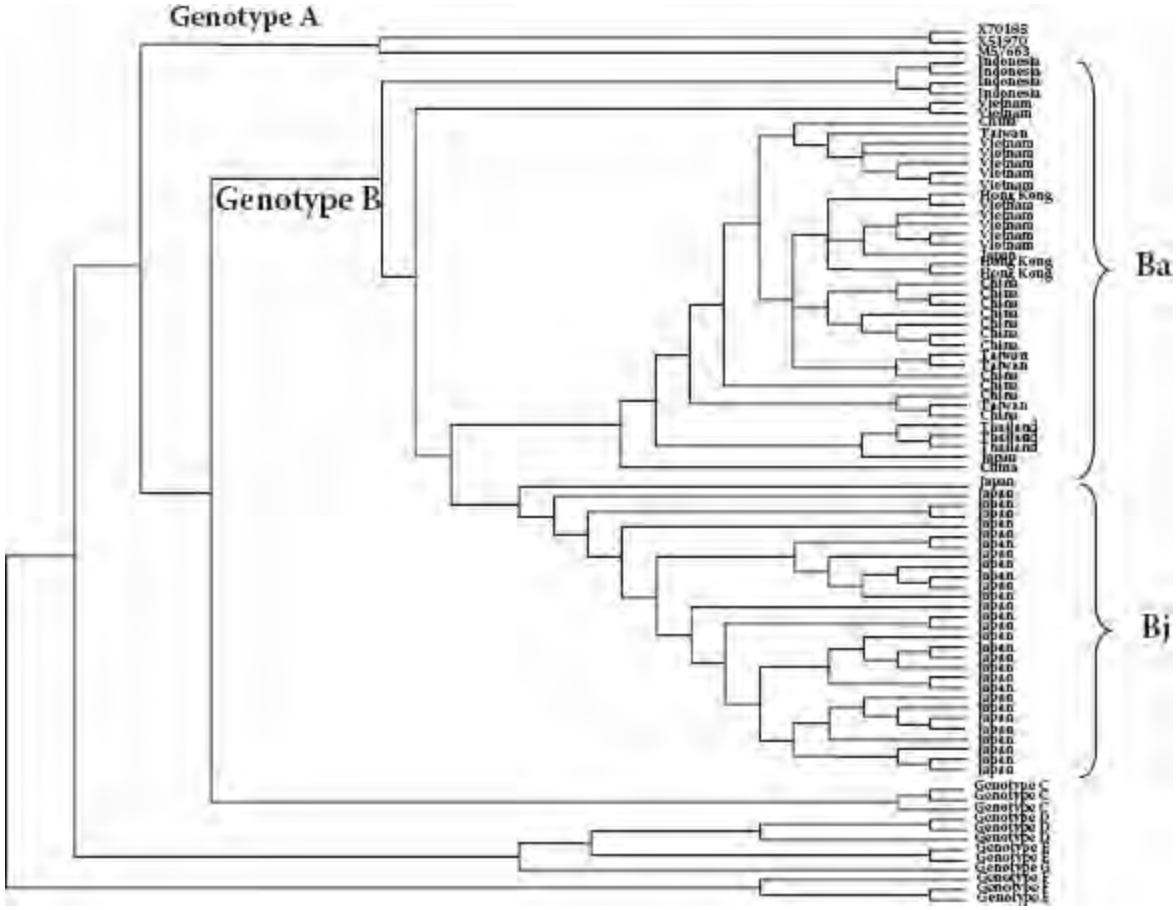

Figure 5. Phylogenetic tree constructed with 70 HBV isolates (based on the entire sequence). All 70 HBV isolates of genotype B can be subdivided into HBV subtype Bj, "j' for **J**apan, and Ba, "a" for **A**sian countries except Japan.

HBV genotype A is reported to be distributed in Africa, India, Philippines, Europe, and North and South America. HBV genotype A infection has been reported to have a better response to interferon therapy compared with HBV genotype D infection, which is also highly prevalent in Europe and America. In South Africa, HBV genotype A infection is the major type. Although seroconversion of HBe antigen occurs more commonly in young patients with HBV genotype A infection, hepatocellular carcinoma develops more commonly in these patients [4]. Again, other confounding variables may play a role in determining this epidemiologic profile and more studies are required to address this etiologic link. HBV genotype A infection is also subdivided by molecular evolutionary classification into subtypes Aa with high prevalence in Asia/Africa, such as Africa, India, and the Philippines, and subtype Ae which is prevalent in Europe and America [22] (Figure 6). It is also interesting to note that the frequent occurrence of seroconversion of HBV genotype Aa HBe antigen in young people is related to a specifically high frequency of mutation of the base sequence immediately before the initiation site of the precore region in this HBV genotype [1].

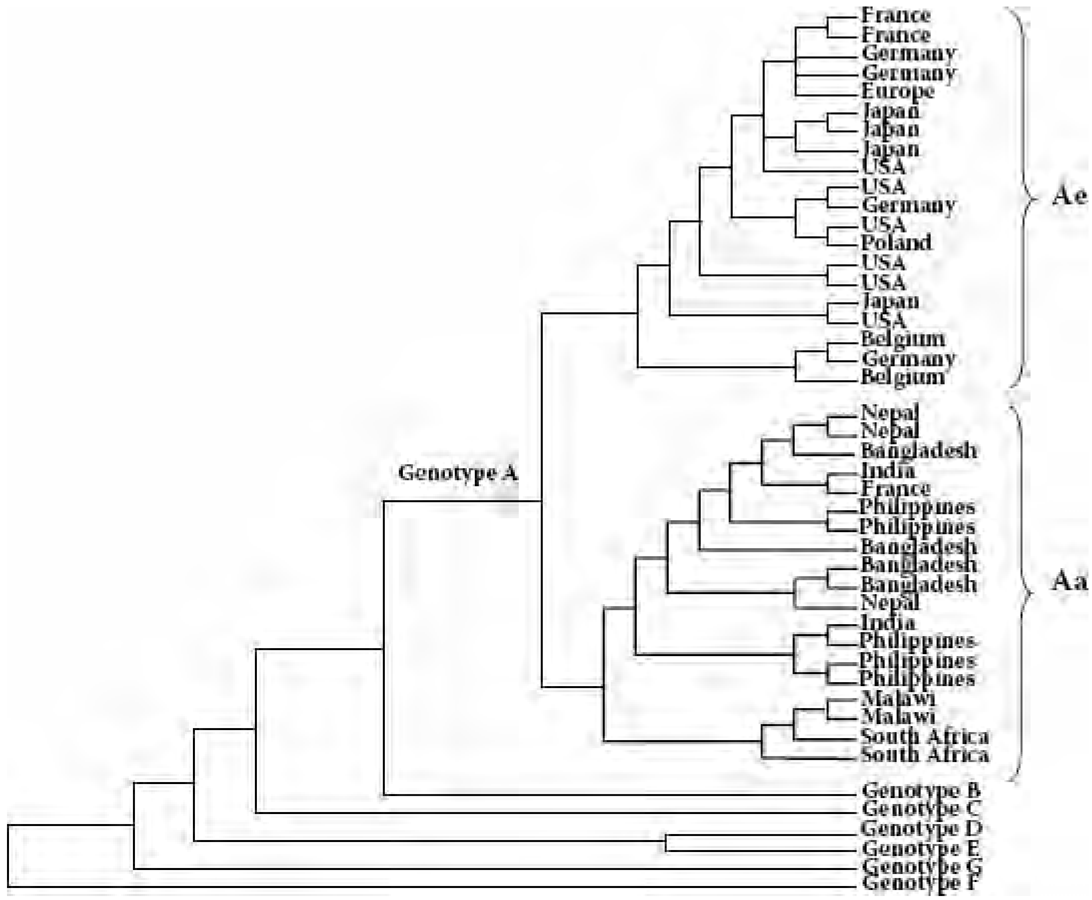

Figure 6. Phylogenetic tree constructed with 45 HBV isolates (based on the entire sequence). All 39 HBV isolates of genotype A are further subdivided into HBV subtype Ae, "e' for **E**uropean genotype A, and Aa, "a" for **A**frican and **A**sian genotype A.

The incidence of hepatocellular carcinoma is high in patients infected with HBV genotype E, which is highly prevalent in West Africa. Seroconversion of HBV genotype E HBe antigen occurs early, and the blood viral level after seroconversion is very low. Because of the low viremia level, it is believed that mother-to-child vertical transmission is less frequent, and people are mainly infected horizontally during childhood in this population [4].

HBV genotype F is reported mainly in South America. HBV infection in that region, when co-infected with hepatitis D virus infection, is associated with a high frequency of development into fulminant hepatitis.

HBV genotype H infection has been reported in Central America with similar geographic prevalence as HBV genotype F infection. As discussed earlier, further molecular evolutionary analysis has suggested that HBV genotype H should be reclassified as a HBV genotype F subtype, because the complete nucleotide sequence divergence of HBV genotype H is less than 8% compared with HBV genotype F.

HBV genotype G infection was recently reported in patients from America and France. The major virologic characteristic of HBV genotype G is the presence of a 36-base insertion in the core genomic region, which is absent in other HBV genotypes [20]. Although this insertion produces a stop codon in the core region, resulting in its inability to produce HBe antigen, HBe antigen was still detected in patients' blood. To solve this puzzle, we have

obtained samples from an American patient infected with HBV genotype G infection, and showed that HBV genotype G infection co-exists with HBV genotype A infection. It is through the HBV genotype A infection that HBe antigen is produced. The clinical evolution in this patient is that HBV genotype G infection finally predominated, and HBV genotype A became a minor species [7,8]. The virologic implications of HBV genotype G infection, including the notion as to (1) whether HBV genotype G represents a dominant defective interfering particle, (2) whether this genotype represents a new variant through evolution, and (3) whether this 36-base insertion represents viral or cellular gene recombination and what role this insertion plays in the virobiology of HBV remains to be determined.

Conclusions

Recent advances in molecular biology and molecular evolutionary analysis have allowed a closer examination of the HBV sequences variations, and different HBV genotypes have been identified. Virologic variants have been identified and new assays that allow rapid HBV genotyping have been developed. We are still in the early phase of evaluation of the virologic and clinical implications of these HBV genotypes. It is likely that a number of important molecular evolution patterns related to different HBV genotype infections in different geographic areas and populations will be revealed in the near future.

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Hepatitis E Virus Infection in Japan

Shunji Mishiro, M.D., PhD.

Toshiba General Hospital, Japan

Introduction

Hepatitis E virus (HEV), a non-enveloped RNA virus molecularly cloned in 1990 [8], causes acute self-limiting hepatitis in humans, with fatal “fulminant” hepatitis on rare occasions. HEV infection, whose major transmission mode is the fecal-oral route, is endemic in the developing countries of Asia and Africa with insufficient sanitary environments, and has caused frequent outbreaks mostly via so-called “water-borne” transmission (i.e., due to contamination of drinking water). Infrequent cases found in industrialized countries were mostly as “imported” infections that developed in individuals who had traveled to HEV-endemic areas. It was recognized in the late 1990s, however, that some cases were of “domestic” infections with a thus far unknown genotype of HEV first reported from the United States (U.S.) followed by European countries [9,10,18]. In addition, an HEV strain that segregates to the same genotype of Euro-American HEV strains (the genotype III) was also identified in swine from the U.S., suggesting that HEV is a zoonotic virus [4].

In Japan, HEV had been underrepresented or mostly overlooked until a “Japan-indigenous” strain (HEV-JRA1) was recovered in 2000 from a patient having no history of traveling abroad [11]. Thereafter, we began to uncover “hidden cases of hepatitis E”, and now we know that a substantial proportion (up to 30% in Hokkaido) of non-ABCD acute and/or fulminant hepatitis cases in Japan are ascribable to HEV infection. Although the mystery about the transmission routes of HEV in Japan has not been fully uncovered yet, we have obtained a line of evidence for zoonosis, as shown below.

HEV Strains from Japan

HEV consists of 4 major genotypes whose worldwide geographical distributions are: genotype I in Asia and Africa; II in Mexico; III in industrialized countries (or in non-endemic areas); and IV in China and neighboring countries. As shown in Figure 1, HEV strains recovered from domestically-infected Japanese patients segregate to genotype III and IV [5,12]. Even within the same genotype, Japanese strains are closely related to each other and remotely related to those from other countries. The fact that the HEV isolates from Japanese swine *did* co-segregate with those from Japanese patients [7,13] recapiturating previous findings reported from the U.S. [4] and Taiwan [16], adds to the indirect evidence for HEV infection to be a zoonosis.

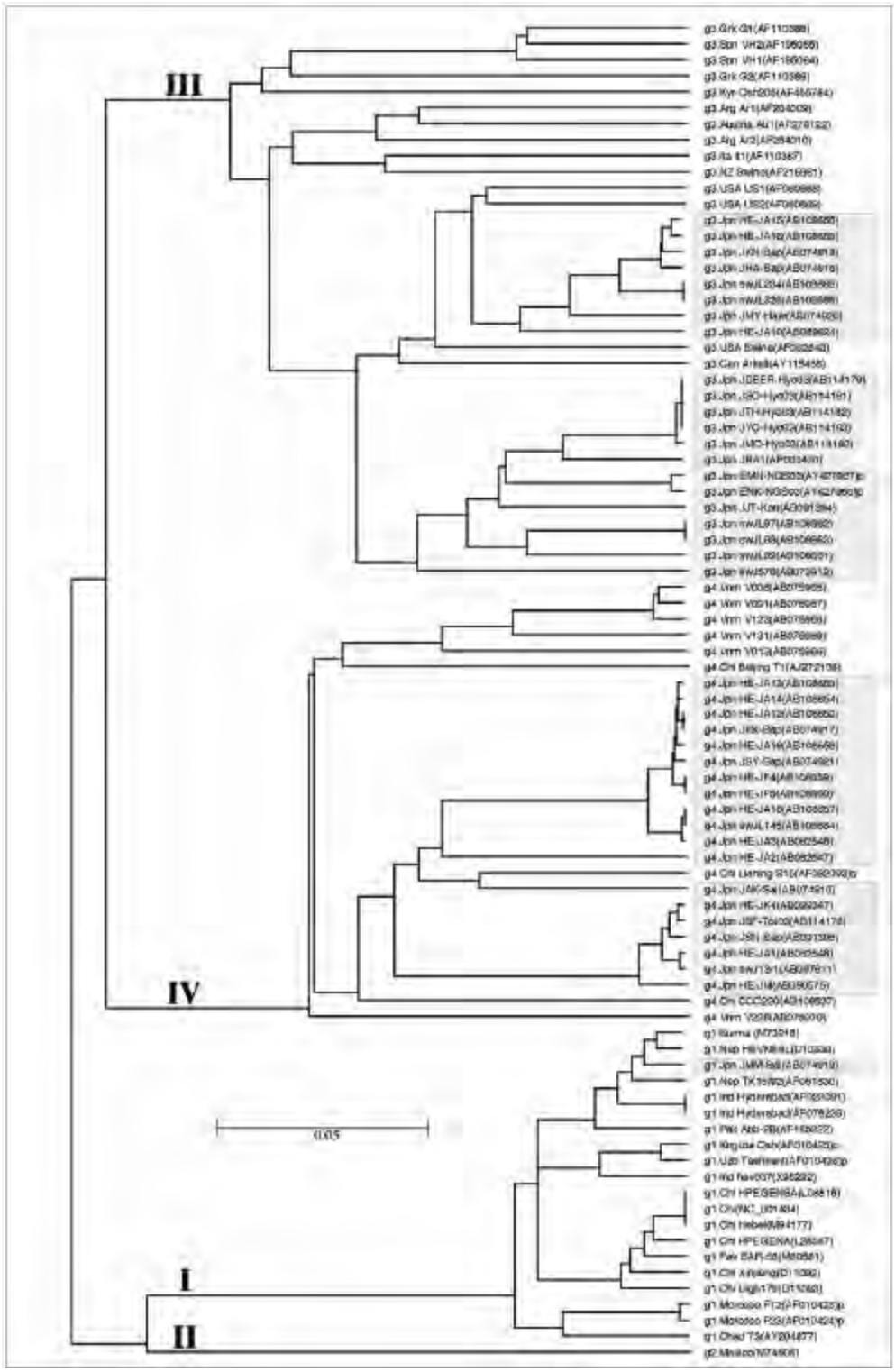

Figure 1. Phylogenetic tree (by UPGMA method) of HEV. A 326-nt sequence within the ORF1 of HEV RNA was used [12]. Japanese human and swine isolates are indicated by shaded boxes. Database accession numbers are in the parentheses.

Direct Evidence of Zoonosis: From Deer to Humans

Since HEV or like viruses and anti-HEV antibodies were found in a wide variety of animals represented by swine, it has been hypothesized that zoonosis is involved in the transmission of HEV, particularly for cases observed in non-endemic areas. Unfortunately, however, all of these findings have served as only indirect evidence.

In 2003, we experienced a series of cases of HEV infection, findings from which provided first direct evidence of zoonotic transmission [15]. As shown in Table 1, all patients involved in this intrafamilial mini-outbreak of hepatitis E had eaten uncooked deer meat (*sashimi* of *Sika* deer) 6 to 7 weeks before disease onset, while patients' family members who ate none or very little of the deer meat remained uninfected. The most important point of this case was that a frozen, leftover portion of the deer meat was positive for HEV RNA upon testing and had an identical nucleotide sequence with those from the infected patients.

Indirect Evidence: From Boar to Humans

In March 2003, 2 patients with acute hepatitis were admitted separately to 2 hospitals on the same day in a city of Tottori prefecture, and both were diagnosed with hepatitis E by detecting either or both HEV RNA (genotype IV) and anti-HEV antibodies. One patient died of fulminant hepatic failure while the other survived despite similarly severe hepatitis. What was later surprisingly revealed was that the 2 patients were friends and had enjoyed eating uncooked boar liver together a total of 5 times approximately 1 month before disease onset [2].

In April 2003, another case of boar-related HEV infection occurred in a mountain village of Nagasaki prefecture. A golden age club of the town held a barbecue party where grilled boar meat was served. Two of the 12 participants developed acute hepatitis E about 1 month later and 9 of the remaining 10 participants showed positive anti-HEV response on testing their sera after revelation of the 2 overt hepatitis cases. The nucleotide sequence of HEV RNA (genotype III) was identical between the 2 patients [14].

Unfortunately, boar liver and meat eaten by the patients in the above 2 outbreaks were not stored.

Indirect Evidence: From Swine to Humans

In 2001 and 2002, 10 patients with hepatitis E were sporadically admitted to a hospital in a city of Hokkaido Island. Nine of the 10 patients had ingested grilled or undercooked pig liver 2 weeks to 2 months before disease onset. A total of 363 packages of raw pig liver were purchased by the researchers from grocery stores in Hokkaido to test for the presence of HEV RNA, and 7 (1.9%) had detectable levels of HEV RNA [17]. A pair of swine and human HEV isolates with 99% nucleotide identity over the entire genome was revealed [6].

Table 1: A Case of Intrafamilial Mini-Outbreak of Hepatitis E Caused by Eating Uncooked Deer Meat

	Family-O					Family-H	
Subject	O-1	O-2	O-3	O-4	O-5	H-6	H-7
Age (yrs)/Sex	44/Male	69/Male	42/Male	38/Male	35/Female	61/Male	30/Male
Relation	Index patient	Father of O-1	Brother of O-1	Brother of O-1	Wife of O-4	Friend of O-3	Son of H-6
22 Feb 2003	Ate deer meat-1* (100g)	(100g)	(100g)	(none)	(none)	(100g)	(very little)
05 Apr 2003	Ate deer meat-2* (100g)	(100g)	(100g)	(100g)	(100g)	(100g)	(100g)
13 Apr 2003	Ate deer meat-3* (none)	(100g)	(100g)	(none)	(none)	(100g)	(100g)
16 Apr 2003	Fever, nausea, malaise ALT=2163 U/L	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
25 Apr 2003	ALT=394 U/L	Fever, nausea, malaise ALT=3906 U/L	Uneventful	Uneventful	Uneventful	Uneventful	Uneventful
27 Apr 2003	ALT=289 U/L	ALT=1605 U/L	Fever, malaise, arthralgia ALT=666 U/L	Uneventful	Uneventful	Uneventful	Uneventful
29 Apr 2003	ALT=201 U/L Anti-HEV IgM/G +/+ HEV RNA +	ALT=833 U/L +/+ +	ALT=455 U/L +/+ +	Uneventful -/- -	Uneventful -/- -	ALT=521 U/L +/+ +	Uneventful -/- -
16 May 2003	ALT=58 U/L	ALT=45 U/L	Uneventful	Uneventful	Uneventful	ALT=27 U/L	Uneventful

*Leftover portions of the deer meat-1, -2, and -3 were tested for HEV RNA, and only the "deer meat-1" was positive. ALT stands for alanine amino transferase and its values are of international units. Originally reported in Tei et al. Lancet 2003;362:371-3 (Reference 15).

Direct Evidence: From Human to Human

In 2002, a case of transfusion-transmitted hepatitis E was experienced for the first time in Japan. Of 23 volunteer blood donors, one, a 24-year-old female, tested positive for HEV RNA in retrospective analyses of stored sera. The patient-donor pair showed complete identity of the nucleotide sequence of HEV RNA PCR products. Since the donor showed negative HEV RNA in her sera obtained 15 months before and 5 months after donation and had normal ALT levels even at the time of donation, it seemed that she was a case of asymptomatic transient infection, and that she caused the transfusion-transmitted HEV infection by donating her blood during the window of infectivity [1].

Conclusion

The zoonosis hypothesis is now supported by substantial evidence. It remains possible that still more species of animals not mentioned above might also serve as the reservoirs for HEV transmission, as suggested by Meng [3]. At present, the boar-deer-swine trio (Figure 2) should be highlighted, at least in Japan.

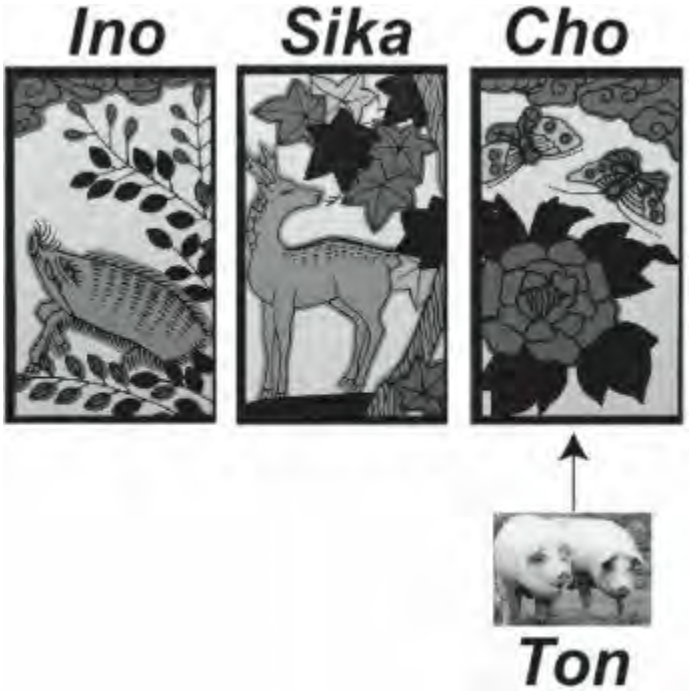

Figure 2. *"Ino-Sika-Ton"* is the key word for HEV zoonosis in Japan. *Ino-Sika-Cho* (boar-deer-butterfly) is one of the most special combinations in the Japanese card game *Hanafuda*. As for hepatitis E, however, *Cho* is replaced by *Ton* (swine).

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A National Project for the Management of Viral Hepatitis toward Prevention of Hepatocellular Carcinoma in Japan

Hiroshi Yoshizawa, M.D. and Junko Tanaka, Ph.D.

Hiroshima University Graduate School of Biomedical Sciences, Japan

Introduction

In Japan, deaths due to hepatocellular carcinoma (HCC) numbered 9,442 in 1970 and increased by 3.7-fold to 34,637 in 2002. HCC ranks third among malignancies in Japan, next only to lung cancer (56,405) and stomach cancer (49,213). Approximately 93% of HCC is caused by hepatitis viruses, including hepatitis B virus (HBV) and hepatitis C virus (HCV); the etiology is unknown for the remainder of cases [26]. Although cases of HCC due to HBV infection have been constant since 1970, those attributed to HCV infection keep increasing and amounted to 81% in 2002.

By far the majority of HCC cases develop in the background of fibrosis and cirrhosis in the liver as sequelae of chronic necro-inflammation induced by HBV or HCV infection. Most cases of HCC caused by persistent HBV infection present in individuals in their early 50's, while HCC caused by persistent HCV infection present somewhat later in the late 50's to early 60's [26]. These distributions reflect carriers of HBV and HCV who cluster in the cancer-baring ages (40's, 50's, and 70's) in Japan.

To cope with escalating rates of HCC due to hepatitis virus infections, a national project was started in Japan in April 2002, targeting individuals aged 40 years or older. It is based on information regarding seroepidemiology of persistent HCV infection combined with epidemiological profiles of HCC. Additionally, it is aided by recent remarkable advances in the diagnosis of HCC by means of imaging modalities, such as ultrasonography, computed tomography, and angiography, as well as tumor markers like alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II: also known as DCP [des-γ-carboxyl prothrombin]). An increased life expectancy of patients with HCC by virtue of markedly improved treatments would make this project rewarding as well.

In this review, we will focus on: (1) the seroepidemiology of persistent HCV infection and HCC induced by this infection; (2) present status and achievements of a national program in the management of HCV infection aimed at preventing HCC; and (3) imminent issues that have surfaced during the first year of national screening and recommendations for going forward.

Shifting Patterns of HCC in Japan during the Past 50 Years

In Japan, the outbreak of HCV infection occurred some 50 years ago during the chaos that surrounded the termination of World War II [26]. The Japanese are now facing long-term ramifications of this HCV epidemic as seen by the surge in deaths due to HCC in recent years which as shown in Figure 1, is breathtakingly evident. Mortality attributable to HCC started to increase in 1975, and numbers 27.1/100,000 population per year at the latest survey in 2001. An abrupt rise in the number of HCC cases in 1993 is attributed to changes in the diagnostic criteria identified by the WHO International Classification of Diseases (ICD), from ICD9 to ICD10 in that year. Men suffered from HCC 2.3 times more frequently than women at the last survey. Furthermore, deaths due to HCC in men have almost quadrupled in remarkable contrast to those in women where the death rate has doubled. Thus, the speed at which HCC has increased in men is twice that seen in women.

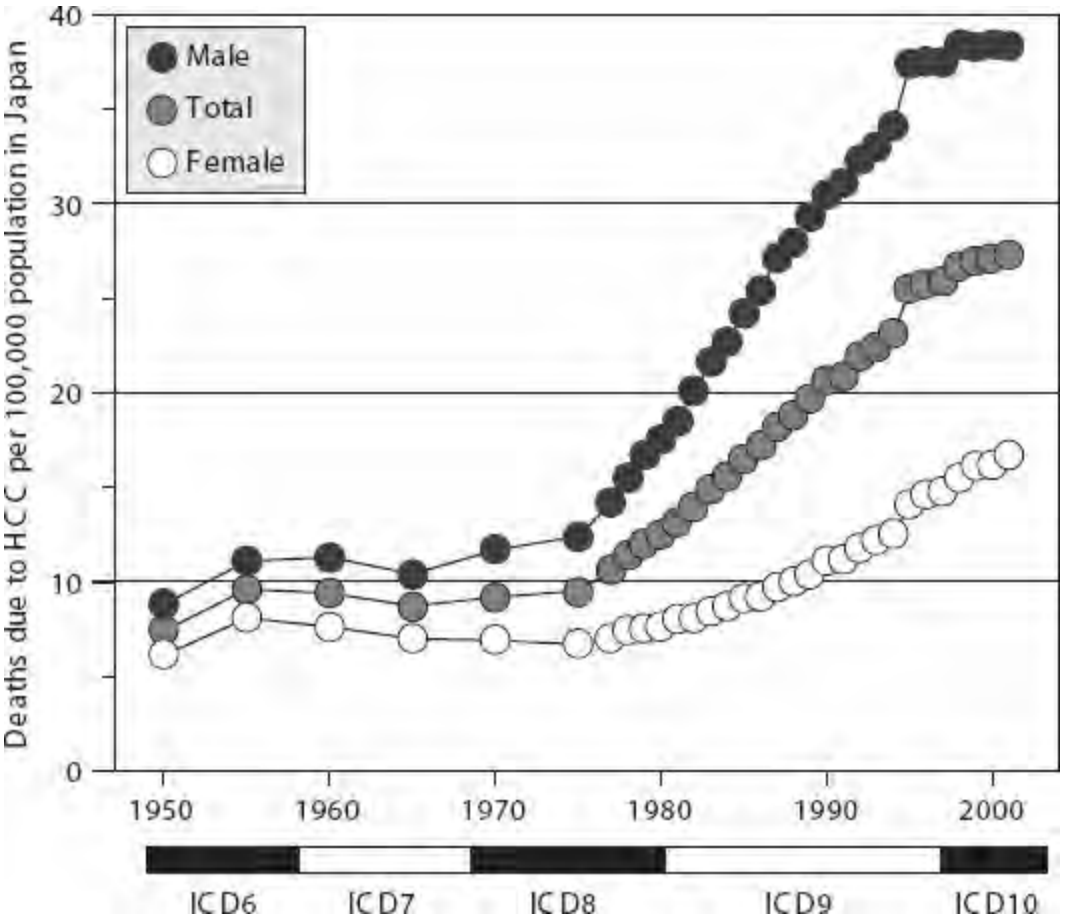

Figure 1. Increasing deaths due to HCC in Japan during the past 50 years (modified from Reference 26 with addition of recent data).

Shifting causes of deaths due to HCC during the past 20 years are illustrated in Figure 2. Unlike most countries in Asia and Africa where persistent HBV infection has been the leading cause of HCC, deaths due to HBV infection have stayed almost the same throughout the past 2 decades. HCC categorized as due to Non-A, Non-B viral infection surfaced at the same time but kept increasing. As the serological diagnosis of HCV infection became feasible in 1990 [13] in the immediate wake of the landmark discovery of HCC by Choo *et al.* in 1989 [4], most HCC of a Non-A, Non-B etiology has turned out to be induced

by persistent HCV infection. There remain, however, approximately 10% of HCC cases in Japan for which etiology is not ascribed to persistent infection with HBV or HCV, and henceforth these cases are classified as Non-A, Non-B, Non-C.

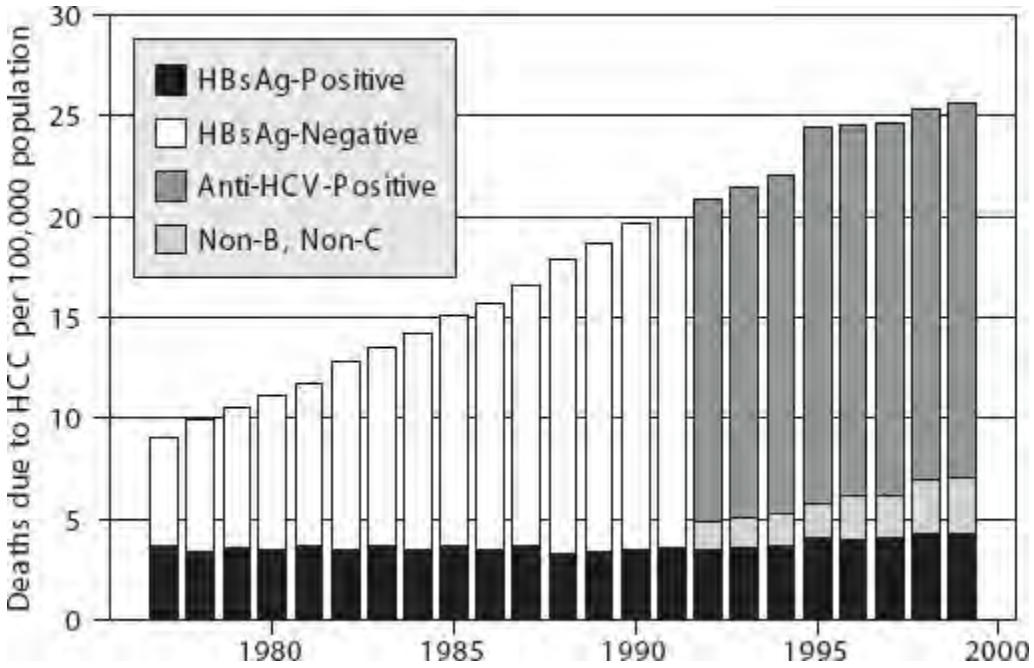

Figure 2. Causes of HCC shifting with time in Japan (modified from Reference 26 with addition of recent data).

Due to an iatrogenic nature of the transmission of HCV, its distribution in the world is extremely uneven [5]. Even in the same country, HCV tends to prevail more in inhabitants of urban districts compared to rural regions. Even in Japan, there are marked differences in the prevalence of persistent HCV infection. An increasing gradient in the frequency of HCC from northeast to southwest along the axis of the Japanese Islands was noted some time ago. Thus, deaths due to HCC in excess of 30/100,000 population per year occur only in southeastern prefectures, represented by those in Shikoku and Kyushu Islands. Yamanashi in the eastern part of Japan is a single exception with 31.7 deaths of HCC per 100,000 population per year. The mass treatment of *Scistosomiasis japonicum* with intravenous injection since 1982 is implicated in the local spread of HCV there. As the incidence of HCC increased, over time the distribution of HCC has become more diffused over Japan.

Figure 3 compares the distribution of HCC in men between the mid-1970s and the new millennium. The distribution of HCC was fairly even throughout Japan in 1995, although scattered districts with a high rate of deaths due to HCC stood out in the south coast of Kyushu Island. Although the hardest hit areas have remained the same throughout the last 30 years, the distribution became more uneven in the survey taken in 2001. Thus, northern Kyushu, Hiroshima in the west of Honshu Island, and districts surrounding Setonaikai basin with Osaka on the coast of Honshu, as well as Yamanashi in the center of Honshu, became most strongly infiltrated with HCC.

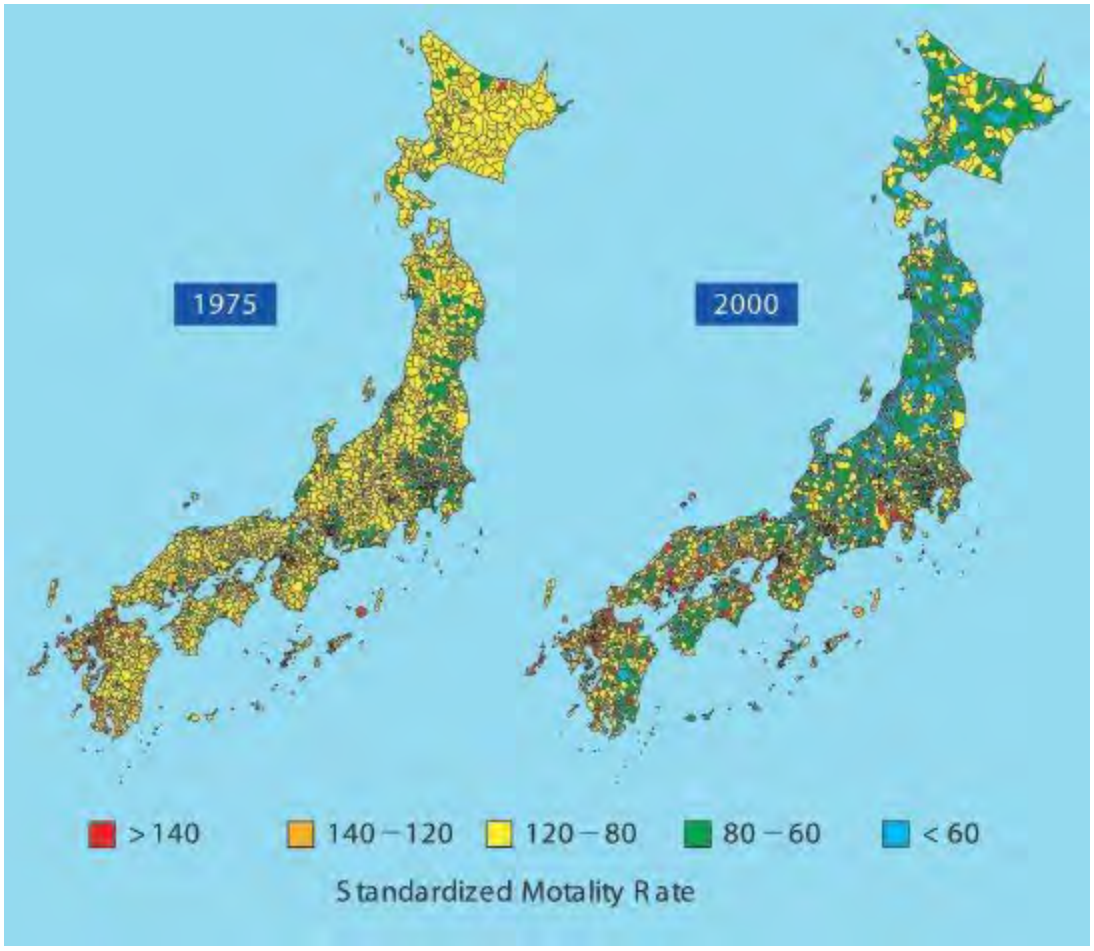

Figure 3. Changes in the distribution of deaths due to HCC in men during the past 30 years in Japan. These maps were produced by Dr. Yoshihiko Mirua of Saitama Prefectural University.

Prevalence of Persistent HCV Infection in Japan

Volunteer blood donors are the best population for estimating the prevalence of persistent infections with any microbe, including HBV and HCV, although they may not faithfully mirror national sex and age distributions. Since blood donors found to have ongoing HBV or HCV infection in previous donations have been deferred from future donation, first-time blood donors need to be tested for avoiding sampling and selection biases. Figure 4 illustrates the age-specific prevalence of antibody to HCV (anti-HCV) in 3,485,648 Japanese individuals who donated blood for the first time during a 6-year period from January 1995 to December 2000 [23]. For convenience of comparison, the ages of blood donors were extrapolated to those in the year 2000. The prevalence of anti-HCV was very low in the blood donors younger than 30 years, but stayed rather high, around 1.5%, in those up to 45 years of age, and started to increase at the age of 50. From that point on, anti-HCV prevalence increased almost exponentially and exceeded 10% among cohorts in their late 60's.

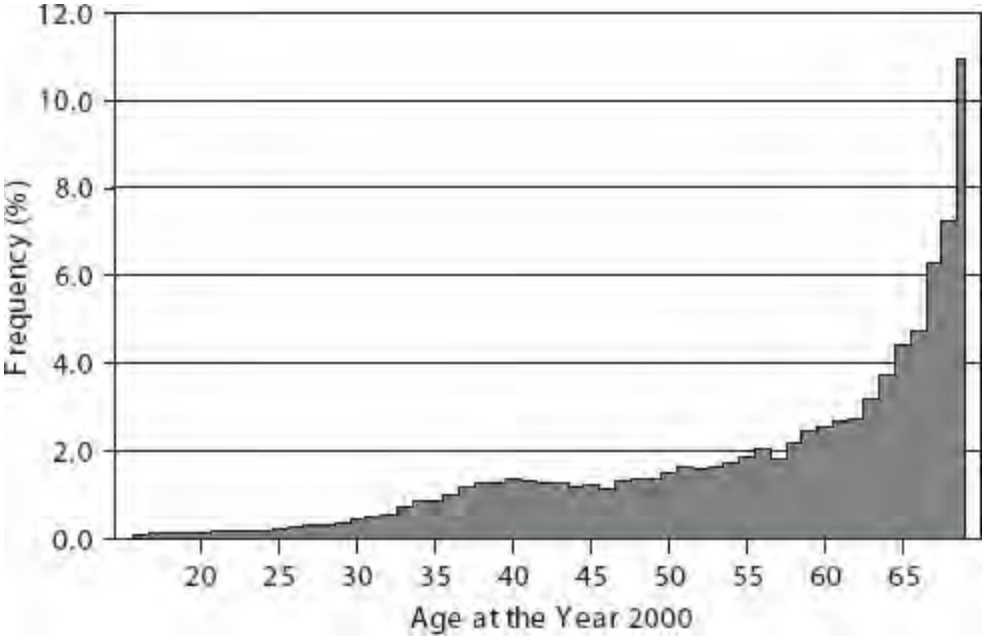

Figure 4. Age-specific prevalence of anti-HCV in a large-scale survey on the first-time blood donors in Japan (reproduced from Reference 23 with permission).

Figure 5 depicts age-specific distributions of anti-HCV in first-time blood donors across the Japanese Islands [23]. Although the pattern of increasing prevalence of anti-HCV with age was maintained in the first-time blood donors at each of the 8 jurisdictions of Japanese Red Cross Blood Centers, there were some differences in the magnitude of HCV diffusion that are reflected in the prevalence of anti-HCV among them. This is most evident in the highest prevalence group – blood donors in their 60's – where anti-HCV prevalence rates ranged widely from 2.3% in the Chubu/Tokai district to 5.0% in the Chugoku district. Anti-HCV prevalence rates in this age group were also high in the Kyushu, Kinki, and Shikoku districts. These differences in HCV infection are mirrored in the distribution of deaths due to HCC in Japan in recent years (Figure 3).

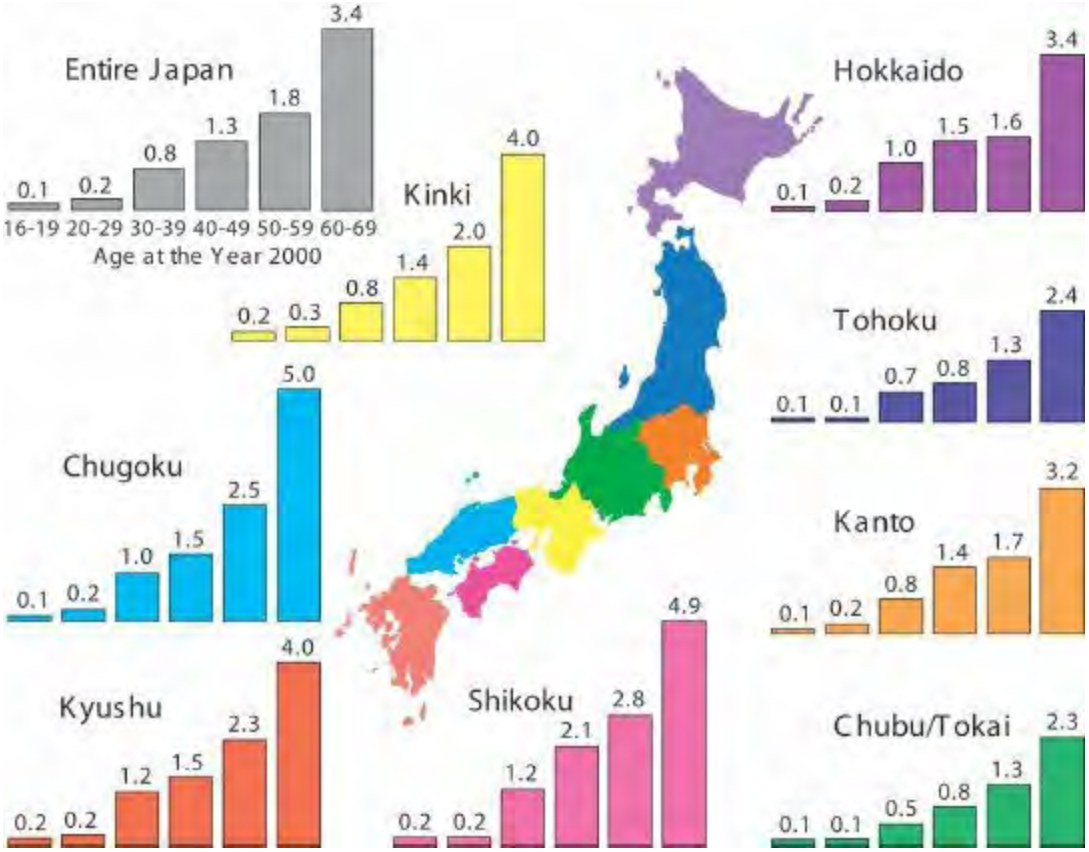

Figure 5. Age-specific distributions of anti-HCV in the first-time blood donors at the 8 jurisdictions of Japanese Red Cross Blood Centers (reproduced from Reference 23 with permission).

The pattern of age-specific anti-HCV in Japan reflects the exposure to HCV in the past and suggests that de novo HCV infection would be rare. This has been proven in prospective studies on different populations in various districts in Japan [26]. The incidence of HCV infection in blood donors was very low and ranged from 1.8/100,000 person-years in Hiroshima during 1988-1992 to 3.4/100,000 person-years in Osaka during 1992-1997. The incidence of HCV infection was also low in company employees (0 in 3,079 during 1992-1995) as well as among patients in mental health institutions and elderly in nursing homes (combined: 0 in 678 during 1992-1995) who are at increased risk for viral infections. Hence, horizontal transmission of HCV has been contained in Japan and only rarely are there new cases nowadays.

With regards to perinatal HCV transmission, only 2 of the 86 babies (2.3%) born to carrier mothers were infected [15]. This incidence is on par with that of mother-to-baby transmission of HBV of less than 3 to 4% for babies born to mothers expressing hepatitis B e antigen in serum [11,17]. The rarity of mother-to-baby transmission of HCV is reflected in the extremely low prevalence of anti-HCV in infancy; it was detected in merely 240 of the 400,000 (0.06%) children aged less than 6 years [26]. Hence, there are no pressing needs for taking measures to cope with the mother-to-baby transmission of HCV.

The incidence of post-transfusion HCV infection has decreased to practically nil since screening of blood donors by the second-generation immunoassay for anti-HCV was introduced in 1992 [26]. The remaining risk has been reduced further by the introduction of

nucleic acid amplification testing (NAT) that has been in place since October 1999 [16]. HCV rarely, if ever, transmits by sexual contact, unlike HBV for which this mode of transmission occurs frequently. Fortunately, illicit intravenous drug use is still very infrequent among the Japanese youth. Each of these factors have contributed to a very low incidence of new HCV infection in Japan, which is exceptional for developed countries.

Clinical Courses of HCV Infection

The prevalence of anti-HCV in first-time blood donors increases sharply with age (Figures 4 and 5) [23], which represents the long-term sequelae of exposure to HCV in the distant past that was accelerated by a number of factors [26]. Insofar as *de novo* HCV infection is contained in Japan, the age-specific prevalence of anti-HCV will keep shifting toward the elderly in the future. Because HCC in Japan occurs predominantly in HCV carriers older than 50 years of age, deaths due to HCV-associated HCC will likely plateau around 2000–2010 and decrease thereafter.

We are left with an estimated 880,000 HCV carriers older than 40 years [23], however, who are at increased risk of developing HCC. How soon and how often this group develops HCC is our utmost concern. Figure 6 illustrates clinical diagnoses among the 912 HCV carriers who were identified at the time of blood donation in Hiroshima. Surprisingly, approximately two-thirds of them have already developed chronic hepatitis and only one-third were without clinical liver disease. Furthermore, liver cirrhosis was detected in 5 carriers (0.5%) and HCC in 1 (0.1%). In a prospective study, 362 of this cohort received a second medical examination at least 5 years later (average 8.2 years [range: 5.0 to 10.3 years]) (Figure 7). Clinical diagnoses remained unchanged in most of this group. However, 4 men and 1 woman had developed HCC at the ages of 62, 63, 64, 68, and 67 years, respectively. In addition, liver cirrhosis had emerged in 11 of the 214 (5.1%) blood donors who were originally found to have chronic hepatitis, and in 2 of the 144 (1.4%) who were without clinical illness at the outset. Notably, HCV infection resolved through the use of interferon therapy in 42 blood donors who had presented with chronic hepatitis C.

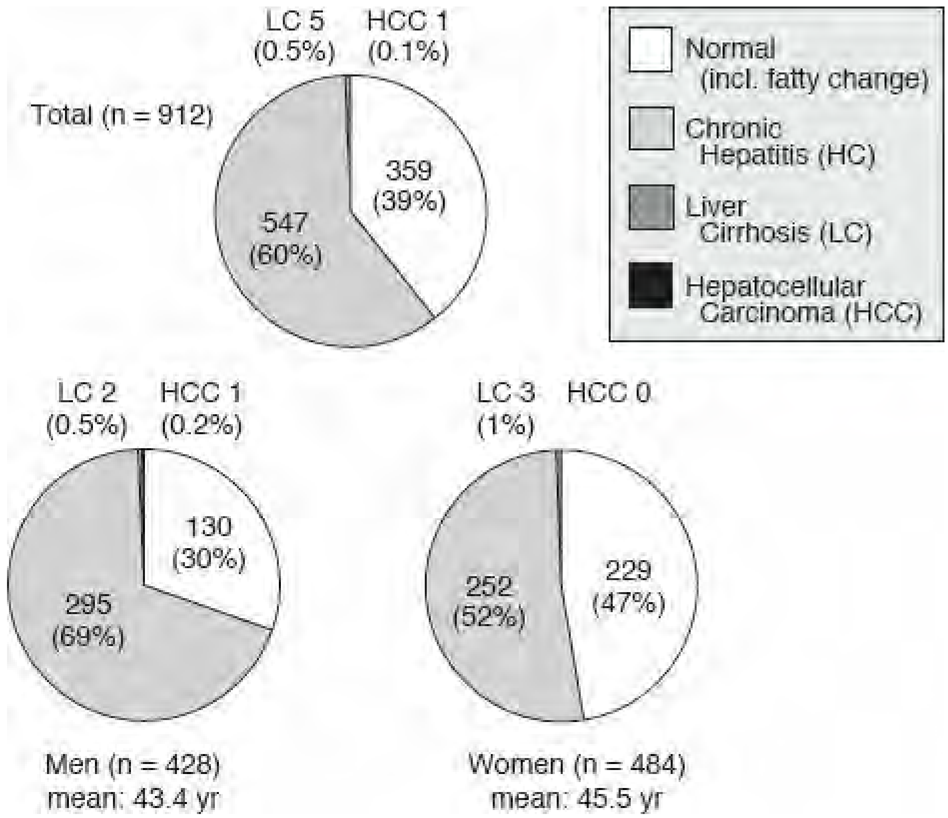

Figure 6. Distribution of liver disease in blood donors in Hiroshima who were found positive for anti-HCV.

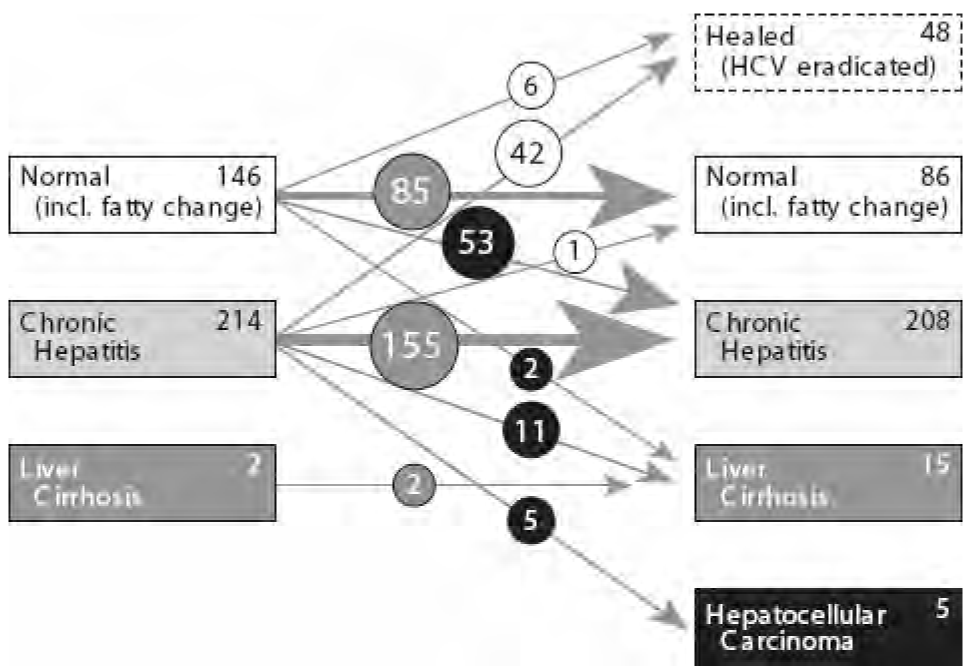

Figure 7. Evolution of liver disease in blood donors with anti-HCV in Hiroshima during follow-up for at least 5 years.

These observations warrant the need for systematically identifying HCV carriers hidden in the general population. These individuals need to be identified, evaluated for the presence of liver disease and the attendant implications for treatment, and then followed closely even if immediate medical intervention is unnecessary. By coordinated efforts of the government,

co-medicals, and doctors, it would be reasonably expected that deaths due to HCV-associated HCC will decrease.

The natural history of HCV infection is influenced by many factors and can hardly be generalized [1,19]. It is subject to cohort effects, with the evolution of liver disease faster in patients who visit hospitals [10]. In contrast, children and young women fare much better and infrequently develop clinical disease during long-term follow-up [9,25]. Furthermore, the progression of liver disease is influenced by a number of factors, such as ethnicity, obesity, and alcohol intake. Since it takes decades before liver cirrhosis and HCC to develop in a proportion of HCV carriers, the clinical course is difficult to be seen by a single doctor in the same hospital. In addition, it would be unethical to observe the natural disease course among HCV carriers without offering antiviral treatments of proven efficacy when such individuals are diagnosed with clinical disease.

The Markov chain model is very instrumental for simulating the natural history of chronic diseases [20], and we have applied it to the natural history of HCV infection [22]. The probability of transition between any 2 of the 4 stages, i.e., asymptomatic carrier state, chronic hepatitis, liver cirrhosis, and HCC or death, were calculated on the basis of 2,251 patient-year data obtained from rigorous medical examinations performed on this group at least once a year. HCC was defined as the absorbing state from which no transitions occur, since it has become infrequent for patients with liver cirrhosis in Japan to die of hepatic decompensation or gastrointestinal bleeding. With use of probability matrices constructed on 6 subsets of individuals with HCV infection (asymptomatic carrier state, chronic hepatitis, and liver cirrhosis in men and women) in their 40's, 50's, and 60's, long-term outcomes of HCV infection were simulated.

The outcomes of men and women who are asymptomatic carriers at the age of 40 years are illustrated in Figure 8. After 30 years, male asymptomatic carriers are expected to remain in the asymptomatic carrier state in 2.6%, evolve into chronic hepatitis in 48.4%, progress to liver cirrhosis in 14.6%, and develop HCC in 34.4%, when they reach 70 years of age. The corresponding rates for these outcomes among female asymptomatic carriers aged 40 years were 1.9%, 43.5%, 32.8%, and 20%, respectively. The validity of these simulations was verified in 153 HCV carriers who were identified among blood donors and followed for 5 years [22].

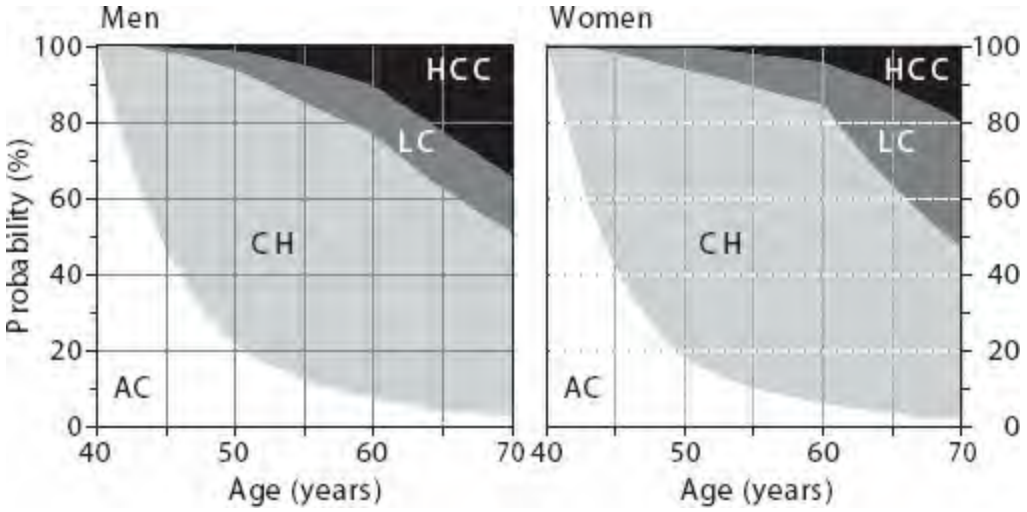

Figure 8. Simulation of clinical courses of 40-year-old male and female asymptomatic carriers of HCV during 30 by the Markov model (reproduced from Reference 22 with permission).

People in Japan, especially the elderly, have been exposed to HBV in the past, although most of them have resolved infection and cleared hepatitis B surface antigen (HBsAg) from the circulation by the present time. HBV remains in the liver, however, even after HBV infection is terminated, accompanied by the seroconversion to antibody to HBsAg in serum [24,27]. Eventually, the antibody to hepatitis B core (anti-HBc) remains as the only serological marker of resolved HBV infection. A high prevalence of anti-HBc in patients with HCV-associated HCC has been reported repeatedly and proposed as evidence for synergetic effects of the past HBV infection on the frequent development of HCC in HCV carriers in Japan [12,18]. The resolved HBV infection common in aged patients with HCV-associated HCC, however, does not contribute to their hepatocarcinognesis. It represents a cohort effect rather than a casual relationship. In support of this view, anti-HBc was detected significantly more frequently in blood donors of the same age with anti-HCV than without it (56.3% vs. 25.5%, $p<0.001$) [7]. The prevalence of anti-HBc in the Japanese with anti-HCV was no different, however, between those with and without HCC (54.0% vs. 56.3%), thereby indicating that a resolved HBV infection does not contribute to the development of HCC in HCV carriers.

Strategies for Preventing Evolution of Chronic Hepatitis C into HCC

More than 90% of HCC develops in patients with advanced chronic hepatitis and liver cirrhosis, and 80% of it represents the long-term sequel of HCV infection in Japan. Hence, it is very effective to identify HCV carriers in the general population who are at increased risk for developing HCC and to take measures to prevent the occurrence of this outcome. Three options for preventing HCC among HCV carriers are listed in Table 1. The first choice, of course, is to terminate HCV infection by antiviral therapy, such as interferon with or without ribavirin, in patients for whom such treatment is indicated. The indication is judged from viral factors, such as viral load and genotype, as well as host factors including grade and stage of liver disease. For those who do not respond to antiviral therapies or for whom this therapy is not indicated, and in whom necroinflammation in the liver is active with accompanied by elevated serum transaminase levels, conservative treatments are an option. Stronger Neo-Minophagen C (Minophagen Pharmaceutical Co., Tokyo, Japan) and ursodeoxycholic acid, as well as phlebotomy for decreasing iron loads in the liver, can be given to suppress the inflammation and retard or prevent the progression to fibrosis. Such treatments have proven to be effective in decreasing the development of HCC [2].

Table 1. Three Options for Preventing and Controlling Hepatocellular Carcinoma (HCC) Arising in Carriers of Hepatitis C Virus (HCV)

1. **Causative Treatment**
   Antivirals such as interferon combined with ribavirin for clearing HCV infection and removing the high risk of developing HCC
2. **Conservative Treatment**
   Anti-inflammatories such as Stronger Neo-Minophagen C as well as ursodeoxycholic acid and phlebotomy for retarding the development of HCC
3. **Early Diagnosis of HCC**
   Close surveillance for HCC toward radical treatment enabling a prolonged survival

There is a third option for patients of cancer-bearing ages who cannot receive these 2 therapeutic options for any reason and in whom fibrosis has progressed. These patients need to be followed closely by imaging modalities and tumor markers in serum (AFP and PIVKA-II). Such surveillance helps in an early diagnosis of HCC with the possibility of radical treatment by surgical resection. For those in whom surgery is not indicated, percutaneous ethanol injection therapy (PEIT), percutaneous microwave coagulation therapy (PMCT) and radiofrequency ablation (RFA), as well as transcatheter arterial embolization (TAE) and continuous infusion with anticancer agents through hepatic artery feeding HCC, can be performed.

Two major points that should be kept in mind for improving the effectiveness of any

project for preventing HCC are: (1) establishment of an effective system to detect HBV or HCV carriers; and (2) systematic and logical management of identified hepatitis virus carriers for prevention and surveillance of HCC as well as prompt treatment of these patients as required.

Methods for Detecting HCV Carriers on the National Scale

The national project for screening Japanese for HBV and HCV infection was launched in April 2002. It is performed on 2 distinct populations. Health check-ups are offered to Japanese citizens every 5 years starting at the age of 40 years until the age of 70 years. By screening at 5-year intervals, all carriers of hepatitis viruses can be identified who are aged 40 years or older. Beginning with the 6^{th} year, only individuals who reach 40 years in that year need to be screened. This project is based on an extremely low incidence of HBV or HCV infection in Japan [26], which makes it sufficient to screen for viral markers only once in a lifetime.

Those who do not receive regular health check-ups are given the opportunity to receive tests at least once. These include individuals at high risk of HCV infection: (1) who were found with abnormal liver function tests in the past; (2) who had a major operation or a history of massive hemorrhage at delivery, and are not receiving regular monitoring for liver function; and (3) in whom liver function tests gave abnormal results at regular health check-ups.

Individuals older than 70 years and not receiving regular health check-ups are also offered testing for viral markers upon request. In addition, individuals testing negative for viral markers but who later produce abnormal liver function test results, with or without clinical symptoms of hepatitis, can consult with their doctors to determine the indication to screening for viral markers.

Anti-HCV is detected in sera from individuals who have ongoing HCV infection as well as from those who have resolved infection. These 2 categories of anti-HCV need to be distinguished from each other. When the national screening was started in April 2002, individuals were screened for anti-HCV, and those with anti-HCV in middle titers only were evaluated for the presence of HCV RNA in serum by NAT to detect ongoing HCV infection. When the ability of this method in efficiently identifying HCV carriers was evaluated in a model area, however, rare persons with low anti-HCV titers tested positive for serum HCV RNA. These individuals would not have undergone NAT evaluation under the original plan.

As a result, a 3-stage screening process has been developed as illustrated in Figure 9. Starting in April 2003, individuals testing positive for anti-HCV in low or middle titers were tested for the HCV core protein, and those with negative results only are tested for HCV RNA by NAT. Individual testing positive for the HCV core protein are deemed carriers.

Testing for HBsAg is performed simultaneously as a serum marker of ongoing HBV infection, for identifying HBV carriers who are at risk of developing HCC as well.

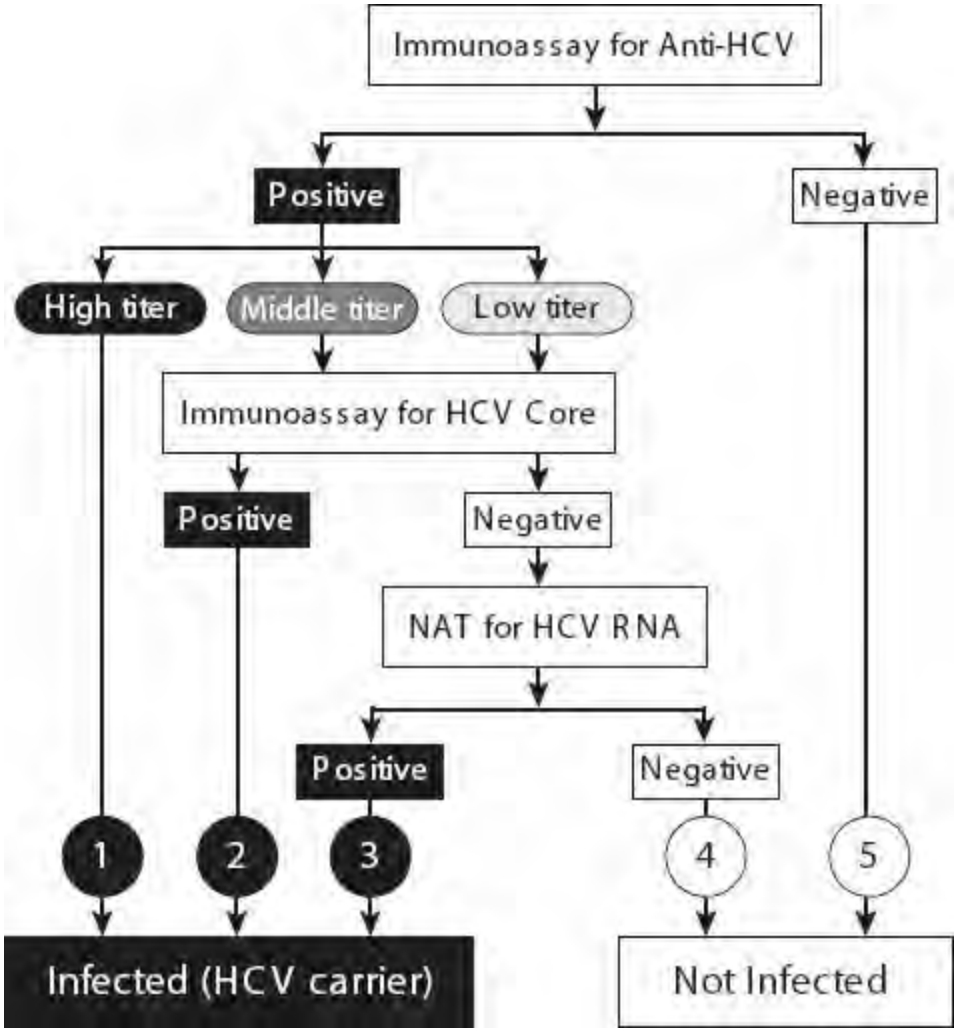

Figure 9. Strategy for the national screening for persistent HCV infection with immunoassays for anti-HCV as well as HCV core protein combined with nucleic acid amplification testing (NAT).

Results of the National Screening Project for Detecting Hepatitis Virus Carriers in the First Fiscal Year (April 2002 – March 2003) and Prospects for the Future

The Japanese Ministry of Health, Labor, and Welfare has reported results of the national screening project in the first fiscal year from April 2002 to March 2003. Of the 3,212 cities and towns in Japan, 2,923 (91%) began HCV screening with good compliance. The Department of Welfare for the Aged compiled results in the first fiscal year and identified 1,923,480 individuals who underwent testing of whom 31,393 (1.6%) were found to have ongoing HCV infection. There were 1,298,746 individuals who received tests at regular health check-ups, and 14,672 (1.1%) of these were found to be HCV carriers. In contrast, 16,721 (2.7%) of the 624,734 high-risk persons who received tests on other occasions were found to have ongoing HCV infection. This reflects a frequency twice as high as that among those receiving regular health check-ups. HCV carriers were found in 3.2% and 1.3% of the individuals aged 65 to 70 years at high risk and those on check-ups, respectively, and more often in individuals aged 70 years or older (4.0% and 2.1%, respectively).

The numbers of HCV carriers who will be identified during the 5-year project for national screening can be estimated by the prevalence of anti-HCV in first-time blood donors (Figure 4). HCV carrier state is considered to be present on average in 70% of blood donors testing positive for anti-HCV. Sex- and age-specific prevalence rates of anti-HCV were determined individually in 8 jurisdictions of Japanese Red Cross Blood Centers. The estimated total number of HCV carriers in each jurisdiction was obtained by multiplying these prevalence rates by the sizes of the corresponding subpopulations stratified by sex and age extrapolated to reflect the population size in 2000. There are an estimated 850,500 carriers of HCV in Japan who are aged 15 to 69 years, with little difference in numbers between men and women. Targets of the national screening program, who are aged 40 to 70 years, encompass 760,000 HCV carriers and account for 86% of those aged 15 to 65 years (Figure 11).

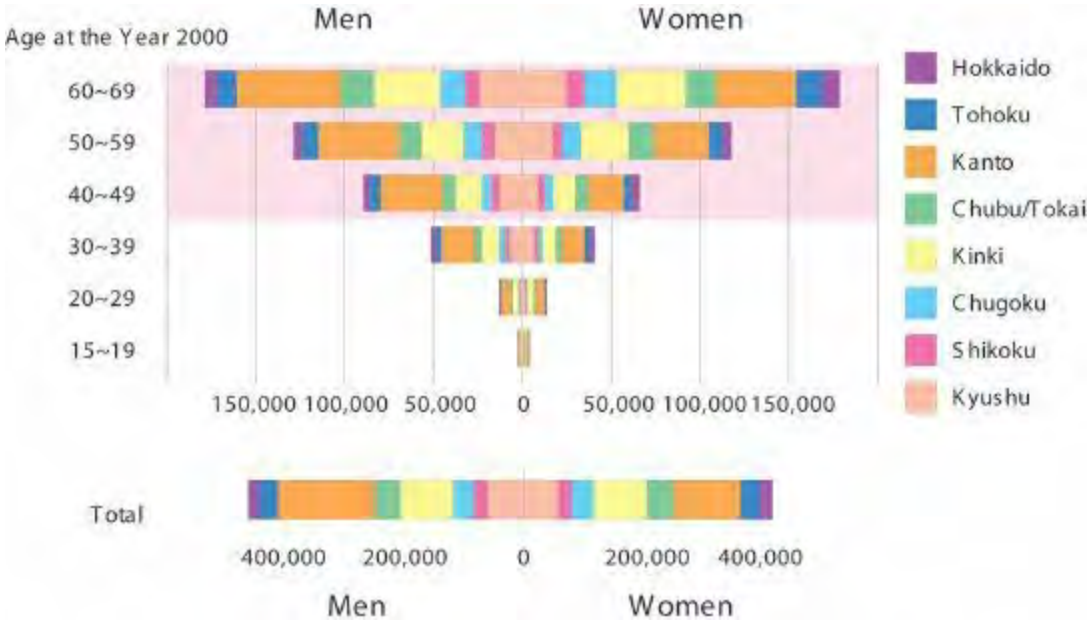

Figure 11. Carriers of HCV stratified by sex and age in Japan estimated by the prevalence of anti-HCV in the first-time blood donors and corresponding subpopulations registered at the Census 2000 (from Reference 23 with permission).

Detection of 29,809 HCV carriers amongst the 1.8 million receiving screening during the first fiscal year may have unfolded only a fraction of persistent HCV infection in the target individuals. Further efforts to get the Japanese populace to be aware of the risk of HCC associated with HCV infection and for orienting them to receive screening are necessary. High compliance and increased reception rate will only be achieved by concerted efforts among the government, medical community, and public.

HCV Infections Throughout the World

The age-specific prevalence of HCV infection in the year 2000 is compared between Japan and Kiev, Ukraine as well as with the United States (U.S.) in Figure 12. These data are based on 270,000 blood donors in Hiroshima for Japan, 40,000 blood donors in Kiev for Ukraine, and data reported by the Centers for Disease Control and Prevention for the U.S. The age groups with the highest prevalence of anti-HCV differ markedly among the 3 countries. The prevalence of anti-HCV steeply increases with age in Japan, so that it is the highest in the elderly who scale out of the figure. In contrast, the highest prevalence of anti-HCV occurs in much younger individuals, aged 40 to 44 years, in the U.S., and in those even younger, aged 30 to 35 years, in Ukraine.

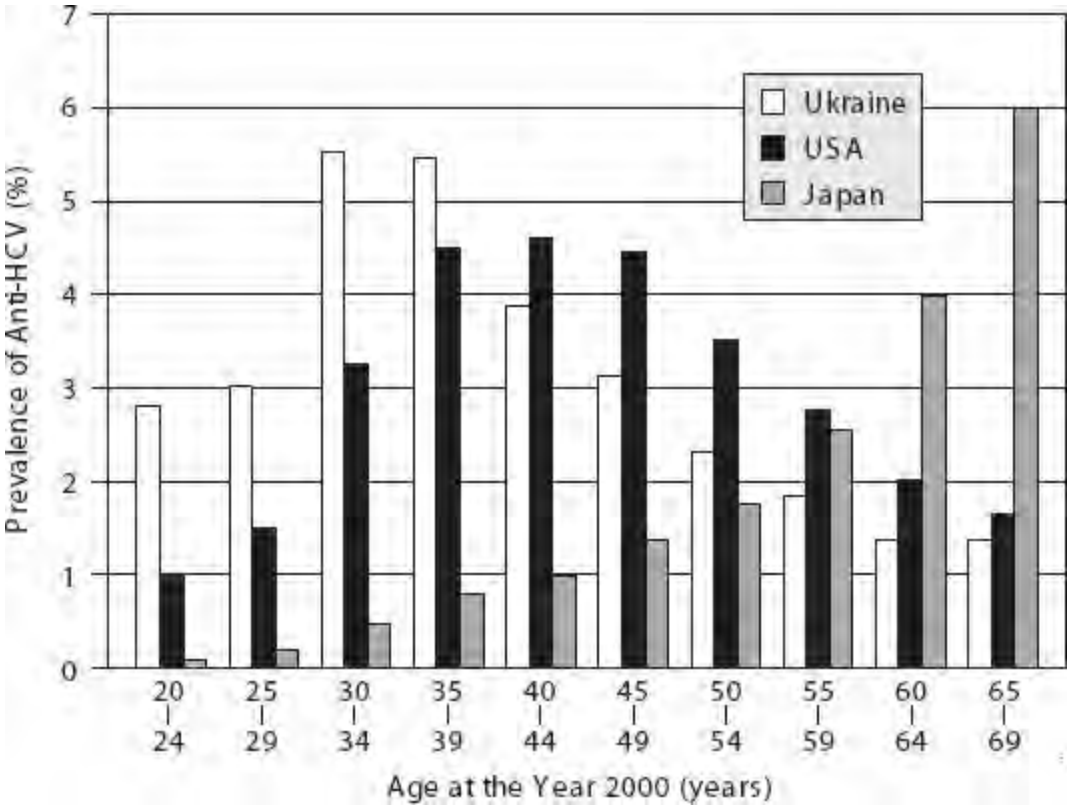

Figure 12. Age-specific profiles of HCV infection in Japan, the Ukraine, and the U.S.

Wide differences in the age at which anti-HCV is most prevalent among the 3 countries are attributed to distinct time points when HCV spread extensively among these populations. In Japan, HCV infection infiltrated the subpopulation of younger generations during the 1950s through 1960 in a vicious cycle [26]. About 2 decades later during the 1970s to early 1980s, HCV infection was widely diffused in the U.S. although it has become less invasive at the present. HCV infection started to spread in the Ukraine since the late 1980s and has continued to involve younger generations. What we witness for the age-specific distribution in the 3 countries at present are long-term sequelae of the shifting peak of age-specific HCV infection with time.

Conclusions

Today, HCC in Japan preferentially occurs among individuals in their late 50's through early 60's. Fortunately, the life expectancy of patients with HCC has been extended by 6 to 7 years with the advent of efficient treatments. Based on the age-specific distribution of anti-HCV in Japan, deaths due to HCC that have increased continuously until the present will reach a plateau shortly and then start to decrease around 2005–2010. By contrast, in the U.S. the individuals infected with HCV in the past are just now entering the cancer-bearing ages. If appropriate measures are not taken there, the steep increase in deaths due to HCC that we have experienced in Japan since 1975 (Figures 1 and 2) is likely to be reproduced there. The same situation is expected in the Ukraine but the peak is likely to occur some 10 to 15 years later than in the U.S. In addition, the incidence of HCC is increasing already in countries where HCV prevails, including Australia [14], France [3], Italy [21], and Sweden [8], and is expected to double in the U.S. over the next 10 to 20 years [6].

It is our sincere hope to accomplish a major success with screening for hepatitis virus infections through the national project in operation begun in Japan in April 2002. Should our 5-year strategy to cope with HCC induced by persistent hepatitis virus infections prove successful, our experience will likely be valuable to other countries throughout the world where HCV infection prevails.

Acknowledgments

We thank members of Hepatitis Research Groups under the auspices of the Japanese Ministry of Health, Labor, and Welfare and persons in charge of screening for hepatitis virus infections in respective jurisdictions of the Japanese government. Our thanks are due, also, to members of the Japanese Red Cross Association who have helped us collect data from blood donors and compiling these data for analyses, and to Dr. Yoshihiko Miura of Saitama Prefectural University for calculating standardized rates of mortality by HCC for each jurisdiction in Japan. The epidemiological surveys for serological markers of hepatitis virus infections and organization of the guidelines for the national survey have been conducted as a part of researches granted by the Japanese Ministry of Health, Labor, and Welfare (H-13-Shinko-6 and H13-21 Seiki (gan)-32).

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Discussion

Viral Hepatitis in Asia

Question for Dr. Mishiro, Toshiba General Hospital, Japan: Given the extent of HEV in the animal population, particularly among swine and rodents, why is there so little communication to humans? In particular, I would think that slaughter-house workers who are in immediate contact with blood and meat would be particularly vulnerable.

Answer by Dr. Mishiro: The Japanese have little data on HEV, but the researchers in India have reported that if you get infected with HEV when you are younger than 30 years old, your symptoms will be very mild. We have many HEV RNA-positive animal handlers but most of them got HEV much earlier in their life. Infection has spread widely but the active disease is rare under 30 years old. Our experience in Japan indicates that most patients are over 50 or 60 years old.

Question for Dr. Lemon, The University of Texas Medical Branch at Galveston, U.S.A.: Are there genetic factors that influence the probability of developing chronic hepatitis B infection?

Answer by Dr. Lemon: There have been studies that have looked at human histocompatibility antigen (HLA types) and have found some association between risk of persistent versus non-persistent infection. My response would be that it's a mixture of both viral and host genetic factors, as well as the age of the host, presence of other infections, and other influences on the immune system that might be the prime determinants.

One additional comment. There was a slide shown from Sherlock's textbook on the liver with a statement that 10% of infections become chronic. That has been one of those generalizations that have become burned at least into American medicine, and I think it's completely wrong. In the follow-up studies of recipients of the contaminated yellow fever vaccine that was received by American troops in the early 1940s, there were very few carriers. We did a study of several hundred American servicemen when I was with the Army 20 years ago that were infected with hepatitis B. Every one of them as I recall cleared the infection in follow-up. That is one of those generalizations that I think comes from looking cross-sectionally at patients coming in with hepatitis B and seeing that some are carriers and some are not. If you look at incident infections in adult individuals who are healthy with normal immunologic systems, there is an extremely low rate of persistence to chronicity.

Question to Dr. Mizokami, Nagoya City University Graduate School of Medical Sciences, Japan: Can you speculate whether our Japanese ancestors carried the HBV subtype Bj from Mainland China to the Korean peninsula or did this subtype evolve after our

ancestors came to Japan?

Answer by Dr. Mizokami: HBV infecting humans are classified into 6 genotypes. Genotypes A and D are globally distributed, genotypes B and C are found mainly in East and Southeast Asia, genotype E is predominantly in West Africa, and genotype F is found among indigenous peoples of Central and South America. Genotype B is an older form, coming from the South to Japan. I tried to determine if this was via our Japanese ancestors or from the Alaskan Eskimo, as this group separated from Mongolia and cross the Bering Strait. If my speculation is correct, perhaps the Alaskan Eskimo is the original source of HBV genotype Bj.

Comment by Dr. Mishiro: Hepatitis B virus can function as an archeological marker because it is transferred from mother to infant vertically like mitochondrial DNA.

Question for Dr. Mishiro: As you know with hepatitis A virus which is culturable, it is not a problem to test sterilants and germicides. But with hepatitis B since it cannot be cultured, it is a problem. There was work in the 1970s at the labs at the CDC and Dr. Kobayashi in Japan using the chimpanzee infectivity model. More recently, the surrogate virus of the duck hepatitis virus was used. For hepatitis C, bovine diarrheal disease virus is used as a surrogate for C. My question is what would you recommend for hepatitis E virus in the context of a surrogate virus?

Answer by Dr. Mishiro: I don't know a model virus that is suitable to serve as a surrogate for HEV in cell culture.

Comment Dr. Lemon: I agree, I don't think we have a good surrogate. I don't think we know enough about the physical characteristics of this virus to say much about it. Certainly there is no other virus that we have in a culturable system that we can use a ready surrogate. I'm also not to sanguine about using bovine diarrheal disease virus as a surrogate for hepatitis C because again there are big differences in the structure, even in terms of the number of envelope proteins and so forth. So I think you need to be very careful when you make extrapolations outside of the immediate genus of the virus you are working with.

Session V

Viral Infections

Chairman: **Takashi Uchiyama, M.D., Ph.D.**
Kyoto University, Japan

Opening Remarks

Takashi Uchiyama, M.D., Ph.D.

Graduate School of Medicine, Kyoto University, Japan

Welcome to this afternoon's session. It is a great pleasure and honor to chair this session on viral infections. As Dr. Hughes described in his Keynote Address, new emerging or re-emerging viruses have been producing global threats to human health, including Ebola virus, Hantavirus, human T-cell leukemia virus type 1 (HTLV-1), human immunodeficiency virus (HIV), human herpes virus 6 and 8 (HHV-6,8), hepatitis E and C virus, and SARS coronavirus.

HTLV-1 was discovered in 1980. It appears that studies on HTLV-1 have been concentrated on a particular viral protein called Tax, a 40kDa nuclear phosphoprotein mainly encoded by pX of the HTLV-1 genome. Tax interacts with various kinds of cellular proteins, which results in the changes of transcription of cellular genes, apoptosis, cell proliferation, and genetic instability. Some of these effects exerted by Tax are considered to be closely associated with the initial changes of HTLV-1 infected T-cells and eventually lead to the development of adult T-cell leukemia (ATL). Dr. Matsuoka will talk about the function of Tax, focusing on genetic and epigenetic changes in the development of adult T-cell leukemia.

Another human retrovirus, HIV, was discovered and identified as a causative agent of AIDS in 1983. Subsequent studies on the genetic structure, life cycle, epidemiology and pathogenesis of the virus have provided us with not only a better understanding of HIV and a deeper insight into the mechanism of the disease, but also effective ways to at least partially control HIV infection. An increasing amount of evidence has been accumulated about viral receptors, cell entry mechanisms, the interaction between viral accessory molecules including Tat, Rev, Nef, Vpr, and Vif host factors, mechanism of latent infection and immuno-pathogenesis. The introduction of highly active anti-retroviral treatment (HAART), has clearly prolonged survival of HIV-infected patients. The most unique biological aspects of HIV are probably the high error rates of reverse transcriptase and high rates of recombination. Dr. Max Essex will discuss genetic variation among emerging HIVs.

In addition to these emerging viruses, Epstein-Barr virus, which has been implicated in Burkitt's lymphoma and nasopharyngeal carcinoma, has recently been found to be causally associated with other types of cancer such as Hodgkin's disease, NK-cell lymphoma, and gastric cancer. Epstein-Barr virus was discovered in 1964 by electron microscopy of cells cultured from Burkitt's lymphoma tissue. Epstein-Barr virus research has progressed slowly as compared with research on other viruses probably due to its large genome size. Dr. Takada will discuss the key role of Epstein-Barr virus encoding small RNA in the maintenance of malignant phenotypes of these tumor cells.

West Nile virus was first isolated in 1937 from the blood of a febrile patient in the West

Nile district of northern Uganda. West Nile virus is a mosquito-borne flavivirus and human, equine, and avian neuropathogen. The virus is indigenous to Africa, Asia, Europe and Australia. In 1999, the virus was first introduced into the New York City area and subsequently spread throughout much of the eastern half of the United States of America. Neither virus specific treatment nor an effective vaccine is available for West Nile virus. Dr. Peterson will discuss the current state of our knowledge about West Nile virus.

Influenza virus is a member of the orthomyxoviridae family. The segmented genome of these viruses facilitates the development of new strains through mutation and re-assortment of gene segments between different human and animal viral strains. This genetic instability is responsible for the annual epidemics and periodic pandemics like that of 1918. Dr. Palese will talk about lessons learned from the 1918 influenza pandemic.

Genetic Variation Among Emerging HIVs

Max Essex, D.V.M., Ph.D.

Harvard AIDS Institute, U.S.A.

Introduction

The acquired immunodeficiency syndrome (AIDS) and its cause, the human immunodeficiency virus (HIV), were not known to exist until 20 to 25 years ago. Based on serological surveys of samples from central Africa, it appears that the virus or viruses first entered the human population only 50 to 100 years ago [15].

Analysis of genetic sequences with calculations of differences between HIV-1 and HIV-2 also suggest a similar time for the entry of HIVs into the human population [24]. Despite this, the AIDS epidemic should soon surpass the bubonic plague of medieval Europe as the largest new epidemic of recorded medical history.

Effective drugs are now available to treat AIDS. They function by inhibiting 2 viral enzymes, the reverse transcriptase and the protease, that the virus requires for replication. No effective vaccines are on the horizon, although several designs are now receiving serious evaluation in human trials. While the drugs work well in carefully selected combinations, drug-resistant variants rapidly emerge when only 1 or 2 are used alone. The greatest impediment to an effective vaccine and the problem of HIV drug resistance are both linked to the same fundamental property of HIV – an unusual ability for very rapid genetic change.

Virus Characteristics

HIVs enter susceptible cells by initially attaching to a receptor, CD4, that is present on helper T lymphocytes, and in lower densities, on some other cells such as macrophages. Subsequently, the virus attaches to a co-receptor, usually CCR5 or CXCR4, – 2 molecules in the chemokine receptor family that are normally found on the same cells [2,5].

After the virus enters the cell, the RNA genome, which contains 2 complete copies of the genetic information, is transcribed into strands of proviral DNA. The DNA then integrates into the chromosomal DNA of the host cells. These characteristics allow extreme versatility. First, the diploid nature of the genome and the mechanism of transcription allow recombination between different viral parental genomes to occur very effectively when a single cell happens to be infected by viruses of different origin. Second, because transcription of RNA is very prone to accumulation of mutational errors, the process of reverse transcription allows for very rapid generation and accumulation of genetic changes. Third, because the DNA proviral copy then integrates into the chromo somal DNA using the viral endo-nuclease, it can remain in a latent, inactive form for long periods of time. When taken together, these properties allow the virus to evade elimination in individuals and to adapt to successful survival and spread in populations. During production and assembly of progeny virus particles, 2 different genotypes of RNA may be packaged in the same virion when completing the cycle of replication.

The HIV has a genome of about 10,000 nucleotides and several areas seem particularly important for genetic changes and selection pressure. The outermost surface of the virus is covered by projectile glycoproteins, gp120, that allow the virus to form specific bonds between the receptors and coreceptors for attachment. Because gp120 is also the portion of virus that could then serve as a target for neutralizing antibodies, the genetic changes in this gene allow for vigorous immunoselection pressure to occur. The area of gp120 that serves as the primary target for neutralizing antibodies is the third variable loop, V3. As a result, this region of the gene sequence of the virus often reflects more rapid mutational changes than other regions. In contrast to some of the regions of the genome that encode enzymes, such as the reverse transcriptase and protease, gp120 shows great plasticity in allowing full functional activity with extensive changes in the amino acid sequence.

The internal core proteins of the virus, which are encoded by the *gag* gene, show more constraint for genetic conservation than gp120, but immune epitopes linked to the cytolytic T lymphocyte (CTL) response also show variation due to immunoselection [1]. The long terminal repeat (LTR) sequences are another area of particular interest which appears to evolve to adapt to changes in the *tat* regulatory gene and host-cell-related DNA binding proteins [12].

When a person gets infected with HIV, there is a period during which the body becomes seeded with infected cells. After a peak phase of viremia that occurs at about 4 to 6 weeks, a combined CTL response targeted at cells that are replicating virus, and neutralizing antibodies directed at free virus, effectively lower viral titers by several logs. While some positive T lymphocytes are killed, a rebound occurs as virus levels are greatly diminished. Gradually, after months and years, virus levels again rise as neutralizing and

CTL mutant escape variants take control. Eventually, HIV levels become very high, and CD4 cell levels become very low, causing severe impairment of the immune system. As CD4 counts fall below 200 cells, opportunistic infections such as extrapulmonary tuberculosis and cryptococcal meningitis become much more common.

Global Burden

Of approximately 42 million current infections with HIV, as estimated by UNAIDS for the end of 2002, 70% are attributed to sub-Saharan Africa [28]. Of 6 million new infections, 70% were estimated to have occurred in sub-Saharan Africa, 14% in South and Southeast Asia, and 16% in all of the rest of the world combined.

At least 6 countries have rates of HIV infection that exceed 20% in adults aged 18 to 49 years. All of these countries are in southern Africa, where mean rates of infection in adults now exceed 20% [4]. In countries like Botswana and Zimbabwe, for example, it has been estimated that life expectancy by the year 2010 could drop by as much as 40 to 45 years unless changes occur [25]. Rates in West Africa and East Africa are somewhat lower, but still substantially higher than for all the other regions of the world.

Although much attention has been devoted to the possibility of an impending epidemic in Asia, adult rates in India were estimated to be below 1% in 2002, and in China were estimated to be only about 0.1% [28]. Rates of infection in the United States (U.S.), Europe, and South America are also below 1%. Outside of Africa, only a few countries, such as Cambodia or Haiti, have rates of 3% or above.

Variation Among HIVs

Soon after the recognition of AIDS and the link to a retrovirus cause, it became clear that a wide range of lentiretroviruses were already present in the human population. Some of the HIVs in people apparently represent different events of entry from non-human primates. Others represent rapid diversification of HIV-1 following what was presumably a single source entry.

HIV-1s presumably came from chimpanzees or other apes [3]. Most subtypes or clades of HIV-1 are within the major or M group, where nucleotide sequence relatedness shows 20 to 30% differences within the envelope gene and 10 to 15% differences within the *gag* core genes for different subtypes or clades. Only 4 subtypes – A, B, C, and D – have accounted for ≥ 1 million infections in people. HIV-1 B and D are more closely related to each other than are other subtypes, such as HIV-1A and HIV-1C [21].

Two other HIV-1 viruses from the M group that account for more than a million infections are actually intersubtype recombinants. These are designated as circulating recombinant forms (CRFs). CRF 01 A/E was initially described as HIV-1 subtype E because the envelope of the virus is from a distinctly different subtype or clade, designated E. This virus is predominant in Thailand and adjacent regions of other countries in Southeast Asia where it has spread much more rapidly than the HIV-1 B that was introduced earlier in that region [7]. The second CRF that has spread to large numbers of people is CRF 02 A/G, a recombinant between HIV-1G envelope and the HIV-1A viral core [20]. CRF 02 A/G is now the second most common HIV in the world, accounting for the majority of infections in western and central Africa [4].

HIV-2 also appears to have entered the human population relatively recently, but perhaps before HIV-1. HIV-2 almost certainly entered from mangabey monkeys in West Africa because it is almost indistinguishable from simian immunodeficiency viruses (SIV) that can be regularly found in a large fraction of the healthy wild monkeys of this species [6]. Although SIVs were first identified in captive, ill Asian macaque monkeys, they are not native to this species and were presumably introduced through experimental procedures [8]. HIV-2s are only about 40% related to HIV-1s at the gene sequence level, but were almost certainly derived from the same ancestral virus.

HIV-2 is less virulent than HIV-1. By the criteria of disease induction, HIV-2 causes clinical AIDS, but less efficiently than HIV-1 [11]. By the criteria of transmission, HIV-2 is much less efficiently transmitted from infected mothers to their newborn infants and also less efficiently transmitted between adults by sexual contact [9].

HIV-1 Subtype C

HIV-1C now accounts for the majority of infections in the world, more than the other HIV-1 subtypes combined [4]. While HIV-1C accounted for a relatively small fraction of the world epidemic in the 1980s, the rampant epidemic of HIV-1C that began in southern Africa in the late 1980s and early 1990s now accounts for the highest rates of infections. Although no studies have been conducted to compare sexual transmission rates of HIV-1C to other subtypes, 1 report suggests that HIV-1C was more efficiently transmitted from infected mothers to newborn infants than was HIV-1D in the same population of mothers in Tanzania [22]. Another group found that viral RNA load levels in the blood of HIV-1C-infected people was higher than for infections with other subtypes [16].

An analysis of viral genotypes suggested that HIV-1C was undergoing genomic variation more rapidly than were the other common HIV subtypes [19]. Whether this is related to higher replication rates or higher error rates during transcription, or both, is unclear. HIV-1Cs from southern Africa, however, usually have an extra nuclear factor kappa B site (NFκB), which is associated with more efficient transcription and/or activation from latency [14]. Among the factors that induce more efficient transcriptional activation of HIV-1C with 3 NFκB sites is tumor necrosis factor alpha (TNFα), a common inflammatory cytokine that is released in large amounts when tissue damage occurs [13].

The enhanced activation of HIV-1C by factors such as TNFα may be particularly important for the sexual transmission of HIV-1C. Sexually transmitted infections, when concurrently present with HIV, are known to increase the risk of HIV transmission. Herpes simplex type 2, which causes lytic lesions, shows an even greater association with HIV transmission than other sexually transmitted diseases [29]. Hypothetically, at least, we might postulate that this effect would be greater for enhancing HIV-1C transmission as compared to other HIVs because of the potential interactions of the HIV-1C NFκB and the TNFα.

Still another characteristic of HIV-1C that is different from the other major subtypes is the highly preferential use of CCR5 as the only major coreceptor used [27]. For all HIVs, CCR5 coreceptor use almost always occurs at the time of transmission and initial infection. After the infections progress to disease, however, a large fraction of the viruses of HIV-1B switch via mutation to preferentially use the coreceptor CXCR4 instead of CCR5 [26]. With HIV-1C, this apparently does not occur, except perhaps under rare circumstances. The significance of this for the epidemiology of HIV-1C is unclear.

Design of Vaccines and Drugs

Almost all of the current data that have established the efficacy of antiretroviral drugs and drug resistance are based on studies with HIV-1B, the only virus that occurs at high levels in the U.S. and Europe. Early results suggest that the same classes of drugs will also work effectively for other HIVs, such as HIV-1C, but subtle differences in drug sensitivity have been observed *in vitro* [10].

For vaccines, most of the early prototypes designed for human trials have also been made with HIV-1B immunogens, but recently more designs have been made with other subtypes, including HIV-1A and HIV-1C. Many vaccine researchers have argued that the variation seen between subtypes should not be important enough to require the use of different subtypes in vaccine designs, at least when CTL vaccine designs, as opposed to neutralizing antibody designs, are considered. However, the extent of genomic variation between subtypes is substantially greater than within subtypes.

When conducting surveys to map CTL epitopes among HIV-infected, asymptomatic people in southern Africa, it was clear that the immunodominant regions were distinct for HIV-1C-infected Africans as compared to HIV-1B-infected people in the West [17]. This is further complicated by the presence of different frequencies of major histocompatibility alleles involved in the presentation of the HIV peptides [18]. For HIV-1C in southern Africa, the most immunodominant region is in the p24 section of the *gag* gene protein [17]. In contrast, for the HIV-1B in western populations, the most immunodominant region is the p17 of *gag*. Most of the CTL epitopes that are most immunogenic in HIV-1C-infected populations do not overlap the immunodominant epitopes detected in HIV-1B-infected populations.

Recombinant HIVs

In some regions of the world, especially in sub-Saharan Africa, HIV epidemics caused by different viral subtypes have occurred together. Perhaps the most dramatic example is in Tanzania, where the earlier East Africa epidemic of the HIV-1A and HIV-1D viruses fused with the HIV-1C epidemic that was moving northward from southern Africa [20]. This resulted in an elevated risk for many people to be concurrently infected with more than 1 subtype, creating an optimal environment for the generation of intersubtype recombinants. By evaluating the genotypes of viruses present in infected newborn infants, it was also possible to estimate the fractions of different intersubtype recombinants present in this population. In a survey conducted in 1998 in Dar es Salaam, more than 35% of the viruses surveyed were intersubtype recombinants [23].

While the generation of recombinants, like the generation of mutants, is presumably random, the viable viruses observed represent the results of selection for fitness within the host. Efficiency of sexual transmission then allows selection at the population level – an important step for those viruses that become CRFs.

Of those intersubtype recombinants recently generated in Dar es Salaam, 3 patterns of genomic organization have emerged. First, intersubtype recombinants that have HIV-1C as 1 parent appear to retain the V3 section of gp120 of HIV-1C origin. This implies that the HIV-1C V3 gives a selective advantage, perhaps at the level of evasion from antibody neutralization and/or coreceptor attachment. The second class of intersubtype recombinants that appear more often than we might expect due to chance alone are those with an incorporation of the non-coding long terminal repeat (LTR) sequences of HIV-1C. Since the HIV-1C LTR has an amplification in the NFκB promotor region, we can postulate that this acquisition gives recombinant viruses a selective advantage for transcriptional efficiency.

The third region that shows selective retention in intersubtype recombinants with an HIV-1D background is retention of the viral core *gag* gene of HIV-1D. Whether this is related to more efficient viral packaging, the retention of plasticity for CTL epitopes, or some entirely different function is unclear. Future studies of such recombinants as CRFs should allow a better understanding of these issues.

Conclusions and Projections

HIV-1C, which already accounts for about half of the world's infections, has infected more than 20% of adults in Southern Africa. As the global epidemic of HIV continues to expand, many new HIV-1 variants are emerging as CRFs. Such CRFs occur at the highest rates in those regions where epidemics of different subtypes have fused, such as Tanzania, where the earlier epidemic of HIV-1A and HIV-1D was overlapping with the newer epidemic of HIV-1C moving northward from Southern Africa.

Most of the current research on HIVs, including the development and testing of drugs and vaccines, has been with HIV-1B, the virus that predominates in the U.S. and Europe. The extent to which the newer viruses, such as HIV-1C and several CRFs, will show differences in epidemic transmission efficiency, drug resistance profiles, and overlap in immune epitopes potentially involved in protection has not been determined. The genetic diversity of existing and newly evolving HIVs has presented major challenges for the prevention and control of HIV infection.

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Human T-cell Leukemia Virus Type I Infection and Adult T-cell Leukemia

Masao Matsuoka, M.D., Ph.D.

Institute for Virus Research, Kyoto University, Japan

Introduction

Adult T-cell leukemia (ATL) is a highly aggressive neoplastic disease caused by human T-cell leukemia virus type I (HTLV-I) infection. ATL was identified as a distinct clinical entity by Takatsuki *et al.* on the basis of its unique distribution among patients and clinical and immunological features. Its causative virus, HTLV-I, was the first retrovirus shown to be associated with human disease, and it's discovery led to further understanding of the virological, immunological, and epidemiological aspects of ATL. However, the mechanisms of leukemogenesis have not been clarified.

Molecular Virology of HTLV-I

HTLV-I belongs to the Delta type retroviruses which also include bovine leukemia virus (BLV), human T-cell leukemia virus type II (HTLV-II), and simian T-cell leukemia virus (STLV). BLV and STLV have also been associated with neoplastic diseases. Like other retroviruses, the HTLV-I proviral genome has *gag*, *pol,* and *env* genes, flanked by long terminal repeat (LTR) sequences at both ends [65]. A unique structure was found between *env* and the 3'-LTR, denoted the pX region, that encodes the regulatory proteins, $p40^{tax}$ (Tax), $p27^{rex}$ (Rex), p12, p30, and p21 (Figure 1). Among these, Tax protein is thought to play a central role in the leukemogenesis of ATL because of its pleiotropic actions (Figure 2) [85]. Tax potently increases the expression of viral genes through the viral LTR and also stimulates the transcription of cellular genes through cellular signaling pathways of NF-κB, CREB, SRF, and AP-1. Tax does not bind to promoter or enhancer sequences by itself, but interacts with cellular proteins that are transcriptional factors or modulators of cellular functions.

Transcriptional Activation

Tax can activate the NF-κB pathway by interacting with IKKγ. IKKα, β, and γ form a 700kDa complex, in which IKKγ functionally adapts Tax into this large complex [33,35]. The activated complex phosphorylates IκB, that detaches NF-κB, results in activation of NF-κB. Activation of NF-κB induces transcription of various cytokines and their receptor genes as well as numerous genes associated with apoptosis and the cell cycle. For example, Tax can activate the transcription of interleukin (IL)-2Rα and IL-2 genes through the NF-κB pathway [8,79]. In addition, transcription of IL-6 [52], IL-15 [7], and GM-CSF [25] genes can be activated by the Tax protein via NF-κB. Such activation of genes associated with cell proliferation seems to be involved in the growth of HTLV-I infected cells both *in vitro* and *in vivo*. Tax can also induce expression of Bcl-X_L via activation of NFκB, which renders ATL cells apoptosis-resistant [75]. As well as HTLV-I transformed cell lines, increased expression of Bcl-X_L was observed in fresh ATL cells. This finding may account for the resistance of ATL cells to chemotherapy [54].

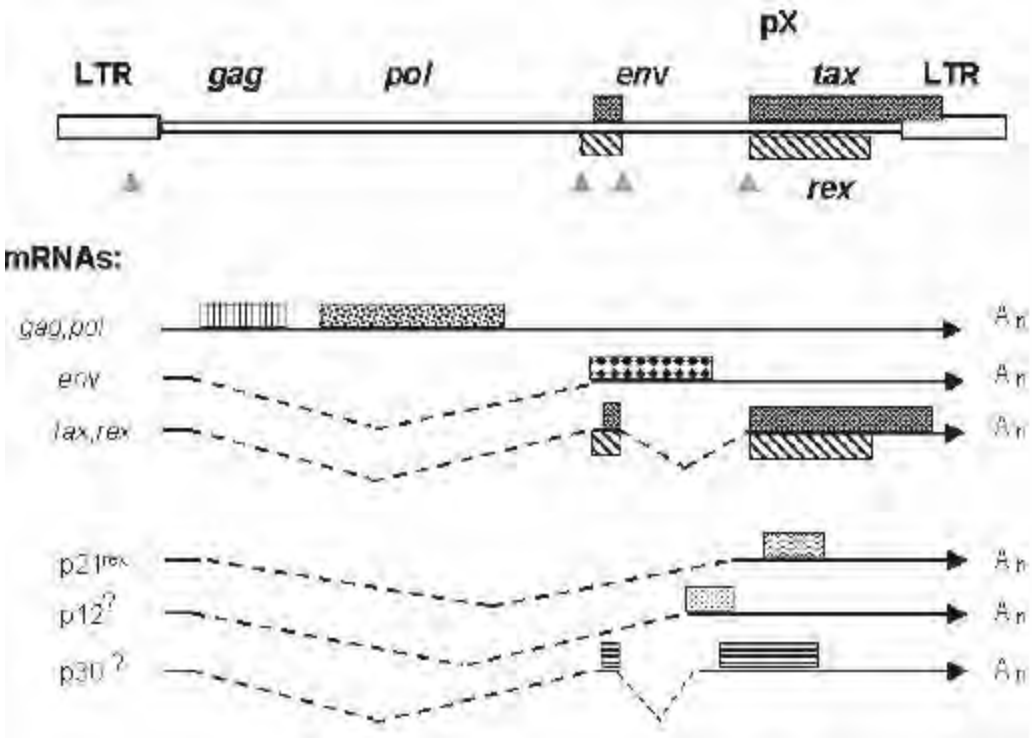

Figure 1. Structure of HTLV-I provirus and its encoding genes. The HTLV-I provirus genome is shown at the top of the figure. The structure of mRNAs and locations of proteins (boxes) encoded by the provirus are shown underneath.

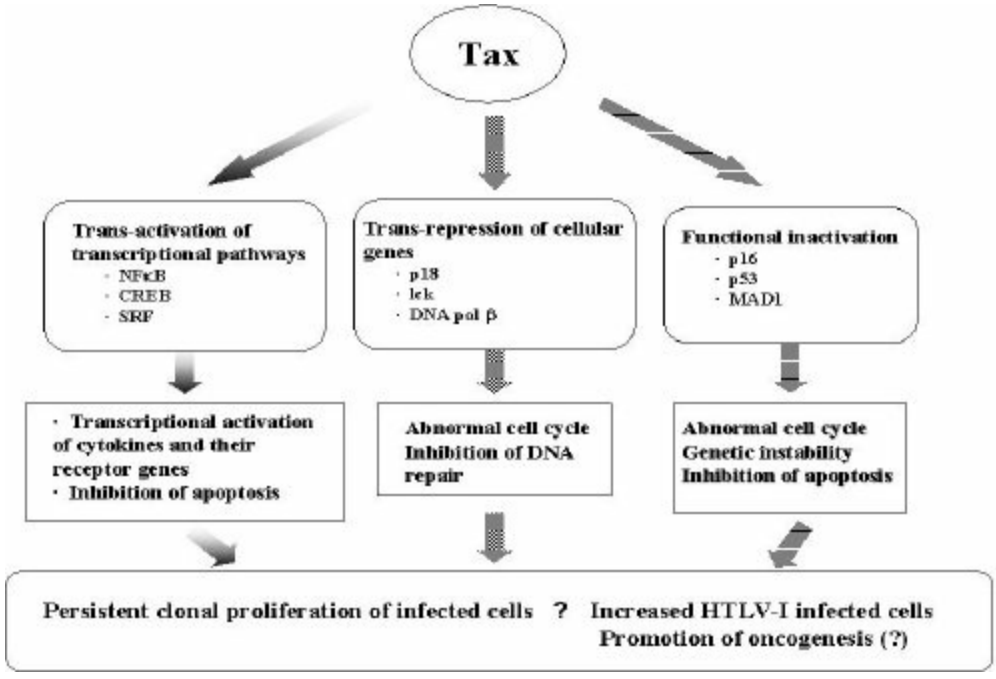

Figure 2. Pleiotropic actions of Tax proteins are summarized.

For activation of the viral LTR, Tax requires at least 2 21-bp enhancers containing an imperfect cAMP-responsive element, to which binds cyclic AMP response element binding protein (CREB) [81]. Tax can bind to both CREB and CREB-binding protein (CBP), the latter of which is a transcriptional co-activator [43]. Under physiological conditions, only phosphorylated CREB induced by stimulation can bind to CBP. Tax shunts this pathway, resulting in stimulation-independent activation of the CREB pathway. CBP acetylates histone

and opens the nucleosome structure around the transcriptional site [87].

Transcriptional Repression

Conversely, Tax can trans-repress the transcription of certain genes, such as DNA polymerase β [32], *lck* [46], *p18,* and *p53* [69] genes. For trans-repression of *p18* gene transcription, the E-box, which binds to transcriptional factor E47 is critical. Tax protein itself could not bind to E-box or E47, but interfered with the binding of E47 to the transcriptional co-activator p300, resulting in repression of transcription. *p53*-dependent transcription is also repressed by Tax protein. Similar to trans-repression of *p18*, Tax does not bind to *p53* or *p53* binding site, but rather it inhibits the recruitment of CBP to *p53* on the *p53*-binding sites [6,69]. This mechanism of trans-repression contrasts with that of trans-activation of CREB pathway by Tax protein. Although both mechanisms depend on binding of Tax protein to the transcriptional activator CBP/p300, their effects on transcription are quite different.

Functional Inhibition

Apart from transcriptional regulation, Tax can influence the function of cellular factors. Tax protein can interact with a negative inhibitor of cyclin-dependent kinase (CDK) 4, $p16^{INK4a}$, via its ankyrin motif and impair its function [68]. Since $p16^{INK4a}$ is an inhibitor of CDK4, its functional inactivation leads to activation of CDK4/6, phosphorylation of Rb, and finally G1/S transition.

Transforming growth factor β (TGF-β) is an inhibitory cytokine that plays an important role in development, the immune system, and oncogenesis. Since TGF-β generally suppresses the growth of tumor cells, most tumor cells acquire escape mechanisms to inhibit signaling from TGF-β, which include mutation of its receptor and mutation of Smad molecules that transduce the signal from receptor. Tax is also reported to inhibit the signal of TGF-β binding to Smad2, 3 and 4 or to CBP/p300 [45,51]. Inhibition of TGF-β signaling by Tax enables HTLV-I infected cells to escape TGF-β–mediated growth inhibition.

ATL cells are well known to show remarkable chromosomal abnormalities, which are thought to reflect chromosomal instability. Tax has been reported to interact with the check-point protein, MAD1, which forms a complex with MAD2 and controls the mitotic check-point. The functional hindrance of MAD1 by Tax protein causes chromosomal instability, suggesting the involvement of this mechanism in oncogenesis [34].

Molecular Biology of p12

Among accessory genes encoded by pX region, *p12* is well studied in addition to *tax* gene. Open reading frame (ORF) 1 of pX region encodes *p12* (Figure 1), which is present in endoplasmic reticulum and the Golgi apparatus [13]. *p12* has been shown to play an important role in establishment of HTLV-I infection and optimal viral infectivity *in vivo* [12] and quiescent primary lymphocytes [2]. Several mechanisms of *p12* have been suggested

to facilitate infectivity; *p12* interacts with calreticulin and calnexin and increases cytoplasmic calcium, leading to NFAT activation in T-lymphocytes [13,14]. This action of *p12* facilitates host cell activation and establishment of persistent infection. Although the expression of *p12* protein *in vivo* has remained obscure, cytotoxic T-lymphocytes (CTLs) against *p12* has been demonstrated in individuals infected with HTLV-I, indicating that *p12* protein was expressed *in vivo* [60].

Therefore, HTLV-I has redundant strategies to increase infected cells through its encoded proteins, such as Tax and *p12*, which promote cell proliferation, inhibit apoptosis, and increase genetic and chromosomal instability. With these strategies, HTLV-I increases its copies *in vivo* by increasing infected cell numbers and causes ATL as a consequence.

Natural Course of HTLV-I Infection and Clonal Expansion of HTLV-I Infected Cells

Throughout the world, about 20 million people are estimated to be infected with HTLV-I. HTLV-I is endemic in southwest Japan, the Caribbean islands, countries surrounding the Caribbean basin, and parts of Central Africa. In addition, epidemiological studies of HTLV-I revealed high seroprevalence rates in Melanesia, Papua New Guinea, the Solomon Islands, and among Australian aborigines [9]. In Japan, approximately 1.2 million individuals were estimated to be infected by HTLV-I, and more than 800 cases of ATL are diagnosed each year [70]. The cumulative risk of ATL among HTLV-I carriers in Japan was estimated at about 6.6% for men and 2.1% for women, indicating that most of HTLV-I carriers are asymptomatic throughout their whole life [4].

Transmission of HTLV-I

HTLV-I is transmitted by 3 routes: vertical (mother-to-infant in breast milk or transplacentally), horizontal (sexual), and parenteral (blood transfusion or intravenous drug use). Via any of the routes, infected cells are essential for transmission of HTLV-I, which has been shown by the absence of seroconvertors among recipients of fresh frozen plasma transfusions [59]. Similarly, infection with HTLV-I could be established by co-culture of infected cells *in vitro*. Thus, HTLV-I requires infected living cells for transmission. It has been demonstrated that cell-to-cell contact induces polarization of the cytoskelton of the infected cell to cell-to-cell junction, and Gag protein complexes along with HTLV-I genome accumulate at the cell-to-cell junctions. Then, such complexes transfer into the uninfected cells [29]. With this mechanism, HTLV-I transmits from infected cells to uninfected cells. After infection, HTLV-I increases its infected cell numbers *in vivo* as described later.

HTLV-I can infect many types of cells including B-lymphocytes, dendritic cells, fibroblasts, rat cells, and mouse cells, indicating that its receptor is ubiquitously expressed on cell surfaces. Recently, the glucose transporter GLUT-1 has been identified as the receptor of HTLV-I [48]. However, HTLV-I provirus was predominantly found in the CD4+ T-lymphocytes [84]. This suggested that HTLV-I could increase the number of infected CD4+ T-lymphocytes *in vivo* after infection, coinciding with the finding that HTLV-I could transform CD4+ T-lymphocytes *in vitro*. Even a retrovirus vector expressing only Tax could transform T-lymphocytes *in vitro* [1]. Taken together, these findings suggest that Tax promotes proliferation and inhibits apoptosis of HTLV-I-infected CD4+ T-lymphocytes *in vivo*.

HTLV-I Provirus Load

HTLV-I provirus load, which is correlated with the number of HTLV-I infected cells, varies by more than 100-fold among HTLV-I carriers. Whether the provirus load is constant or variable over time in individuals is an important issue. To address this question, sequential DNA samples from peripheral blood mononuclear cells of HTLV-I carriers who were

followed in a Miyazaki cohort study were analyzed. Provirus loads fluctuated only 2- to 4-fold in most carriers, showing that provirus loads were relatively constant over time for up to 7 years in individual carriers [16].

What determines the provirus load in carriers? As shown in the previous studies, age and sex did not influence provirus load [16]. A quantitative study of HTLV-I provirus load in seroconverters revealed that the same virus sequences were identified in married couples (infected persons transmitted virus to partners). Their provirus loads, however, were quite different, suggesting that provirus load was determined not by HTLV-I itself but rather by host factors [28]. It is assumed that immune responses, especially CTLs against HTLV-I, control the number of HTLV-I infected cells. As described above, CTLs in asymptomatic carriers control the proliferation of HTLV-I infected cells and suppress the development of ATL [82]. Another explanation for differences in provirus load is that genetic factors, other than MHC, influence the provirus load. Polymorphism of tumor necrosis factor α (TNF-α) has been shown to be associated with ATL in comparison with asymptomatic carriers, suggesting that genetic polymorphism that increases the production of TNF-α is associated with susceptibility to ATL [76]. Such genetic analysis utilizing rapidly accumulating knowledge and developing technology will clarify the genetic basis of familial clustering of ATL patients.

Clonal Expansion of HTLV-I Infected Cells

In HTLV-I carriers, HTLV-I provirus is randomly integrated in the host genome. In other words, the integration site is specific to each HTLV-I infected cell [66]. Part of the LTR and flanking genomic DNAs were amplified by inverse PCR, with each detected band representing a clonal proliferation of HTLV-I infected cells. Using this assay to analyze clonal proliferation of HTLV-I infected cells in HTLV-I carriers, some clones were shown to persist over 7 years in the same individuals [10,15]. These persistent clones were CD4+ lymphocytes, which is consistent with the finding that HTLV-I predominantly immortalizes CD4+ T-lymphocytes *in vitro*.

The HTLV-I provirus is genetically very stable, especially compared with the other major human retrovirus, human immunodeficiency virus (HIV). It has been postulated that increased HTLV-I load is achieved not by replication of virus but by clonal proliferation of infected cells. Since reverse transcriptase is an error-prone DNA polymerase, a higher replication rate generates the vast diversity in the virus genome. In HIV, a higher rate of mutation generated in the viral replication results in acquisition of drug resistance and escape from the host immune system. Conversely, HTLV-I increased its copies by proliferation of HTLV-I infected cells. In such situations, HTLV-I provirus in the host cells is replicated by cellular DNA polymerase with proofreading activity. Therefore, HTLV-I provirus is genetically stable in striking contrast to HIV.

tax Gene in ATL Cells

A long latent period of about 60 years precedes the onset of ATL, suggesting the multi-

step mechanism of leukemogenesis [58] (Figure 3). Tax is considered to play a critical role in clonal expansion of HTLV-I infected cells, however, its expression is frequently undetectable in the leukemic cells, and ATL cells frequently lose the expression of Tax by several mechanisms. The 5'-LTR is a viral promoter for transcription of viral genes, including the *tax* gene. The 5'-LTR was reported to be lost in 39% of cases examined (21/54 cases), indicating that ATL cells with such a provirus could no longer produce Tax [72]. The second mechanism is the nonsense or missense mutation of the *tax* gene in fresh ATL cells. Interestingly, in some cases, ATL cells had mutations in the class I MHC recognition site of Tax protein resulting in escape from immune recognition [17].

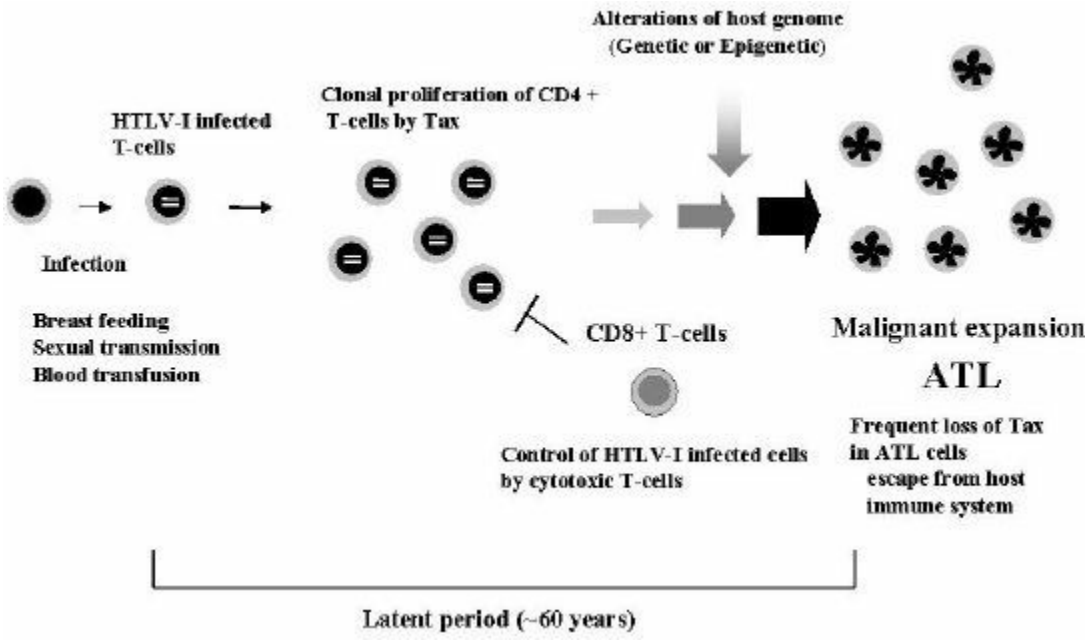

Figure 3. Natural course from the infection of HTLV-I to onset of ATL. HTLV-I is transmitted via 3 routes, and infected cells are necessary in each of them. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax. Proliferation of HTLV-I infected cells is controlled by cytotoxic T-cells *in vivo*. After a long latent period, ATL develops in about 5% of asymptomatic carriers. The expression of Tax is inactivated by several mechanisms, suggesting that Tax is not necessary in this stage. Alternatively, alterations in the host genome accumulate during the latent period, finally leading to onset of ATL.

The third mechanism is epigenetic change of the 5'-LTR. The 5'-LTR was selectively methylated, which silenced the transcription of viral genes [41]. We performed the detailed analysis of *tax* gene in leukemic cells from 47 cases of ATL and found genetic changes of *tax* gene (5 cases: 11%), loss of 5'-LTR (14 cases: 30%), and complete methylation of 5'-LTR (4 cases: 9%). The *tax* gene transcripts were detected in 14 of 41 fresh ATL cases (34%) by RT-PCR. With these mechanisms, ATL cells lost Tax expression and could escape from the host immune system.

Tax promotes the proliferation of HTLV-I infected cells by its pleiotropic actions; however, it is the major target of CTL. Thus, the presence of Tax in HTLV-I infected cells provides advantages and disadvantages for survival of HTLV-I infected cells. It is speculated that Tax

plays an important role in persistent proliferation of HTLV-I infected cells during the carrier state. Genetic and epigenetic changes then accumulate in the host genome mediated by mutator phenotype of Tax [38], which finally leads to Tax-independent proliferation and escape from the host immune system by inactivation of the *tax* gene.

Alternations in the Host Genome of ATL Cells

Various mutations of oncogenesis and tumor-suppressor genes have been demonstrated in cancers, and it has been established that multiple changes are necessary for the appearance of malignant disease. In ATL cells, previous studies have demonstrated mutations of *p53* gene in about 30% of patients [11,64], while we observed such mutations in 11% of patients (Takeda *et al.,* unpublished data). *p53* mutations were detected in more aggressive disease states. Moreover, deletion or mutation of the $p16^{INK4A}$ gene was also reported in ATL [24]. Again, those abnormalities were observed in acute or lymphoma-type ATL, suggesting that somatic DNA changes in *p53* or $p16^{INK4A}$ genes are associated with the progression of ATL. Mutations of *Fas* gene are also reported in patients with ATL cells [47,73]. However, such genetic changes were not frequently detected. In this regard, epigenetic change of $p16^{INK4A}$ gene was more frequent in ATL cells, and accumulated according to disease progression [56]. This finding suggests that epigenetic change, including DNA methylation, plays an important role in the leukemogenesis of ATL.

Molecular Pathogenesis of ATL

In the immune system, CD4+ T-lymphocytes play a central role by controlling the immune cells, such as B-lymphocytes, dendritic cells, and CTLs, and their responses. Helper T-lymphocytes secrete various cytokines to control the immune response and express adhesion molecule or chemokine receptors that influence their migration and co-stimulatory molecules that mediate differentiation and activation of various cells. Since ATL cells are derived from helper T-lymphocytes, they exhibit various phenotypes similar to the original cells.

Cytokines

ATL cells have been shown to produce various cytokines, including IL-1 [78], IL-6 [77], TGF-β [55], IL-5, M-CSF, GM-CSF [79], and parathyroid hormone related protein (PTH-rP) [80]. Such cytokines can modify the clinical manifestations of ATL. For example, enhanced production of IL-5 is associated with eosinophilia which is frequently observed in patients with ATL. Monocyte numbers in the ATL patients are increased by a high level of M-CSF.

Chemokine Receptor

Chemokines and their receptors have been implicated in the migration and tissue localization of lymphocytes. ATL cells are well known to infiltrate into various organs or tissues, frequently invading skin or lymphoid tissues. The differential expression of chemokine receptors might determine the migration of ATL cells. Analysis of chemokine receptor expression revealed that CCR4 was frequently expressed on HTLV-I transformed cell lines and fresh ATL cells [86]. CCR4 positive T-lymphocytes contain skin-seeking memory T-cells, suggesting that expression of CCR4 accounts for frequent infiltration of

ATL cells into skin. On the other hand, expression of CCR7 was reported to be associated with involvement of lymphoid tissues and lymph node enlargement [23].

A subtraction strategy between ATL cell lines and activated T-cells identified I-309 as a secreted chemokine from ATL cells, and I-309 expression was remarkably enhanced in ATL cell lines [63]. I-309 showed an anti-apoptotic effect via its receptor, CCR8, suggesting that an autocrine mechanism via I-309/CCR8 allowed ATL cells to survive *in vivo*.

RANK Ligand

The high frequency of hypercalcemia is the most striking feature of ATL; about 70% of ATL patients have high serum Ca^{2+} levels during the clinical course of the disease, particularly during the aggressive stage of ATL [40]. Such a frequency is the highest among hematological malignancies, and hypercalcemia is more severe in patients with ATL than those with other hematological malignancies [62]. In hypercalcemic patients, serum Ca^{2+} levels are often > 20 mg/mL, and accordingly, most patients are in a state of coma. Several pathological studies of ATL patients with hypercalcemia have indicated that high serum Ca^{2+} levels are due to an increased number of osteoclasts (OCL) and accelerated bone resorption [18,40]. Several cytokines, such as IL-1 [78], TGF-β [39,55], and PTH-rP [80] have been implicated in ATL-associated hypercalcemia. Among these factors, PTH-rP is considered to play an important role by stimulating OCLs, resulting in increased bone resorption. Immunodeficient mice implanted with leukemic cells from patients with ATL exhibited hypercalcemia and overexpressed PTH-rP [71]. However, PTH-rP cannot directly induce the differentiation of hematopoietic precursor cell (HPC) to OCL [50]. Furthermore, high serum levels of PTH-rP are not always associated with hypercalcemia in patients with ATL, suggesting that another factor is involved in the pathogenesis of hypercalcemia [83].

Bone is constitutively remodeled by osteoblasts (matrix synthesis) and OCLs (bone resorption). OCLs are derived from HPCs and belong to the monocyte macrophage lineage. During differentiation of OCL, precursor cells sequentially express c-Fms (a receptor of M-CSF) followed by receptor activator nuclear factor κB (RANK) [3]. M-CSF and RANK ligand (RANKL) have been shown to be critical factors for the differentiation of OCL, which are physiologically produced by stromal cells and osteoblasts [44]. Kong and colleagues identified abnormal expression of RANKL on activated T-lymphocytes and postulated that this gene is associated with bone loss and joint destruction in inflammatory arthritis [42]. Among the genes associated with hypercalcemia, increased expression of the RANKL gene correlated with hypercalcemia in ATL. ATL cells from patients with hypercalcemia, which highly expressed the transcripts of the RANKL gene, induced the differentiation of HPCs into OCL *in vitro* in the presence of M-CSF. In contrast, ATL cells from patients without hypercalcemia did not induce such differentiation, suggesting that the induction of differentiation correlated with expression of the RANKL gene in ATL cells. Cell differentiation was suppressed by osteoprotegerin/Fc (OPG/Fc), an inhibitor of RANKL, indicating that such differentiation occurred via the RANK-RANKL signal. In addition, direct contact between ATL cells and HPC was essential for the differentiation, suggesting that membrane-bound RANKL, but not the soluble form, played a role in this process [57].

These results suggested that ATL cells induce the differentiation of HPC to OCL via RANKL expressed on their surface, in co-operation with M-CSF, and ultimately cause hypercalcemia.

OX40

OX40/OX40L, members of the TNFR/TNF superfamily, play an important role in activation of T-cells that transduce the co-stimulatory signal. The OX40 signal suppressed apoptosis of T-cells by promoting expression of Bcl-X_L and Bcl-2, resulting in survival of CD4+ T-cells [61]. OX40 is expressed on HTLV-I-infected T-cells, and was also isolated as a molecule that served in adhesion to endothelial cells [30], suggesting that OX40/OX40 system is implicated in survival and tissue infiltration of ATL cells.

Thus, ATL cells express various molecules that can modify their phenotypic properties, thereby modifying the clinical disease manifestation and facilitating the survival of ATL cells.

Immunological aspects of HTLV-I infection

Immune Response Against Tax

Whereas Tax evokes the clonal proliferation of HTLV-I infected cells, it has been shown to be a major target of CTLs *in vivo* [31,36]. Therefore, Tax-expressing cells are considered to be eliminated *in vivo*. Indeed, depletion of CD8+ T-lymphocytes from the peripheral blood mononuclear cells of HTLV-I infected individuals *in vitro* promoted Tax expression in the CD4+ subpopulation, indicating that CD8+ CTLs suppressed Tax expression *in vivo* [21]. Thus, the survival of HTLV-I infected cells depends on the balance of proliferative actions of Tax and the host immune system. The enigma about CTLs against Tax is that, in patients with HTLV-I associated myelopathy (HAM)/Tropical spastic paraparesis (TSP), a high frequency of CTLs against Tax was observed in spite of a high provirus load (increased number of HTLV-I infected cells). Analysis of CTLs with fluorescent-labeled tetrameric complexes of HLA-A*02/β2 microglobulin/Tax 11-19 peptide showed that CTL response is effective in asymptomatic carriers; however, it is ineffective (weak CTL response) in patients with HAM/TSP [82]. These findings suggest that in asymptomatic carriers, CTLs against HTLV-I can control the growth of cells carrying the HTLV-I provirus resulting in prevention of ATL development.

Immunodeficiency in ATL Patients and HTLV-I Carriers

ATL is a highly aggressive neoplastic disease. The mean survival time of patients with acute ATL is less than 1 year regardless of intensive chemotherapy. Major obstacles in the treatment of patients with ATL include drug resistance and the development of opportunistic infections caused by various organisms such as *Pneumocystis carinii*, cytomegalovirus, *Strongyloidiasis*, and a variety of fungi. Such infections indicate that cell-mediated immunity is severely impaired in these patients [49,67]. Although the frequency of opportunistic infections is much higher in patients with ATL than in patients with other hematological malignancies, the underlying mechanism(s) of this phenomenon remains obscure. Opportunistic malignancies, Kaposi's sarcoma [19], and Epstein-Barr virus (EBV)-associated lymphoma [74], have also been reported in patients with ATL. This suggests a state of immunodeficiency in these patients, which is similar to that observed in acquired immunodeficiency syndrome (AIDS). Impaired cell-mediated immunity, such as a suppressed T-cell response to EBV [37], and seronegativity against purified protein derivative (PPD) [81], have been reported in HTLV-I carriers, also indicating a relationship to immunodeficiency.

Several mechanisms were reported to account for the immunodeficient state of HTLV-I infected individuals. One is that HTLV-I infects CD8+ T-lymphocytes impairing their function [53,84]. Indeed, the immune response against Tax, via HTLV-I infected CD8+ T-cells, renders these cells susceptible to fratricide mediated by autologous HTLV-I-specific CD8+ T lymphocytes. Fratricide amongst virus-specific CTLs could impair the immune control of HTLV-I [22].

Another mechanism of immunodeficiency associated with HTLV-1 is based on the observation that the number of naive T-cells is decreased in individuals infected with HTLV-I [84]. Naive T-cells were identified by 3-color fluorescence with CD62L and CD45RA coexpression either with CD4+ or CD8+ T-cells. The number of naive T-lymphocytes was markedly suppressed in patients with ATL, particularly in those with acute form, compared with uninfected control individuals. The number of naive T-cells was low in HTLV-I infected individuals under 50 years of age compared with uninfected individuals, whereas the number of memory T-lymphocytes was greater in HTLV-I infected individuals. T-cell receptor rearrangement excision circles (TREC), which are generated by DNA recombination during early T-lymphopoiesis, were quantified to evaluate thymic function in HTLV-I infected individuals. TREC levels were lower in HTLV-I infected individuals compared with uninfected individuals. The suppressed number of naive T-lymphocytes was due to suppressed production of T-lymphocytes in the thymus, which might account for the immunodeficient state observed in HTLV-I infected individuals.

Immunodeficiency and Development of ATL

Such immunodeficiency might be associated with leukemogeneisis of ATL by allowing proliferation of HTLV-I infected cells. A prospective study of HTLV-I infected individuals identified carriers who later developed ATL, showed that anti-Tax level was low in all ATL

cases for up to 10 years preceding their diagnosis, independent of the level of anti-HTLV-I titer. This finding indicates that HTLV-I carriers with a higher anti-HTLV-I titer and a lower anti-Tax reactivity may be at greatest risk for developing ATL [26]. The levels of anti-HTLV-I antibody and soluble IL-2 receptor (sIL-2R) have been shown to be correlated with HTLV-I provirus load [16], and high antibody titers and high sIL-2R were risk factors to develop ATL among carriers [5]. Taken together, these findings suggest that a higher proliferation of HTLV-I infected cells along with a low response against Tax might be associated with onset of ATL. Given this finding, potentiation of CTLs against Tax by a vaccine strategy might be useful in preventing the onset of ATL [20].

EBV-associated lymphomas frequently develop in individuals with an immunodeficient state associated with transplantaion or AIDS. Does such an immunodeficient state, which abrogates the immune function suppressing HTLV-I infected cells, affect the onset of ATL? Among 24 patients with post-transplantation lymphoproliferative disorders (PT-LPD) after renal transplantation in Japan, 5 cases of ATL were reported [27]. Considering that most of PT-LPDs were of B-cell origin in the Western countries, this frequency of ATL was quite high. Although HTLV-I was thought to be transmitted via blood transfusion during hemodialysis, the immunodeficient state might promote the onset of ATL.

Conclusion

After establishment of ATL as a distinct clinical entity, and identification of HTLV-I as a causative agent of this disease, extensive studies on ATL and HTLV-I in the field of virology, immunology, and molecular biology have clarified many aspects of this viral infection and related diseases (ATL and HAM/TSP). However, the precise mechanism of leukemogenesis remains unsolved. Most importantly, successful therapy for ATL has not been established. Future research will be directed at clarifying the mechanism of leukemogenesis, including alterations of the host genome, and based on this knowledge, new therapeutic strategies against this highly malignant disease should be formulated.

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Epstein-Barr Virus-Associated Malignancies

Kenzo Takada, M.D., Ph.D.

Hokkaido University, Japan

Introduction

Epstein-Barr virus (EBV) is a member of the herpes virus family and contains a 170kbp DNA genome. Most people are infected with EBV by the time they become adults and carry the virus in a latent state [16]. In most people, EBV is maintained without specific manifestations but is associated with the various malignancies. These include Burkitt's lymphoma, nasopharyngeal carcinoma, T/NK cell lymphoma, Hodgkin's disease, lymphoma in immunodeficient hosts, and gastric carcinoma. A role of EBV in the development of malignancies, especially about a role of EBV-encoded small RNA, EBER [13], has been described.

EBV-Induced B-cell Immortalization

B-lymphocytes are primary targets of EBV infection. EBV binds B-cells via CD21 molecules, which are preferentially expressed on the B-cell surface. MHC class II molecules act as co-receptors and are necessary for penetration of the virus to the B cell [16].

The most striking activity of EBV *in vitro* is its ability to transform B-lymphocytes into indefinitely-growing lymphoblastoid cells. After a 2- to 3-day lag period, EBV-infected cells begin an exponential growth. EBV-immortalized B-lymphocytes carry the entire EBV genome as a plasmid and express 11 EBV genes as shown in Figure 1 [6]. This pattern of EBV expression is termed type III latency. Among the 11 EBV genes, latent membrane protein 1, LMP1, is important for immortalization.

The mechanism of LMP1's action has been extensively studied, and it is known that the LMP1 protein binds TRAF and TRADD molecules and constitutively activates signaling from CD40 in B-cells [2].

EBV-cell Interactions *in vivo* and Proliferation of EBV-infected B-cells in Immunocompromised Hosts

Figure 2 shows EBV-cell interaction during primary infection and persistence [16]. EBV is transmitted by saliva. B-lymphocytes in the oropharynx are the primary target of EBV infection. EBV-infected B-cells have 2 fates: latent infection and lytic infection. Latent infection induces proliferation of infected B-cells, and lytic infection leads to production of progeny viruses and the death of infected cells. The produced viruses infect surrounding epithelial cells and B cells. Infection of epithelial cells is largely a lytic infection, and the viruses produced are shed to the oropharynx. Both latent and lytic infections induce a cytotoxic T-lymphocyte (CTL) response and EBV-infected cells are eliminated. During this process, some latently EBV-infected B-cells enter the memory B-cell pool and down-regulate viral gene expression so that the cells can escape from CTL attack. This latency pattern is named latency 0 and expresses only EBER and BamHI-A rightward transcripts (BARTs).

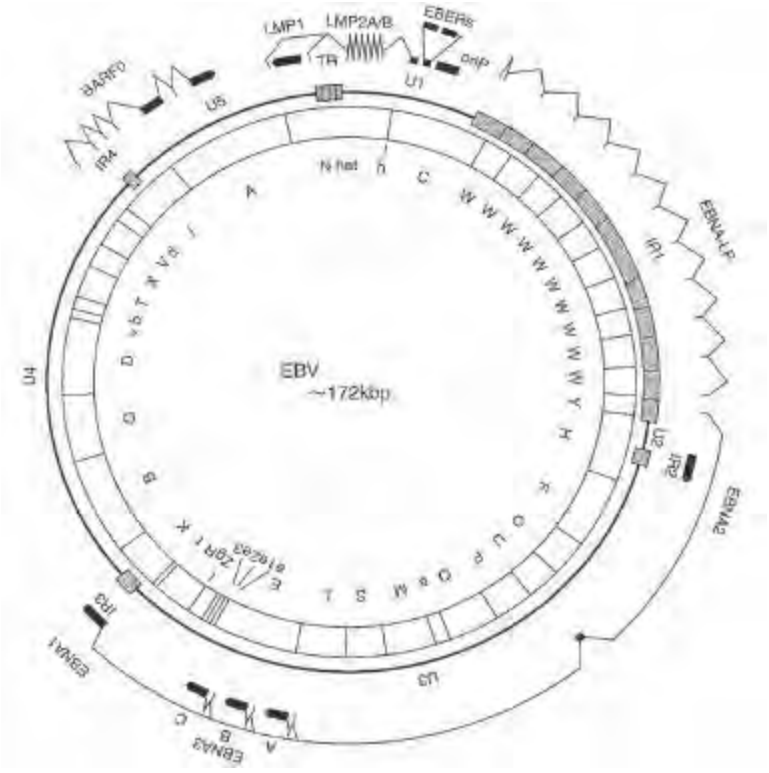

Figure 1. Structure of EBV genome and EBV genes expressed in EBV-immortalized B-lymphocytes. EBV genes expressed are 6 EBV-determined nuclear antigens (EBNA1, EBNA2, EBNA3A, EBNA3B, EBNA3C and EBNA-LP), 3 latent membrane proteins (LMP1, LMP2A and LMP2B), EBV-encoded small RNAs (EBER1 and EBER2), and rightward transcripts of the BamHI-A fragment (BARTs).

The number of EBV-infected cells in the peripheral blood was measured by real-time PCR and spontaneous outgrowth of EBV-immortalized cells. It is estimated that about 1 cell per hundred thousand B cells is infected with EBV. These cells are in latency 0 type

infection and express only EBER and BARTs as EBV products.

Latently EBV-infected cells are potentially oncogenic and start to proliferate under immunosuppressed conditions like AIDS and post-transplantation. Initially it is polyclonal proliferation, and called post-transplant lymphoproliferative disorder (PTLD) or B-lymphpproliferative disorder (BPLD). Occasionally it turns to B-cell lymphoma due to monoclonal proliferation of EBV-infected cells. Proliferation of EBV-infected cells is an important complication that determines the prognosis of transplantation.

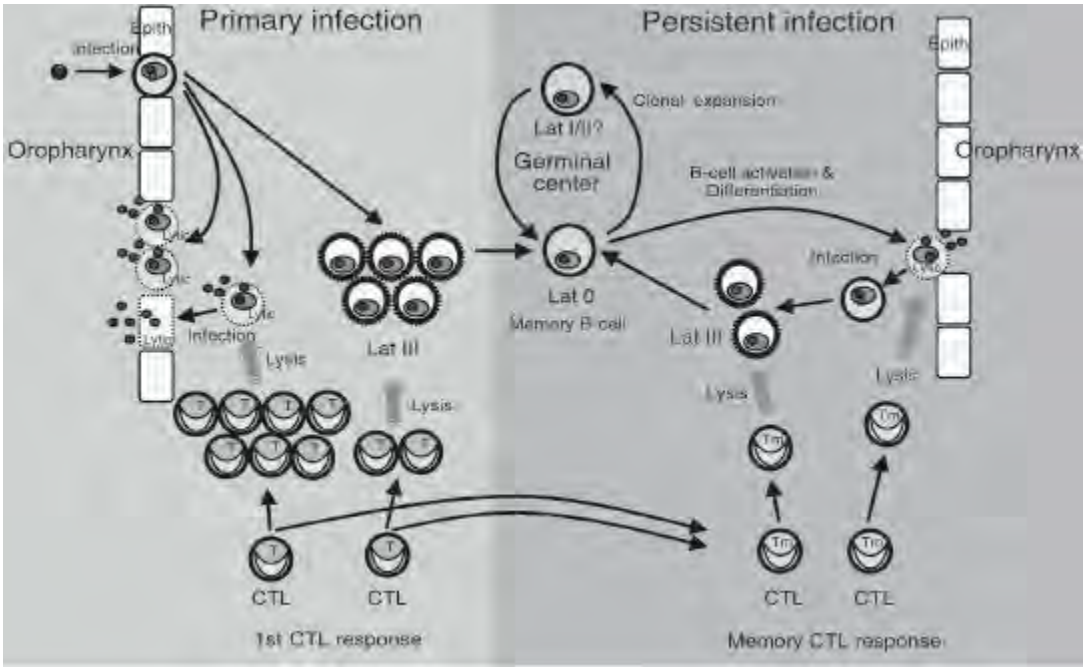

Figure 2. A model of EBV-cell interactions during primary infection and persistence.

EBV-Associated Malignancies

Figure 3 shows the pattern of EBV expression in various EBV-associated malignancies [16]. EBV-immortalized lymphocytes and AIDS lymphoma express identical EBV genes. On the other hand, Burkitt's lymphoma and gastric carcinoma cells express EBV-determined nuclear antigen 1 (EBNA1), but do not express other EBNAs or LMP1. Since it is known that EBNAs other than EBNA1 become targets of CTL, it is important for tumor cells not to express these antigens in order to survive under CTL surveillance.

Burkitt's lymphoma is a B-cell lymphoma that is endemic in Africa. It represents type I latency characterized by expression of EBNA1, EBER, and BARTs and always carries translocation and transcriptional activation of the c-myc gene.

Hodgkin's disease is also associated with EBV, with about half of the cases EBV-associated. Reed-Sternberg cells and Hodgkin cells are infected with EBV and express restricted EBV genes such as EBNA1, EBER, BARTs, and LMPs.

Nasopharyngeal carcinoma (NPC) is common in males indigenous to Southeast China. The histology is poorly differentiated and accompanies strong lymphoid infiltration. NPC cells represent type I or type II latency. Carcinomas with similar histological profiles occur with a low incidence in organs such as the salivary glands, thymus, and lungs, mainly in Chinese and Inuits. These carcinomas are termed lymphoepithelioma-like carcinomas or carcinomas with lymphoid stroma, and most cases are EBV-positive. The association of EBV and gastric carcinoma was first demonstrated in this particular type. Subsequently, EBV infection in gastric adenocarcinomas of ordinary histology was reported [19]. In contrast to observations on Burkitt's lymphoma and NPC, EBV-positive gastric carcinoma is a non-endemic disease distributed throughout the world; however, there are some regional differences. The highest incidences, 16 to 18%, are in the USA and Germany, and the lowest, 4%, in China. There seems to be a reverse correlation with the incidence of gastric carcinoma. Gastric carcinoma is one of the most common carcinomas and the worldwide occurrence of EBV-positive gastric carcinoma is estimated to be more than 50,000 cases per year.

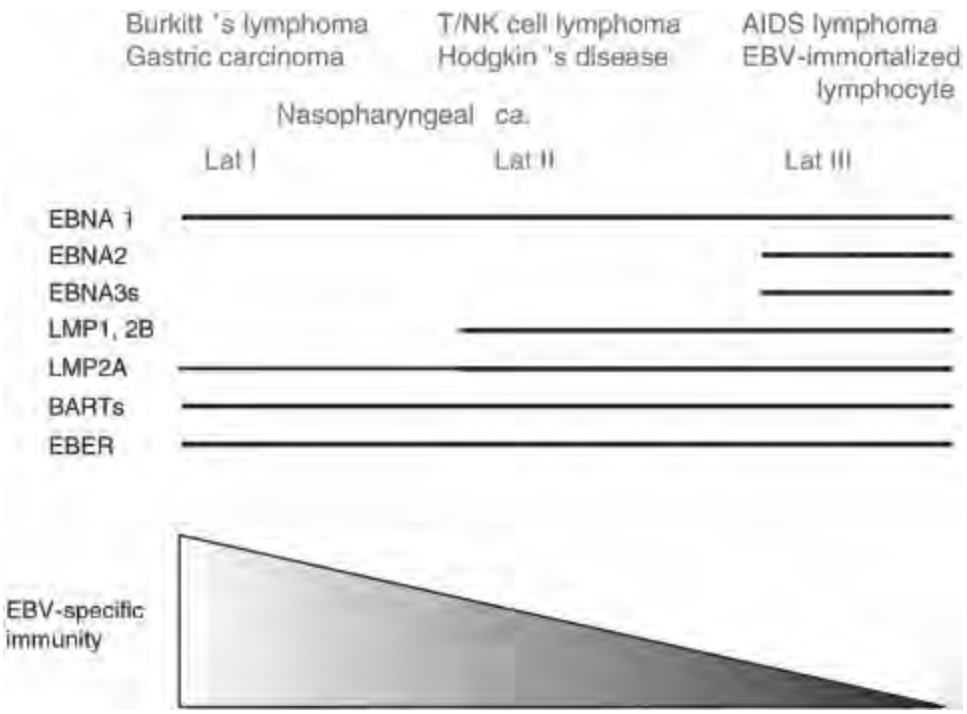

Figure 3. EBV expression in EBV-associated malignancies.

EBV Infection of Epithelial Cells

EBV infection of B-lymphocytes is mediated by binding of envelope protein gp350 to the CD21 molecule. However, epithelial cells are negative for CD21 and refractory to EBV infection *in vitro* despite of frequent detection of EBV in various epithelioid tissues.

The use of recombinant EBV carrying a selectable marker has enabled this difficulty to be overcome. We generated a recombinant EBV carrying the EGFP gene and the neomycin-resistance gene. Both genes were inserted at the BXLF1 region of Akata EBV by homologous recombination. The resultant virus was used for EBV infection of various carcinoma cell lines. As a result, most epithelial cell lines examined were susceptible to EBV infection, but the RT-PCR assay revealed that CD21-specific transcripts were expressed in only 4 of the 18 cell lines that were susceptible to EBV infection [4,22]. Pretreatment of cells with an anti-CD21 antibody did not block EBV infection of the gastric carcinoma line AGS, though it blocked EBV infection of EBV-negative Akata cells in a dose-dependent manner [22]. These results indicate that EBV does not utilize CD21 for infection of epithelial cells.

EBER

We have demonstrated that type I EBV infection induces growth acceleration, apoptosis resistance, and tumorigenicity in BL-derived Akata cells, gastric carcinoma-derived NU-GC-3 cells, and primary gastric epithelia [9,14]. EBV-infected primary epithelial cells grow faster and reach higher saturation density than uninfected cells. The next question is what EBV product is responsible for these activities.

Our series of experiments demonstrated that EBV-encoded small RNA, EBER, was

important [5,7,8]. EBER, consisting of EBER1 and EBER2, is expressed in all EBV-associated malignancies with a high copy number of up to 10^7 copies per cell [18]. EBER is a 170-nucleotide-long RNA and is not translated to protein. EBER is expected to have extensively base-paired structures containing a number of short stem loops and binds some cellular proteins such as PKR, LA and EAP/L22 [1,11,20,21] (Figure 4). However, the significance of this binding is unknown.

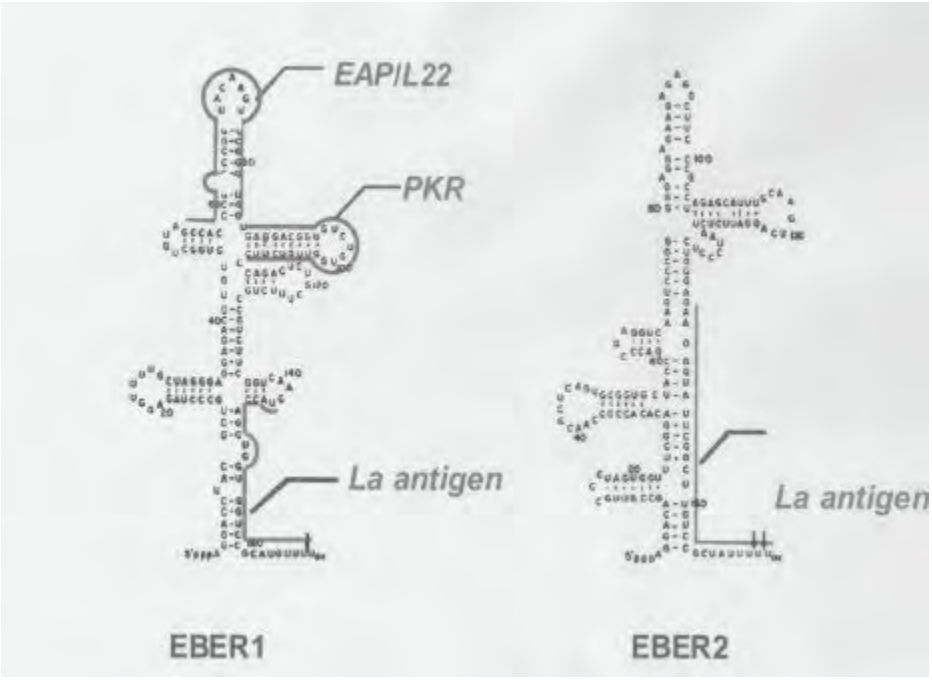

Figure 4. Structure of EBER.

Inhibition of Apoptosis by EBER

PKR (dsRNA-activated protein kinase) is an interferon-inducible serine/threonine kinase and is the key mediator of antiviral activities of interferon [2]. PKR is induced by interferon and phosphorylated by double-stranded RNA. This active form of PKR phosphorylates 2 cellular substrates, protein synthesis initiation factor eIF2α [17] and IκBα [10]. Phosphorylation of eIF2α abrogates translation initiation in the virus-infected cells and stops virus replication. The contribution of IκBα to antiviral defense has not been well studied. Besides its antiviral effects, interferon has antitumor effects, and in some instances cell death is due to apoptosis. A cellular PKR activator, PACT, is known to activate PKR in the absence of double-stranded RNA [15]. In an *in vitro* cell-free system, EBER was shown to bind PKR and inhibit its phosphorylation [18]. Furthermore, studies of dominant-negative PKR demonstrated that PKR has activity as a tumor suppressor.

Based on this background, we studied whether EBER inhibited interferon-induced apoptosis in a cultured cell system [12]. We used 3 BL cell lines, Akata, Daudi, and Mutu, because they retained type I latency, and EBV-positive and -negative subclones could be isolated from parental BL cells by the limiting dilution method. The cells were treated with interferon-α and apoptosis was assessed by flow cytometry. As a result, EBV-negative BL clones were prone to fall into apoptosis without the addition of interferon, and nearly half of

the cells underwent apoptosis after 3 to 4 days of interferon treatment. Conversely, EBV-positive BL cell clones were resistant for induction of apoptosis by interferon.

Next, EBV-negative Akata cells were transfected with individual EBV latent genes, and cell clones that stably expressed levels similar to parental Akata cells were isolated and examined for their susceptibility to interferon. The results indicated that cell clones transfected with EBER exhibited resistance to interferon-induced apoptosis (Figure 5). Furthermore, reinfection of wild-type EBV into EBV-negative Akata cells made them resistant to interferon, but EBER-knockout EBV did not. These results clearly demonstrate that EBER is responsible for resistance to interferon-induced apoptosis.

We further examined whether EBV infection could inhibit PKR phosphorylation. The FLAG-PKR plasmid was transfected into EBV-positive and -negative BL cells. After 48 hours of transfection, FLAG-PKR was immuno-precipitated with an anti-FLAG antibody and subjected to *in vitro* kinase assay. The results indicated that PKR phosphorylation was inhibited in EBV-positive BL cell clones. Furthermore, it was shown that EBER is responsible for inhibition of PKR phosphorylation.

PKR contains a dsRNA-binding domain and kinase catalytic domain. We generated a dominant-negative PKR plasmid lacking the kinase domain, transfected the plasmid into EBV-negative BL cells, and examined whether dominant-negative PKR could confer resistance to interferon-induced apoptosis. As a result, dominant-negative PKR inhibited interferon-induced apoptosis. Our results demonstrated that EBER binds PKR, inhibits its phosphorylation, and confers resistance to apoptosis.

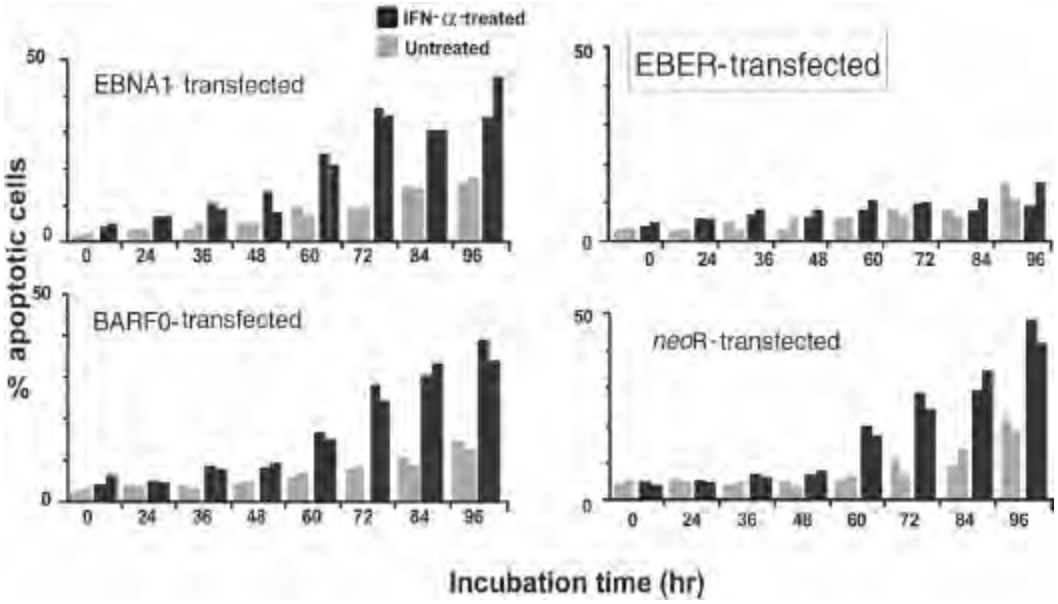

Figure 5. IFN-α-induced apoptosis in EBV-negative Akata cells transfected with an individual EBV latent gene expressed in BL.

Interleukin-10 Induction by EBER

Interleukin-10 (IL-10) induction by EBER has been found by an analysis of Burkitt's lymphoma [7]. We found that expression of IL-10 was higher in EBV-positive BL cell clones than in EBV-negative clones. The results of a RT-PCR assay and an ELISA assay of culture

soup are shown in Figure 6.

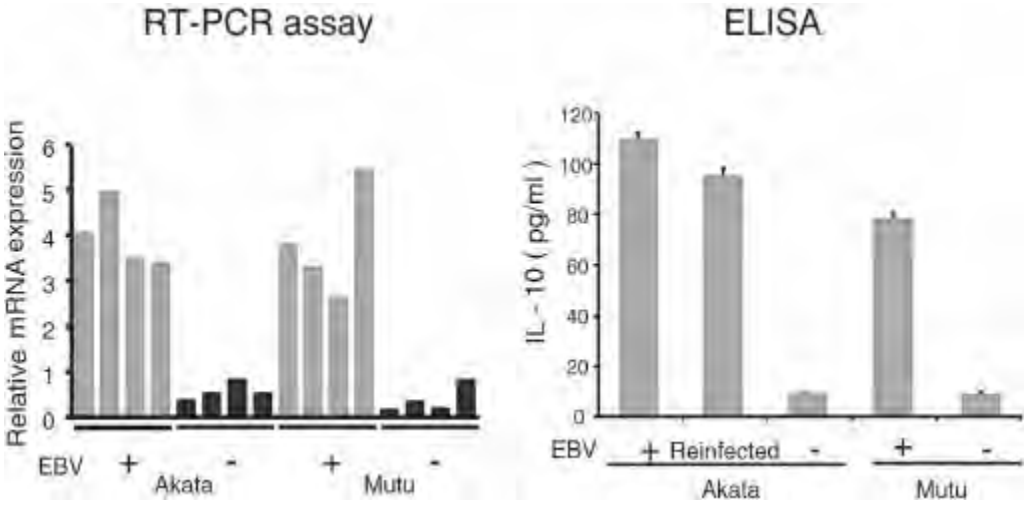

Figure 6. IL-10 expression in EBV-positive and -negative BL cell clones.

Growth of EBV-negative Akata cells was enhanced by recombinant IL-10 and growth of EBV-positive Akata cells was blocked by an anti-IL-10 antibody. These results indicate that IL-10 is an autocrine growth factor for EBV-positive Akata cells.

We next examined which EBV gene among 3 latent genes, EBNA1, EBER, and BARTs, was responsible for IL-10 induction. The EBV-negative Akata cell clone was transfected with an individual EBV latent gene, and cell clones that stably expressed similar levels of EBV-positive Akata cells were selected and analyzed. The results indicated that all cell clones transfected with the EBER gene expressed higher levels of IL-10 than cell clones transfected with the other latent genes. There was a good correlation between the level of EBER expression and that of IL-10 expression. EBV-reinfection of EBV-negative Akata cells induced IL-10 expression, but not when EBER-knockout EBV was used for infection. These results clearly demonstrated that the EBER was responsible for IL-10 induction.

We further examined the IL-10 expression in BL biopsies by the real-time RT-PCR assay (Figure 7). All 4 EBV-positive BL biopsies were positive for IL-10. There was a good correlation between the level of EBER expression and that of IL-10 expression. On the other hand, 9 EBV-negative BL biopsies were negative for EBER expression and did not express detectable amounts of IL-10. These results suggest that the results obtained from the cell culture system is applicable to the *in vivo* situation of Burkitt's lymphoma.

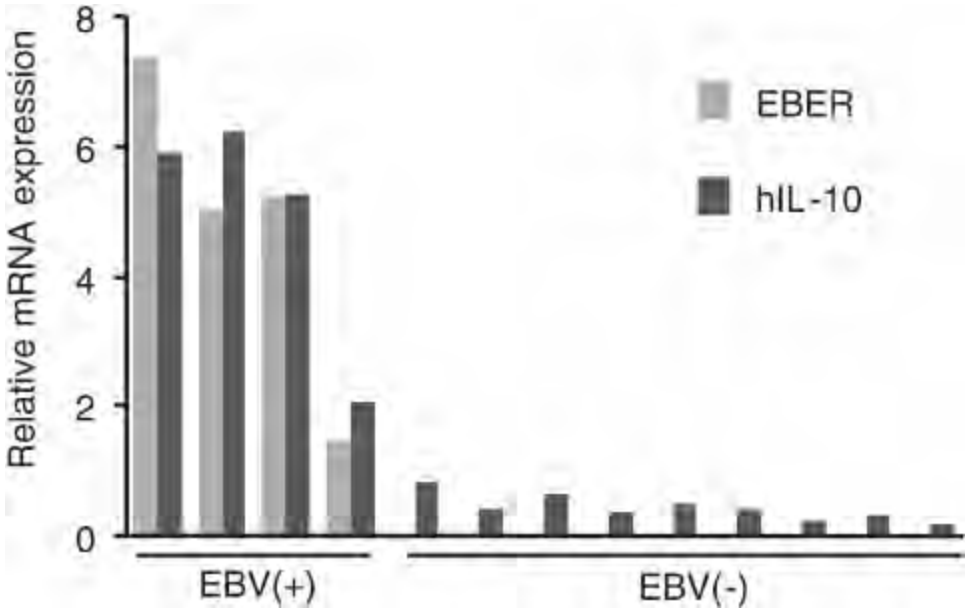

Figure 7. IL-10 expression in BL biopsies.

To examine the half-life of IL-10 mRNA, EBV-positive and -negative Akata cells were incubated in the presence of actinomycin D and IL-10 mRNA was quantified by real-time PCR assay. The results indicated that the half-life of IL-10 mRNA was not different between EBV-positive and -negative Akata cells, thus indicating that increased expression of IL-10 mRNA in EBV-positive Akata cells reflects transcriptional activation of IL-10.

A transient transfection assay of EBV-negative Akata cells revealed that EBER1, but not EBER2, induced IL-10, and dominant-negative PKR could not induce IL-10. Thus, EBER induces IL-10 transcription by a mechanism independent of PKR.

IGF-1 induction by EBER

IGF-1 induction by EBER has been revealed by analysis of gastric carcinoma and NPC [5]. IGF-1 is insulin-like growth factor 1 [3,23]. It consists of 70 amino acids, is produced by many types of cells including hepatocytes, and acts as a growth factor. It is also reported that serum IGF-1 is elevated in various cancers of the breast, prostate, colon, and stomach.

NU-GC-3 is a gastric carcinoma-derived cell line and originally EBV-negative. We made EBV-converted cell clones, and expression of IGF-1 was compared between EBV-positive and EBV–negative NU-GC-3 cell clones. Figure 8 shows the results of RT-PCR assay and ELISA assay of culture soup, and indicates that IGF-1 is induced by EBV infection.

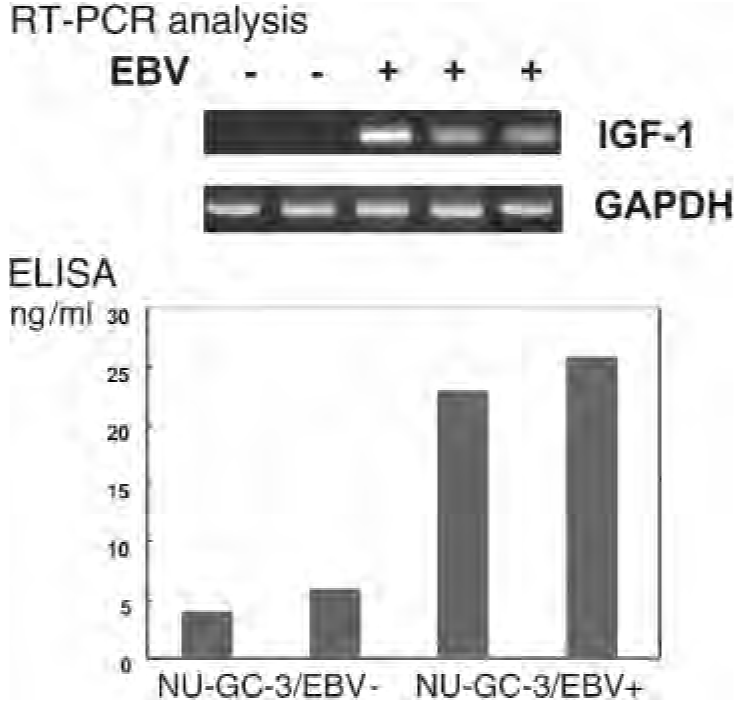

Figure 8. IGF-1 expression by EBV infection in gastric carcinoma-derived NU-GC-3 cells.

Growth of EBV-positive NU-GC-3 cells was inhibited by an anti-IGF-1 antibody. Growth of EBV-negative NU-GC-3 cells, however, was enhanced by recombinant IGF-1 in a dose-dependent manner. These results indicate that EBV infection of NU-GC-3 cells induces IGF-1 expression, and that the produced IGF-1 acts as an autocrine growth factor.

Next, we examined which EBV gene among 3 latent genes, EBNA1, EBER, and BARF0, was responsible for IGF-1 induction. The EBV-negative NU-GC-3 cell clone was transfected with the individual EBV latent genes, and cell clones that stably expressed similar levels of EBV-positive NU-GC-3 cells were selected and analyzed. The results indicated that all cell clones transfected with the EBER gene expressed IGF-1, whereas cell clones transfected with the other latent genes did not. These results clearly demonstrated that the EBER is responsible for IGF-1 induction.

IGF-1 expression of gastric carcinoma tissues was further examined. EBV-positive gastric carcinoma tissues consistently expressed IGF-1, whereas EBV-negative gastric carcinoma tissues did not (Figure 9). These results suggest that the results obtained from the cell culture system are applicable to the *in vivo* situation of EBV-positive gastric carcinoma.

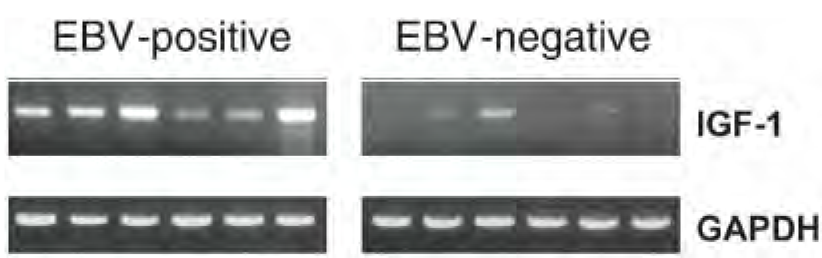

Figure 9. IGF-1 expression in gastric carcinoma tissues.

A reporter assay of the IGF-1 promoter showed that the promoter is activated in EBER-

expressing NU-GC-3 cells. Therefore, IGF-1 induction by EBER is at the transcriptional level. The effect of a PKR inhibitor, 2-aminopurine, on IGF-1 expression in EBV-negative NU-GC-3 cells was examined next which showed that PKR inhibition could not induce IGF-1 expression. These results indicate that EBER induces IGF-1 transcription by a mechanism independent of PKR.

Conclusions

Our series of studies revealed that EBER has many biological functions and suggested that EBER plays important roles in the pathogenesis of EBV-associated malignancies (Figure 10). EBER acts via 2 mechanisms: one is escape from immune attack and the other is oncogenicity. EBER inhibits PKR activation in both B-lymphocytes and epithelial cells, and confers resistance to interferon, which is a non-specific host defense mechanism against viral infection. In tumor cells, however, PKR inhibition contributes to resistance to apoptosis. In the B-cell system, EBER induces IL-10 transcription, and the produced IL-10 promotes growth of tumor cells as an autocrine growth factor. IL-10 is known to suppress T-helper 1 (Th1) cells, which bear specific immunity. In epithelial cells, EBER induces IGF-1, and the produced IGF-1 promotes growth of tumor cells as an autocrine growth factor. In another study, we showed that IGF-1 acts as an co-activator of latent EBV in B-cells. EBV-producing B-cells contribute to the increase of EBV-infected epithelial cells by a cell-to-cell infection mechanism. Recently, small RNAs such as micro RNA have drawn much attention as functional molecules. Our studies on EBER were undertaken as pioneering studies in this field.

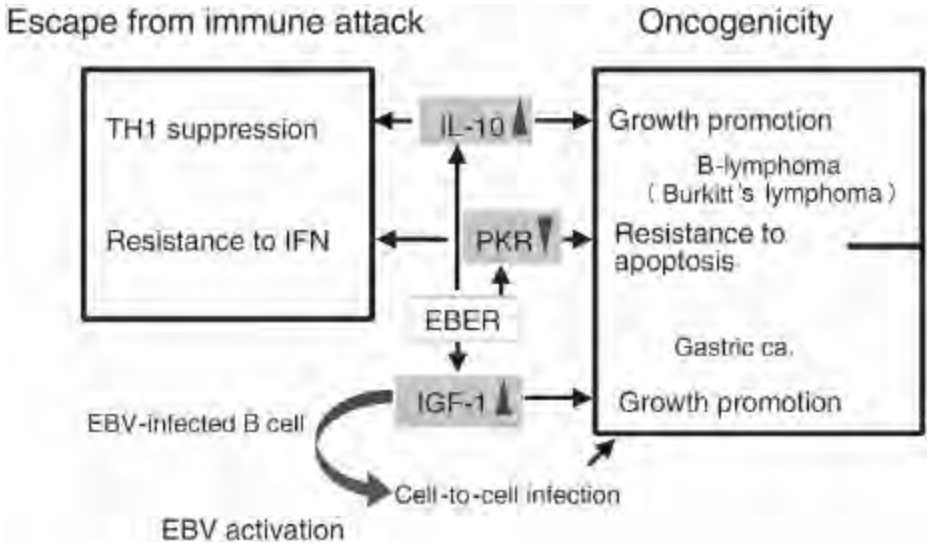

Figure 10. Role of EBER in oncogenesis.

Acknowledgments

I thank my colleagues at Hokkaido University and collaborators.

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Influenza Virus: Lessons Learned

Dmitriy Zamarin and Peter Palese, Ph.D.

Mount Sinai School of Medicine, U.S.A.

Introduction

"…These men start with what appears to be an attack of la grippe or influenza, and when brought to the hospital they very rapidly develop the most vicious type of pneumonia that has ever been seen.… It is only a matter of a few hours then until death comes, and it is simply a struggle for air until they suffocate. It is horrible…" (An excerpt from a letter written by a physician stationed at Camp Devens, Massachusetts in September of 1918) [12].

This is just one of thousands of personal accounts of the 1918 influenza outbreak: the deadliest pandemic recorded in the history of human kind. For many years afterwards, the nature of the virus itself, an agent that claimed more lives than both world wars combined, remained a mystery. While many questions posed by the 1918 influenza pandemic, as well as by other pandemics of the past century, still remain unanswered, recent advances in virology and molecular biology have provided us with tools to dissect the features of pandemic influenza viruses. Lessons learned from the 1918 influenza pandemic put several questions into perspective when we are faced with newly-emerging viruses.

(1) Is there an animal reservoir of the new virus and what are the viral characteristics responsible for crossing the species barrier?

(2) What defines the age-specific distribution of mortality in pandemics/epidemics?

(3) What factors do pandemic/epidemic viruses possess that make them more virulent?

(4) What can we do to circumvent future pandemics?

Learning from the experience with influenza will provide us with some of the answers to these questions.

The Pandemics

Three major influenza pandemics are notable in the last century: the 1918 “Spanish flu” (H1N1), the 1957 “Asian flu” (H2N2), and the 1968 “Hong-Kong flu” (H3N2) [7] (Figure 1). The 1918 pandemic was by far the most severe and spread globally over a short period of time. Although the origin of the 1918 virus remains unknown, sequence analysis of the viral HA, NA, NP, M, and NS genes revealed that the virus closely resembles mammalian (porcine) and human H1N1 viruses [4,35]. This would suggest that the 1918 virus is the precursor to human influenza viruses circulating after 1918 and that it also may have been transmitted to pigs. It is likely that the 1918 virus is either an animal virus that jumped to humans or a new virus that resulted from reassortment of an animal virus with a previously circulating human influenza strain. Further sequence analysis of other human and animal influenza strains circulating at the time will reveal the true genealogy of this virus.

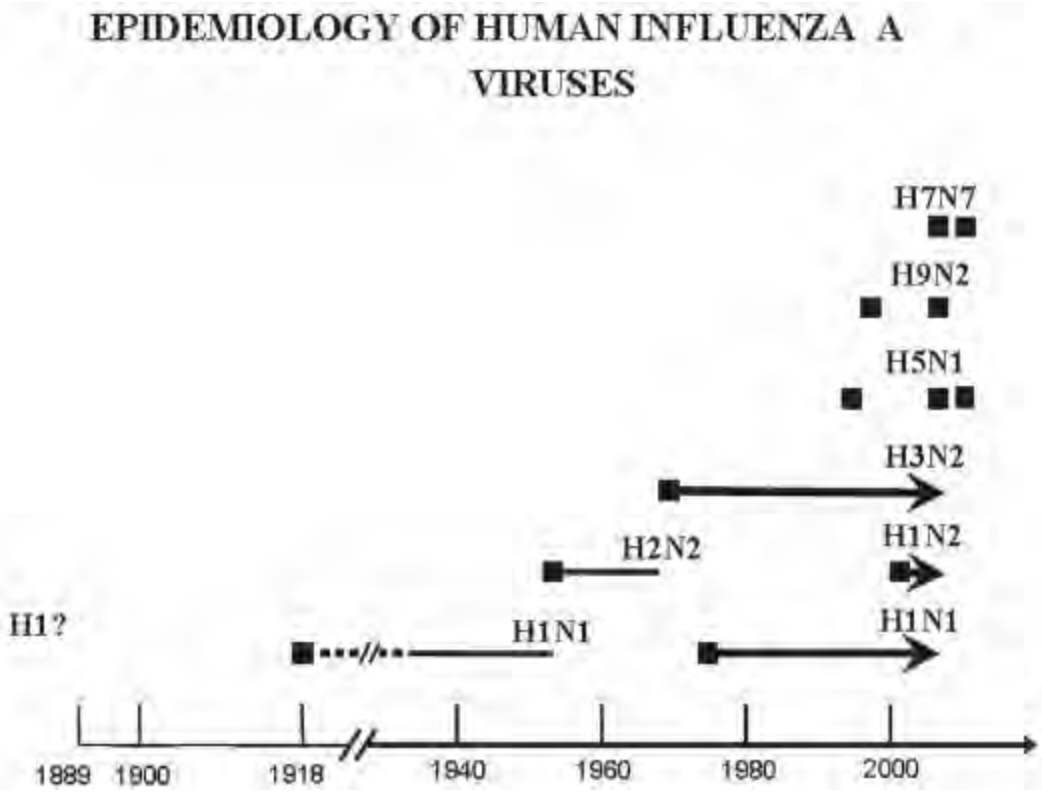

Figure 1. Based on serological evidence and sequence analysis, it appears that human influenza viruses with different antigenic properties (H, hemagglutinin; N, neuraminidase) can emerge frequently. There may also be recycling of strains after specific serotypes have been absent from the population for a prolonged period of time. In addition, emergence of new antigenic serotypes (H5N1, H9N2, and H7N7) has also been observed in the past several years. (Dashed lines indicate that no viral isolates are available).

While the source of the 1918 virus remains unknown, the viruses causing the 1957, 1968, and 1977 pandemics have been characterized in much greater detail [7]. The 1957 H2N2 pandemic virus derived 3 genes (PB1, HA, and NA) from an avian virus, and the remaining genes from the previously circulating H1N1 human virus [17,30-32]. The 1968

H3N2 virus received its PB1 and HA genes from an avian virus and the rest of the genes from the human H2N2 virus [17,30]. In 1977, the H1N1 virus reemerged; the new virus was virtually identical to viral isolates from the 1950s [21]. Recent outbreaks of avian influenza H5N1, H7N7, and H9N2 virus infections in humans are yet other examples of animal viruses crossing the species barrier [1,2,14,20,23] (Figure 1). In the 1997 Hong-Kong outbreak, the H5N1 virus caused infection in 18 people, 6 of whom died. Early in 2003, the H5N1 virus reemerged in Hong Kong and was the cause of at least 1 death [1,27]. In late 2003 and early 2004, further human cases of infection with H5N1 virus with high case-fatality rates were identified in Vietnam and Thailand [37,41]. Outbreaks of H7N7 infections have been reported in The Netherlands since the beginning of 2003, with 83 confirmed cases and 1 death. Interestingly, the H7N7 virus caused conjunctivitis in 79 of the 83 cases [1]. Further human infections with the H7N7 virus were reported in Canada in the spring of 2004 [41]. Reports of human H9N2 influenza virus infections first appeared in 1999, when several cases were confirmed in China [14]. Subsequently, human cases of H9N2 infections were also described in Pakistan and Vietnam [2,23]. Another example of a virus crossing the species barrier is demonstrated by the recent SARS coronavirus outbreak, which is believed to have originated from animal handlers in China [13,18,29,33]. The virus has caused over 8000 infections with over 770 deaths worldwide, bringing the case-fatality ratio to almost 10% [40]. For all of the outbreaks described above, the inefficient spread of these viruses has so far prevented them from starting another pandemic, but each case is a reminder that emerging human diseases caused by animal viruses are a continuing and constant threat (Table 1).

Age Distribution of Mortality in the 1918 Pandemic

In the inter-pandemic years, 80 to 90% of influenza-related deaths occur in the elderly, with some deaths occurring in infants and young children [34]. Severity of the disease is higher in younger children due to lack of previous exposure to the virus and thus lack of protective antibodies. As people acquire immunity to the viruses, severity decreases for the adult age group and increases again for the elderly because of their waning immune systems and a deterioration of overall health. The mortality distribution thus follows a U-shaped curve, as represented by the influenza mortality data for 1911-1915 shown in Figure 2 [28]. In contrast, while mortality rates rose for both young children and the elderly, nearly half of the influenza-related deaths during the 1918 pandemic occurred among young and healthy adults (20 to 40 years of age), changing the mortality distribution to a W-shaped curve [11,24,28] (Figure 2). Similar trends, albeit to a much lower extent, have been observed during the 1957 and 1968 pandemics. The W-shaped curve raises several questions about the age-dependent severity of viral diseases. In the case of the 1918 influenza pandemic, these questions include:

(1) Why is the disease milder in the 5- to 14-year-old age group?

(2) Why is the 40- to 55-year-old population protected against the disease?

Table 1. Emerging Zoonotic Viral Infections

Virus	Disease	Animal Reservoir	Vector/Route
Influenza virus	Influenza	Birds, domestic animals	Respiratory
SARS coronavirus	SARS	Civet cats?	Respiratory
Ebola virus	Ebola Hemorrhagic Fever	Primates?, Rodents?	Contact with body fluids
West Nile Encephalitis virus	Encephalitis	Birds	Mosquito
Dengue virus	Dengue Hemorrhagic Fever	Primates	Mosquito
Crimean-Congo Hemorrhagic Fever virus	Crimean-Congo Hemorrhagic Fever	Domestic mammals, rodents	Tick
Hantavirus	Hantavirus Pulmonary Syndrome	Rodents	Inhalation
Yellow Fever virus	Yellow Fever	Primates	Mosquito

HIV	AIDS	Primates	Blood
Nipah virus	Encephalitis	Fruit bats, pigs	Respiratory
Hendra virus	Pneumonia	Horses	Respiratory
Rift Valley Fever virus	Rift Valley Fever	Sheep, goats, cattle	Mosquito
Lassa Fever virus	Lassa Fever	Mouse	Ingestion
Equine Encephalitis viruses: Easter Equine Western Equine Venezuelan Equine	Encephalitis	Birds, horses	Mosquito
St. Louis Encephalitis virus	Encephalitis	Birds	Mosquito
California Encephalitis virus	Encephalitis	Mammals, wild rodents	Mosquito
Tick-borne Encephalitis virus	Encephalitis	Rodents	Tick
Marburg virus	Marburg Hemorrhagic Fever	Primates	Contact with body fluids

See References 3 and 40.

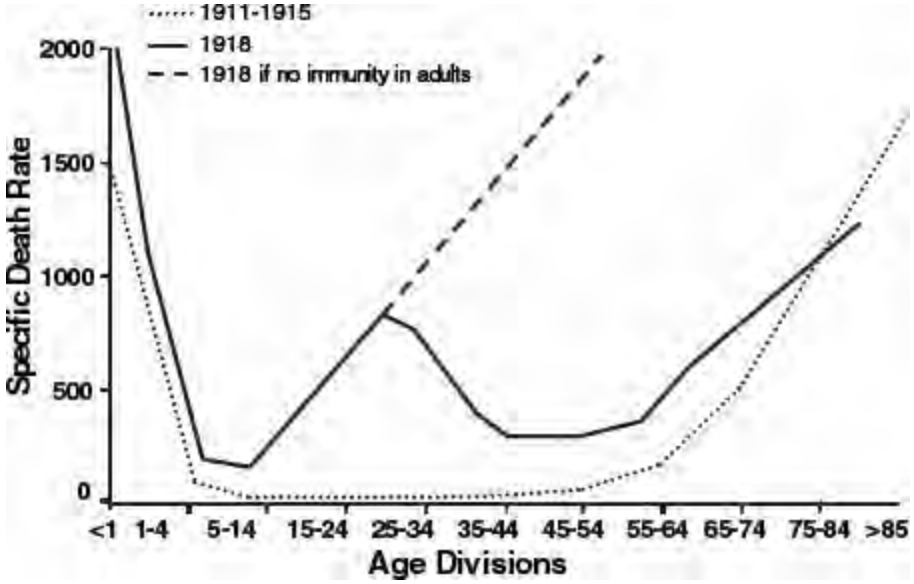

Figure 2. Influenza and pneumonia mortality by age, United States. Average for the inter-pandemic years from 1911 to 1915 is also shown (dotted line). This U-shaped curve shows the severity of disease in a population which has had prior exposure to influenza (pre-existing immunity). Only the very young and the very old show substantial mortality. Dashed line represents predicted age-specific mortality in the absence of preexisting immunity in the population above the age 30. Such a V-shaped course of the disease, with high severity in infants, a minimum in the age group 5 to 14 years and subsequent steady increase in mortality for older age groups is typical for many diseases, including smallpox, mumps, and measles. Adapted from References 11 and 28.

Age-dependent severity of viral diseases in non-immune humans is not limited to influenza. Viruses causing smallpox, mononucleosis, chicken pox, poliomyelitis, hepatitis A, mumps, and measles have all been noted to cause milder disease in younger children than in adults (both being immunologically naïve) [39]. In fact, the course of disease in the non-immune host appears to follow the dashed line in Figure 2, with higher mortality rates in infants, a minimum in the age group of 5 to 14 years, and a steady increase in severity in the older groups.

It has been suggested that the severity of viral diseases is not a reflection of the virulence of the pathogen itself, but is rather due to the maturation of both specific and nonspecific components of the host's immune system including phagocytosis, NK activity, cell-mediated cytotoxicity, and antibody production. The state of maturation and differentiation of the cells targeted by the virus also seems to play a role in disease severity, as seen for coronaviruses, herpesviruses, polyomaviruses, parvoviruses, retroviruses, and arenaviruses (reviewed in Reference 39).

While there is a general increase in severity of disease with age in the immunologically naïve host, this finding does not provide an explanation for the decreased severity of the disease in the older population during the 1918 influenza pandemic (W-shaped curve, Figure 2). For answers to these questions, we turn to the pandemics of 1957 (H2N2) and 1968 (H3N2) as well as the influenza outbreak of 1977 (reemergence of H1N1). Serological

evidence indicates that people born before 1918 had antibodies against both strains in the 1957 and 1968 outbreaks [19]. Partially protective immunity against these viruses may have influenced the severity of the disease in different age groups. Another example of the role of preexisting immunity is demonstrated by the reemergence of the H1N1 virus in 1977. The virus was virtually identical to the strains circulating in the 1950s and caused disease only in people younger than 20 years of age [21]. These examples suggest that lower severity of the disease in the population over 30 years of age during the 1918 pandemic may have been due to the presence of protective antibodies in this population group. This would indicate the possibility of a circulating H1 virus prior to 1889. In the absence of prior exposure, the mortality curve would continue to rise (dashed line in Figure 2), but the presence of immune protection in persons older than 30 years confers resistance to the 1918 virus. Interestingly, in Alaska, entire villages were wiped out by the disease, with the only survivors being young children, suggesting that the adults did not have protective antibodies to the disease due to lack of previous exposure (Figure 3). This seems likely due to the extreme remoteness and isolation of some Alaskan villages, which in the late 1800s could have prevented the population from being exposed to the then circulating H1 viruses.

Virulence Factors

Although influenza pandemics have occurred several times over the past century, the 1918 pandemic was associated with significantly higher morbidity and mortality than the subsequent pandemics. For example, reemergence of the H1N1 virus in 1977 caused pandemic disease in the young, but did not cause significant mortality. This indicates that certain pandemic viruses, the 1918 virus in particular, may possess characteristic mutations in genes other than the surface antigens, resulting in the expression of factors that make the virus more virulent and host-specific. The factors of particular interest are those that seem to be involved in the modulation of host immune responses: the NS1 and the PB1-F2 proteins.

Although attempts to isolate the 1918 influenza virus from tissue samples failed, reconstruction of the viral genome has brought us closer to understanding the virus and its virulence. With the advent of a plasmid-based reverse-genetics system for influenza viruses, we were able to generate viruses possessing the HA, NA, M, and NS genes from the 1918 pandemic strain with the remaining genes derived from the 1933 WSN H1N1 virus [4,38]. Surprisingly, the recombinant virus expressing the 1918 HA and NA genes proved to be highly virulent in mice [38]. This is unprecedented since strains of influenza A viruses typically become lethal in mice only after they are adapted to growth in these animals. In contrast to these findings, a WSN virus expressing the 1918 NS gene proved to be attenuated in mice, indicating that the NS gene may play a role in the determination of species-specificity of the 1918 influenza virus [4]. This hypothesis was further strengthened by the recent finding that an influenza NS1 gene, inserted into a recombinant Newcastle disease virus (NDV), changed the virus' species-specificity [26]. While the wild-type NDV can only efficiently replicate in chicken cells, recombinant NDV expressing the influenza NS1 gene shows an enhanced ability to replicate in human cells [26]. Thus, based on our analysis, while the HA and NA genes may contribute to the virulence of the 1918 virus, the species-specific effect of the NS gene may also be crucial to the pathogenesis of infection. Rescue of the entire virus and further studies are needed to determine functional interactions among all genes of the 1918 virus.

Figure 3. Many children in Alaska, mostly of the native population, lost their parents in the 1918-1919 influenza epidemic. Here, more than 40 influenza orphans from the village of Nushagak wait, under the care of the Alaskan Packers Association, for the government to make arrangements for their permanent care. Printed with permission from the Alaska State Library, Core: Nushagak-People-4. Alaskan Packers Association. PCA 01-2432

The influenza virus NS1 protein possesses several functions (6), but its major role is to suppress the host's interferon (IFN) response [9] (Figure 4). Mutation or deletion of the NS1 gene from the viral genome significantly attenuates the virus, both in tissue culture and in mice, but it does not attenuate the growth of the virus in IFN-deficient systems. Several studies indicate that the NS1 protein may be a virulence factor in pandemic viruses. A single amino acid substitution in the NS1 protein of the 1997 Hong Kong H5N1 virus has been shown to significantly enhance pathogenicity of the virus in pigs [25,33]. Microarray analysis of human cells infected with the 1918 NS1-expressing WSN virus showed stronger suppression of IFN-responsive genes than was found in cells infected with the wild-type WSN virus [10]. The attenuated phenotype of the WSN-1918 NS1 virus in mice provides further evidence for the species-specificity of the NS1 protein for human cells [4].

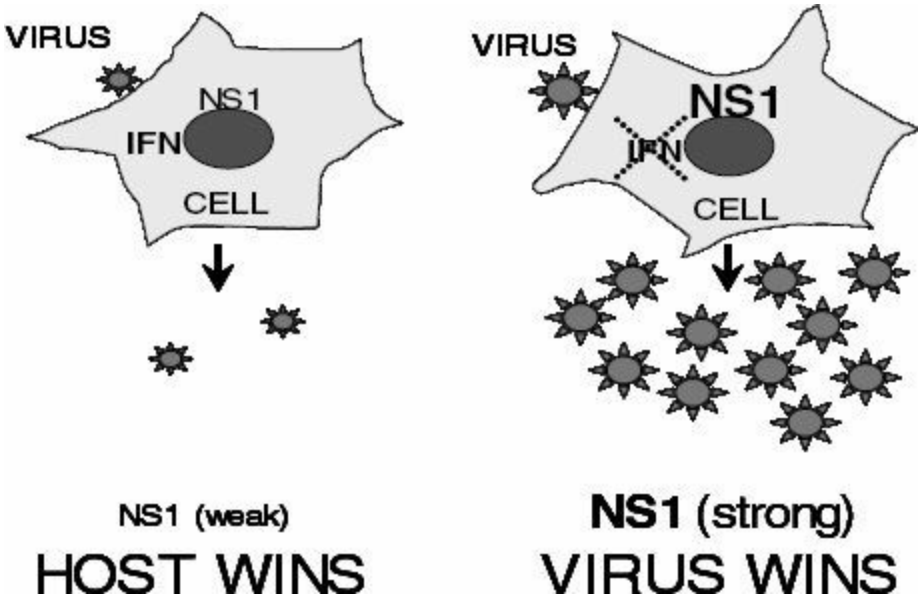

Figure 4. IFN antagonism in influenza viral pathogenesis. Most if not all viruses have evolved strategies to counteract cellular innate antiviral immune responses, including those mediated by type I IFN. An influenza virus encoding a weak NS1 protein (interferon antagonist) is unable to disarm this host antiviral response; this results in decreased viral replication, as in the left panel (host wins). However, an influenza virus encoding a strong IFN antagonist NS1 protein efficiently counteracts the host cell IFN response and viral replication proceeds relatively unimpeded, as in the right panel (virus wins). Adapted from Reference 25.

In 2001, a novel influenza virus protein termed PB1-F2 was identified in an alternate reading frame of the PB1 gene [5]. Subsequent analysis of the PB1-F2 protein from the A/PR/8/34 strain revealed that the protein induces cell death, which is specifically pronounced in immune cells. While the PR8 PB1-F2 protein does not appear to significantly contribute to virulence in tissue culture or wild-type mice, an effect has been shown in SCID (severe combined immunodeficiency) mice, where the wild-type virus proved to be more virulent than the PB1-F2 knockout virus [16]. Also, the PB1-F2 protein of other highly pathogenic influenza viruses may play a role in virulence. In fact, the PB1 gene encoding the PB1-F2 protein is one of the genes that has changed with every new pandemic virus, except for the 1977 virus. Interestingly, at present all of the circulating H3N2 viruses encode the PB1-F2 protein, while all of the H1N1 viruses since the 1977 outbreak only encode a truncated protein. H3N2 viruses are also known to cause disease more frequently than the H1N1 viruses. It would be interesting to determine whether expression of a full-length PB1-F2 protein actually contributes to disease severity in humans.

Rescue of an influenza virus carrying the 1918 HA, NA, and M genes allowed us to determine whether a 1918-like virus would be sensitive to the conventional FDA-approved anti-influenza virus drugs: neuraminidase inhibitors (zanamivir and oseltamivir) and M2 inhibitors (amantadine and rimantadine). Our studies showed that the virus is indeed sensitive to both drug classes, suggesting that current influenza antiviral strategies would be effective in curbing the dangers of a reemergent 1918 or 1918-like virus [38] (Figure 5). In the event of a new outbreak of such a virus, these drugs could provide an efficient method to control virus spread by being used to treat affected individuals and also for general prophylaxis.

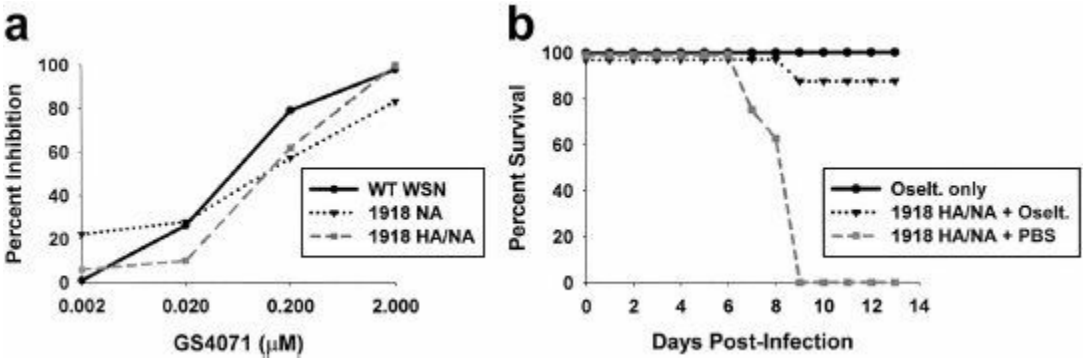

Figure 5. Oseltamivir effectively inhibits replication in tissue culture of recombinant influenza viruses possessing the 1918 NA and prevents lethal infection in mice of the recombinant 1918 HA/1918 NA influenza virus. (a) Inhibition of plaque formation in MDCK cells by GS4071 (oseltamivir carboxylate). (b) Oral administration of oseltamivir protects mice from death due to intranasal infection with the 1918 HA/1918 NA virus. Indicated is the percentage of mice surviving intranasal infection at the indicated times post infection. Adapted from Reference 38.

Despite these findings, influenza drugs have been shown to be most effective during the early stages of an infection, due to the short viral incubation period. Therefore, vaccines remain the best weapon against the virus. Formalin-inactivated influenza virus vaccines are currently widely in use. Their drawback, as seen with other inactivated virus vaccines, is that they are only effective in induction of humoral but not cellular or mucosal immunity. An alternative approach is presented by the FluMist™ vaccine, the first FDA-approved intranasal influenza vaccine which is composed of a formulation of 3 live attenuated influenza viruses: 2 influenza A viruses (H1N1 and H2N3) and an influenza B virus.

Recent advances in the field of reverse genetics for influenza viruses made it possible to generate recombinant influenza A and B viruses entirely from cDNA [8,15,22]. This system allowed the introduction of specific mutations and deletions into the viral genome, with subsequent generation of an attenuated virus that can induce stronger humoral, mucosal, and cellular immune responses without causing disease. We propose to develop second generation live vaccines by the method of reverse genetics. We suggest that targeting

influenza virus virulence factors such as the NS1 protein would provide a more effective strategy for generating live attenuated influenza virus vaccines. In fact, mice immunized with influenza viruses attenuated by deletions in the NS1 gene generate strong immune responses that are protective against subsequent challenge with a lethal dose of the wild-type virus [35]. Should a new strain of influenza viruses emerge, the HA and NA genes of the new virus could be quickly cloned and introduced into the “backbone” of the virus attenuated by NS1 deletion, allowing for efficient generation of vaccines against the emergent strain. Aside from influenza virus, we propose that this approach may also be applicable to the development of vaccines against other viruses, once the anti-IFN genes in these viruses are identified.

Conclusions

While many questions still remain to be answered, we are much further ahead now than we were just a few years ago. Clinical and epidemiological reports collected during the 1918 outbreak are now being supplemented and intertwined with studies of the pathophysiological relationship between the 1918 viral genes and the host cell. Using influenza virus as a model of emerging zoonotic diseases we are trying to understand the causes and mechanisms of cross-species transmission of other viruses, with SARS coronavirus being the most recent example. Finally, identification of the virulence factors has provided us with tools for the development of new vaccines and antiviral drugs, allowing for more effective methods to control future outbreaks. As we get further away from the 1918 pandemic, we are getting closer to solving a puzzle that should provide us with a better understanding of other emerging influenza viruses and with the means to counteract another pandemic.

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West Nile Virus

Lyle R. Petersen, M.D., M.P.H

Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, U.S.A.

Within 5 years of its recognition in North America [46], West Nile virus (WNV) caused the 2 largest arboviral meningoencephalitis outbreaks ever recorded on that continent [48]. By 2003, the virus had spread geographically from its 1999 epicenter in New York City to nearly all of the United States (U.S.) and much of Canada, as well as to the Caribbean, Mexico, and Central America. Although incidence has been variable, newly established endemic areas in the U.S. and Canada have experienced continued transmission to humans from year to year, suggesting that WNV will be a public health threat for years to come.

Virology and Importation to North America

WNV is a single-stranded RNA virus of the family Flaviviridae, genus *Flavivirus* within the Japanese encephalitis (JE) virus antigenic complex. The JE serocomplex contains several medically important viruses that have also been associated with human encephalitis: JE, St. Louis encephalitis (SLE), Murray Valley encephalitis (MVE), and Kunjin (KUN, a subtype of WNV) [52]. All flaviviruses are closely related antigenically, which accounts for the serologic cross-reactions observed in the diagnostic laboratory. Members of the JE complex are even more closely related, often needing specialized tests such as virus neutralization assays to help identify the infecting flavivirus.

Of the 2 known lineages of WNV, only lineage 1 viruses are definitely associated with human disease; lineage 2 consists of African isolates maintained in enzootic cycles. The WNV variant circulating in North America is a lineage 1 virus that is genetically nearly identical to a strain previously circulating in Israel, suggesting Middle Eastern origin [39]. The virus has undergone little genetic evolution since its initial 1999 isolation [38].

The exact means of WNV importation to North America is unknown, but the virus likely arrived in the New World via an infected mosquito, bird, or other animal, or human. Although importation of an exotic arbovirus into New York City was surprising and unexpected, in retrospect, this importation probably reflects New York City's extensive international travel and trade and serves as a model for future importations of exotic arboviral agents into other major metropolitan areas. For example, from August 1988 to July 1999, there were 4.85 million international arrivals into New York City airports, including 154,000 passengers from Israel, where WNV epizootic activity and human transmission have been documented after 1997. Thus, even an improbable event, such as the arrival of a WNV-infected animal or human and subsequent establishment of a local epizootic, becomes more likely given the magnitude of these importations and arrivals.

Ecology

The virus is maintained in a bird-mosquito-bird cycle, with passerine birds serving as the primary amplifying hosts. In temperate regions, this cycle begins in spring when mosquitoes emerge and lasts until early fall when female mosquitoes enter diapause (physiologic dormancy) and infrequently bite. As with SLE virus, Culex mosquitoes are the principal maintenance and amplifying vectors [48,51]. When significant amplification within the passerine Culex cycle occurs, other “bridge vector” mosquitoes that bite both humans and birds may become infected by mid to late summer and pose a human infection threat. Although many of the 46 North American mosquito species shown to harbor WNV as of the fall of 2003 do not bite humans, it is unclear whether most human infections result from bites of the primarily ornithophilic Culex species or from bites of potential “bridge vectors” (e.g., *Aedes* and *Ochlerotatus* species).

Only the U.S., Canada, and Israel have experienced significant avian mortality from WNV [52]. As of the spring of 2003, avian mortality has been documented in 225 native and captive species in North America. Mortality varies by species, but approaches 100% among American crows *(Corvus brachrhynchos)* [35]. Certain common species, such as house sparrows (*Passer domesticus*) are frequently infected during epizootics, develop high viremia for several days, and thus are likely important amplifying hosts [35]. Humans and horses are not thought to be important amplifying hosts since viremia is short-lived and low-grade [8,25,49,59].

WNV can be isolated from bird feces and from oral swabs [36] and bird-to-bird transmission can occur [35]. Although birds can become infected following ingestion of infected mosquitoes, birds, and rodents, the importance of oral transmission in nature is unknown.

Clinical Epidemiology

WNV was first isolated in 1937 from a resident of the West Nile district of Uganda [58]. WNV is extensively distributed throughout Africa, the Middle East, parts of Europe, western Russia, southwestern Asia, and Australia [51,52]. From 1937 to the mid-1970s, human outbreaks, mainly associated with mild febrile illnesses, were reported infrequently from Israel and Africa [32,45,52]. However, after a hiatus of 20 years with relatively little recorded outbreak activity, outbreaks in Romania (1996) [60], Russia (1999) [53], Israel (2000) [17,62], and the U.S. and Canada (2002 and 2003) [15,20,48], have involved hundreds to thousands of humans with severe neurological disease and have coincided with the emergence of new, closely-related WNV strains [38,39,52].

The virus has spread rapidly in North America (Figure 1) [48]. Reported cases were infrequent (62 cases in 1999, 21 in 2000, 66 in 2001), however, until 2002 when a large outbreak, mostly in the Midwest and Mississippi River Delta, resulted in more than 4000 reported cases, mostly with meningoencephalitis [48]. The geographic distribution of cases during 2002 resembled that of a large SLE outbreak in 1975 [43]. In 2003, another large outbreak occurred, with more than 9700 cases reported mostly in the Midwest and western plains states. The large increase in reporting was partially due to reporting of milder cases without invasive neurological disease as a result of wider availability of diagnostic testing. However, the number of cases with meningoencephalitis approximates that of 2002, indicating that the 2002 and 2003 outbreaks were of approximately the same magnitude. In 2003, autochthonous human cases were reported for the first time in Arizona and California, indicating the potential for future epidemic activity in the far western states.

Coincident with the peak transmission within the bird-mosquito-bird cycle, approximately 85% of human WNV infections occur in August and September; however, human illness has been reported in the U.S. from May to December [48,51].

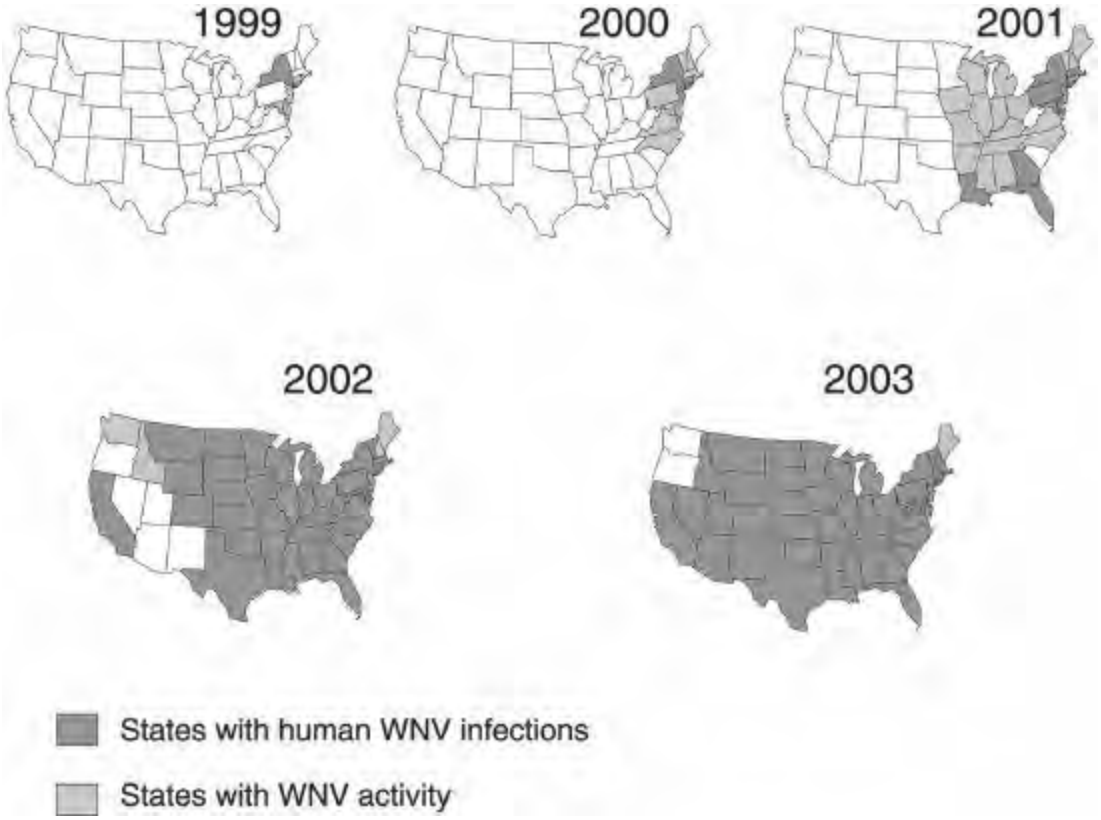

Figure 1. States with reported West Nile virus activity from 1999-2003.

Transmission to Humans

Nearly all human infections result from mosquito bites; however, several novel transmission modalities were recognized in 2002. Transplacental transmission followed a second trimester infection [10]. The infant, delivered at term, had chorioretinitis, severe neurological impairment, and WNV IgM antibody in serum and cerebrospinal fluid (CSF) [2]. Probable breast milk transmission occurred when a lactating mother acquired WNV via blood transfusion [12]. WNV RNA was detected in her breast milk; the infant remained asymptomatic but developed IgM antibodies to WNV. Although infrequently reported in the past, infection of 2 laboratory workers via percutaneous inoculation were reported in 2002 [11]. An outbreak of WNV infection occurred among workers at a turkey breeder farm; however, the means of transmission to these workers was unclear [16].

WNV was transmitted to 4 recipients of organs from a single donor who had been infected from a WNV-contaminated blood transfusion 1 day before organ recovery [31]. Subsequently, WNV transmission was documented via transfused platelets, red blood cells, and fresh frozen plasma donated from both symptomatic and asymptomatic donors [26,49]. Blood-borne transmission risk relates to WNV infection incidence among potential blood donors, and was estimated as high as 21 per 10,000 donations in some cities at the peak of the 2002 epidemic [7]. Routine blood donor screening using nucleic acid-based detection assays was begun in the summer of 2003. As of November 2003, screening assays have detected more than 900 donors with evidence of WNV nucleic acid [14]. At least 2 transmissions to recipients of screened blood have occurred, presumably due to viral levels in the donors below the level of detection using current screening algorithms and assays [14].

Clinical Illness

Most persons infected with WNV remain aysmptomatic. Serological survey data indicate that WNV infection rates are similar by age; however, the frequency and severity of clinical illness increases as age increases [44,46,60]. When clinical illness occurs, the incubation period generally ranges from 2 to 14 days, but prolonged incubation periods of up to 21 days have been observed among immunocompromised persons [31].

Symptomatic illness without invasive neurological disease is named WN fever. The acute symptoms of WN fever generally last 3 to 6 days and often includes sudden onset of malaise, anorexia, nausea, vomiting, eye pain, headache, myalgia, and skin rash [29,41,51]. Upper respiratory symptoms, including rhinorrhea, cough, and sore throat, have been associated with WN fever, but a cause-and-effect relationship has not been proven [11,26,29,41]. Anecdotal reports indicate that many patients with WN fever have prolonged fatigue lasting several weeks after the acute illness. Lymphadenopathy and the erythematous macular, papular, or morbilliform eruption which can involve the entire body were common in earlier outbreaks, but are now less frequently reported [41,46,51]. The proportion of infected patients developing WN fever has not been precisely determined. A household-based, serologic survey in New York City indicated that approximately 1 in 5 infected persons develop WN fever [44]. A serologic survey of patients seeking treatment at hospitals and clinics in Czechland indicated approximately 40% of infected patients developed WN fever [30]. Preliminary results of follow-up of blood donors with positive nucleic acid screening assays indicate that approximately 15% develop WN fever [15].

Despite the increased clinical severity during recent outbreaks, <1% of persons infected with WNV develop invasive neurological disease (encephalitis, meningitis, or acute flaccid paralysis ([AFP]) [13,15,44,60]. In recent outbreaks, encephalitis-meningoencephalitis was more commonly reported than meningitis alone [17,46,60]. More than 90% of patients with severe neurological disease have fever, often accompanied by severe weakness, gastrointestinal symptoms, and headache [8,46,60]. Movement disorders, such as tremor, myoclonus, and parkinsonian features including rigidity, postural instability, and bradykinesia, may occur frequently [8,56,59].

AFP is asymmetric, affects the upper or lower limbs, and can occur without overt meningoencephalitis [1,3,4,9,24,34,40,47,56,57]. In addition to the flaccid paralysis, patients with AFP often do not have paresthesias or sensory loss, but frequenly have diminished or absent deep tendon reflexes, cerebrospinal fluid pleocytosis, acute respiratory distress, and acute changes in bowel or bladder function. Although initial reports attributed profound muscle weakness to Guillain-Barré syndrome [1,3,4], clinical, electrodiagnositic, and pathologic findings from most cases suggest destruction of anterior horn cells similar to poliomyelitis [24,34,40,47,56,57].

Other neurological manifestations described with WNV infection include cranial nerve abnormalities, optic neuritis (23), and seizures. A variety of ocular manifestations, including mulitfocal choroiditis [61], vitritis [5], uveitis [37], occlusive retinal vasculitis [33] and chorioretinitis [5,10] as well as myocarditis, pancreatitis [50], and fulminant hepatitis [22] have been described.

Clinical Outcome

Case fatality rates ranged from 4 to 14% among patients hospitalized during recent outbreaks [17,46,60]. During the 2002 outbreak in the U.S., patients with meningoencephalitis had a 9% case-fatality rate. Advanced age is the most important risk factor for death. Case-fatality rates among persons aged >70 years were 15% and 29% among hospitalized patients in Romania [60] and Israel [17], respectively, and was 20% among those with meningoencephalitis in the U.S. in 2002. Encephalitis with severe muscle weakness, change in the level of consciousness, diabetes, and immunosuppression appear to be risk factors for death and poor neurologic outcome [17,31,46].

Substantial morbidity may follow hospitalization for WNV. At discharge, more than half of the patients hospitalized in New York and New Jersey in 2000 had not returned to their previous functional level, and only one-third were fully ambulatory. A 1-year follow-up of patients reported during the 1999 New York outbreak indicated frequent persistent symptoms (fatigue, 67%; memory loss, 50%, difficulty walking, 49%; muscle weakness, 44%; and depression, 38%). An 8-month follow-up of patients with invasive neurological disease showed that many patients reported continued fatigue, headache, and myalgias, and most had persistent gait and movement disorders [56]. Patients with acute flaccid paralysis did not recover limb strength.

Pathogenesis

Initial viral replication following a mosquito bite is thought to occur in the skin and regional lymph nodes and produces a primary viremia that seeds the reticuloendothelial system [18]. A secondary viremia then occurs which may seed other organs and the central nervous system (CNS).

The pathogenesis of severe infection with WNV is poorly understood, but the pronounced risk of neurologic infection and death in the elderly suggests a role for age-related factors such as immune senescence or changes to the blood-brain barrier. Studies in mice indicate a critical role of the early antibody response in containing viremia and limiting disseminated infection of WNV in the CNS [19]. Other factors such as hypertension and cerebrovascular disease which disrupt the cerebral endothelium, have been suggested to enhance viral entry into the CNS. Three of 4 patients infected via transplanted organs developed meningoencephaltis, indicating that adminstration of immunosuppressive drugs may place persons at very high risk for CNS disease [31]. WNV strains also differ in neurovirulence [6].

Pathologic observations in a few cases of fatal encephalitis showed scattered microglial nodules and mononuclear perivascular inflammatory infiltrates most common in the brain stem but also found in the thalamus, cerebellum, and cerebral cortex [55]. Pathologic findings of 1 case of AFP included focal loss of anterior-horn neurons, with gliosis, occasional macrophages, neuronophagia, and perivascular lymphocytes [34].

Diagnosis

Total leukocyte counts in peripheral blood are mostly normal or elevated [51]. Examination of the CSF of patients with meningoencephalitis or AFP shows pleocytosis usually with a predominance of lymphocytes and elevated protein. Computed tomography of the brain typically shows no evidence of acute disease [17,46,51]. In approximately one-third of patients, magnetic resonance imaging shows enhancement of the leptomeninges, the periventricular areas, or both. One study indicated that most patients with WNV meningoencephalitis have non-specific, abnormal EEG findings consisting of a certain pattern of generalized, continuous slowing, which was more prominent in the anterior (frontal and temporal) regions and at times, had a side predominance [21].

The diagnosis of WNV infection in most clinical settings should be made using serologic methods, particularly detection of IgM antibody in serum or CSF using the IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) [42,51,52]. Since IgM antibody does not cross the blood-brain barrier, its presence in CSF indicates CNS infection; at least 95% of patients with meningoencephalitis have demonstrable IgM antibody in CSF. In addition, at least 95% of serum samples obtained from patients with invasive neurological disease within 8 days of symptom onset test positive for IgM antibody. The timing of the antibody response in patients with WN fever is not well described; however, anectodal reports suggest that a sizeable proportion will not have demonstrable IgM antibody upon clinical presentation.

Persons recently vaccinated with yellow fever or JE vaccines or persons recently infected with a related flavivirus (eg, SLE, dengue) may have a positive WNV IgM antibody test result [42]. The plaque reduction neutralization test (PRNT) can help distinguish false positive results of MAC-ELISA or other assays as well as to help distinguish serologic cross-reactions among the flaviviruses. Among persons with invasive neurological disease, IgM antibody may persist in serum for >6 months; therefore, persistent IgM antibody from a previous infection may be unrelated to current illness [54]. A ≥ 4-fold rise in WN virus-specific neutralizing antibody titer in acute and convalescent sera is confirmatory of acute infection [51].

In otherwise healthy persons, WNV can be isolated from serum several days before illness onset, but viremia rapidly disappears after symptom onset along with concomitant development of IgM, IgG, and neutralizing antibodies. Therefore, the sensitivities of viral detection methods such as nucleic acid amplification or culture are low and are not recommended in most clinical settings. The immuno-compromised patient, however, may develop prolonged viremias documented up to 28 days in duration along with delayed development of IgM antibody [27,28,31]. In this setting, nucleic acid-based assays may be useful for clinical diagnosis.

WNV was used at one time as an experimental treatment for cancer. WNV was isolated in spleen, lymph nodes, liver, and lungs in patients who died within approximately 4 weeks after such treatment.

Treatment and Prevention

No antiviral treatments have proven efficacious for WNV infection. Clinical trials to evaluate the efficacy of ribavirin, intravenous immune globulin containing high anti-WNV antibody titers, and antisense compounds for treatment of severe neurological disease are underway. An inactivated WNV vaccine is available for horses, but human vaccines are unlikely to be available for several years. Personal protection to avoid mosquito exposure, including using repellants containing DEET or permethrin, is the mainstay of prevention. Draining standing water where mosquitoes are likely to breed and community mosquito control programs are recommended.

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Discussion

Viral Infections

Question for Dr. Essex, Harvard AIDS Institute, U.S.A.: What might be the reason or mechanism underlying the growing prevalence of HIV-1C in Africa?

Answer by Dr. Essex: One possibility is that the virus is more transmissible than other HIV-1 subtypes because titers are higher in reproductive tract fluids. Although the mechanisms of why titers would be higher in reproductive tract fluids are not clearly known, we postulate that one possibility is the increased response of HIV-1C to inflammatory cytokines which might be associated with damage to reproductive tract cells by other concurrent sexually-transmitted infections.

Question for Dr. Essex: You mentioned that HIV type 2 is less virulent and less infectious. What is the most important factor that determines the virulence or infectious capacity compared with HIV-1?

Answer by Dr. Essex: There are a number of differences between HIV-2 and HIV-1. One concerns transcriptional activation. HIV-1C has 3 kappa B sites and the most active transcriptional activation. Other HIV-1 subtypes such as HIV-1B and HIV-1D, which are closely related, only have 2 and HIV-2 subtypes only have 1. By transcriptional activation they seem to grow to much lower titers. That is presumably reflected by viremia levels in infected people where, on average, the amount of RNA and viral load at the time of even acute disease is about 2 logs less for HIV-2 than for HIV-1. I suspect there are other mechanisms such as receptor affinity but most of the other mechanisms haven't been well studied.

Question for Dr. Matsuoka, Kyoto University, Japan: What would you think is the mechanism that a virus could cause both hypermaturation in the DNA genome as well as hypomaturation?

Answer by Dr. Matsuoka: This is a tough question. Disregulation of DNA maturation is a common feature of cancer cells. I suspect a long lifetime of infected cells might cause disregulation of DNA maturation, and some DNA hypermaturation is associated with aging.

Question for Dr. Takada, Hokkaido University, Japan: Do you have any information about superinfection of Epstein-Barr virus and *Helicobacter pylori* in gastric malignancy? Does *H. pylori* infection have any effect on transformation by Epstein-Barr virus?

Answer by Dr. Takada: This is a difficult question because most people in Japan are infected with *H. pylori* and it is very difficult to analyze the sequence of Epstein-Barr virus infection and *H. pylori.*

Question for Dr. Palese, Mount Sinai School of Medicine, U.S.A.: Are the new H5N1, H9N2, and H7N7 viruses susceptible to the neuraminidase inhibitors?

Answer by Dr. Palese: All of these neuraminidases have been shown to be sensitive to the neuraminidase inhibitors so it is likely that they would also work in humans. H7N7 just appeared in the Netherlands in March and April of 2003, and about 100 persons had symptoms. The one person who died was actually a veterinarian who did not take a prophylactic neuraminidase inhibitor. The remaining individuals took prophylaxis. Although it is difficult to prove that the neuraminidase inhibitors protected these individuals, it is clear that the one fatality occurred in an individual who did not take prophylaxis.

Question for Dr. Palese: Is there any evidence that a human would develop some immunity to a human-adapted H5 virus if he/she would be repeatedly exposed to an avian H5 or H7 virus.

Answer by Dr. Palese: There would clearly be some protection. There is actually some effort to stockpile and make vaccines against H5, H7, and H9 viruses in the event that such a virus should take off in the human population. The assumption is that these vaccines would work. There is reason to assume that if they are fairly well matched in terms of their overall antigenic virus, they would work. Each of the vaccines that we currently have only work against the H1, H3 and influenza B virus component.

Question for Dr. Petersen, Centers for Disease Control and Prevention, U.S.A.: Is West Nile virus nationally reportable?

Answer by Dr. Petersen: It is. In the United States, every state reports West Nile virus.

Question for Dr. Petersen: What is the methodology of surveillance in each state and is it uniform?

Answer by Dr. Petersen: It is uniform. We do have a national case definition for both animal and human disease. Every state in the country does surveillance for humans for meningoencephalitis to see if it is West Nile virus. We also have a national system for looking at birds. Basically, the public is asked to report dead birds to their local health department where the bird is then tested. We also have a national reporting system for horses with possible West Nile virus disease.

Question for Dr. Petersen: How do you explain West Nile virus seemingly disappearing in 2001 but returning in 2002?

Answer by Dr. Petersen: The epidemic started in 1999 in New York City with 62 cases and in 2000, there were only 21 cases nationwide, mostly in New York State. In 2002, there were more cases which occurred in New York State. The reason is largely related to weather. In 1999, there was an exceptionally warm, hot July summer in New York State. Warm and dry conditions seem to promote the amplification of this virus in nature. The reason is that because Culex mosquitoes like to breed in small, nutrient rich pools of water

which are common when it's hot. For a mosquito to infect a person with West Nile virus, it has to bite a bird. The virus then replicates in the mosquito, the mosquito lays its eggs and then goes out for a second blood meal. It is this second bite that leads to the infection. That period takes about 2 weeks: 1 week to 10 days to replicate, then another 7 to 10 days for the virus to replicate. A mosquito only lives about 2 or 3 weeks, and so that period is very critical. We have shown that when it is warmer outside, that period of viral replication in the mosquito is faster and there is a higher level of virus in the mosquito. So you are more likely to get infected when it's hot. As it turns out, it was very hot and dry in 1999 but in 2000 it was cold and wet. In 2001, it was more normal, and became even more normal in 2002. In 2000, it was not the right climatic condition to promote viral replication in nature.

Question for Dr. Petersen: Do any other countries screen for West Nile virus in blood donors?

Answer by Dr. Petersen: Canada is the only country that does blood donor screening for West Nile virus. A number of countries exclude people from donation if they have traveled to the U.S. during the summer months.

Question for Dr. Petersen: What do you think the probability of an outbreak in California is next summer and will it be bad?

Answer by Dr. Petersen: Although it is difficult to determine the severity of the outbreak, I think the probability of an outbreak in California is quite high which actually raises some concern because of all the travel and trade throughout the Pacific Rim countries. A big outbreak in California raises the possibility that the virus could be exported outside the U.S.

Question for Dr. Petersen: Why are many mosquito species vectors of West Nile virus?

Answer by Dr. Petersen: It just happens that West Nile virus can replicate in the midgut of the mosquito of Culex mosquitos quite well. The mosquito becomes viremic, and the virus is transported to the salivary glands such that when you get bitten, you get infected. It just happens that West Nile virus is very efficient in doing this in Culex mosquitoes in particular.

Question for Dr. Petersen: Are there any prospects for a vaccine for West Nile virus?

Answer by Dr. Petersen: Yes there are good prospects for vaccines for humans. We already have an inactivated vaccine for horses. For humans, we know that for the other flaviviruses such as yellow fever and Japanese encephalitis, you can develop good vaccines. On a theoretical basis, therefore, a vaccine for West Nile virus is likely to work quite well. There are 2 groups that are developing the vaccine. One is developing a chimeric vaccine using the yellow fever vaccine strain as the backbone for the virus. The other group is using a dengue 4 attenuated backbone and a similar chimeric vaccine strategy. These vaccines are fairly far along in development.

Comment by Dr. Linda Detwiler, U.S. Department of Agriculture Advisor. I'd like to make a comment about the equine vaccine. There have been more documented cases of breaks in what was the labeled prescription of the vaccine and it is not that durable. The recommendation is to vaccinate twice a year, once before mosquito season and once during the height of the season.

Question for Dr. Petersen: In terms of your surveillance for acute central nervous system disease with fever, what percentage of cases are you able to nail down etiologically, particularly the summer cases?

Answer by Dr. Petersen: I don't know what percentage of cases of meningoencephalitis have West Nile virus since we only hear about the positive cases. Historically, only about 20% of patients with meningoencephalitis ever get a diagnosis. It's likely to be much higher in the summer when West Nile virus is there, but I don't have a good denominator for that.

Closing Remarks

Joshua Lederberg, Ph.D.

The Rockefeller University, U.S.A.

I've been asked to make some concluding remarks and I will try to keep them brief. We've had a wonderful course in public health, virology, infectious disease, and infection control. I know I've learned an enormous amount. What an intellectual stretch to keep up with the wide range of information and concepts that have been presented! Thus, it would be foolhardy for me to even attempt to give you a summary of the symposium and that's not what I interpret "concluding remarks" to be. But rather, there were some open questions that were suggested to me by different speakers on different topics. In a rather idiosyncratic way, I just want to throw a couple of them up in the air for consideration.

One of them is, "Why are there zoonoses?" I'll explain that in a little more detail. The last presentation by Dr. Peterson was the most exhaustive treatment of a zoonosis covering the amplifying host, primary host, the vectors, and so on that I've ever seen or ever read, and that mitigated some of my questions. Zoonoses are diseases where you have a pathogen circulating in an animal population for an indefinite period of time. In many, but not all cases, there has been human contact. Why isn't there a stable circulation of these same viruses in humans which would negate the idea of a zoonosis because it would already be present in the human population? For a disease to qualify as a zoonosis there has to be some barrier in transmission from the animal source to humans. What are the factors that may control that? There may be circumstantial geographic or other environmental barriers, hindering contact between the human and the animal species. Besides that, I'm quite confident that we are going to find idiosyncratic host factors that humans are not equally susceptible to infection from a virus arising from a zoonotic source. Another possibility is the heterogeneity of the pathogen. It seems likely — e.g., in the case of the human coronavirus causing SARS — it isn't just exposure to an animal coronavirus, but there is some further genetic alteration which characterizes most of the coronavirus strains in humans compared to the animal source.

A remark oft heard these last few days takes note of limited human to human transmission. We've heard that about monkey pox in Africa, although there might be occasional examples of quite severe human disease. We saw that in the outbreak of the H5N1 avian flu in 1997. With one possible exception, every case of this epidemic could be traced to a direct contact with a bird. How is it that the virus gets from the animal to the human, but doesn't go further and jump from human to human? We heard some answers to this question. In the case of West Nile virus, the level of viremia in the bird host was many thousands of times higher than it was in the human host. And maybe that'll be the example. There again there may be any number of idiosyncrasies that could be looked for; for

example, the human who is infected with an animal virus may be a rare genotype who is then much less likely to find another human of that same genotype for further transmission. These are open questions that bear much closer examination.

Another matter that came up obliquely and has been puzzling me for a very long time is the dynamics of seasonality of respiratory infections. We see this regularly with influenza and with a number of other respiratory diseases. They don't all show exactly the same cycle from month to month, and I've not heard a convincing explanation supported by evidence for why that seasonal variation takes place. Parasite durability, host vulnerability, and crowding are some possible explanations that have been made from time to time. It's a rather banal finding, and I know very little investigation that would help to account for it.

Several times during this Conference questions about how we disinfect surfaces and fomites have come up. While we have a lot of practical knowledge of disinfectants, we rarely know how they work. In the absence of a good theory of viricidal and microbiocidal activity, it is really very hard to be certain how applicable the disinfectant will be for any new virus or for an existing virus or bacterium in a matrix of different material that may not lend itself to penetration. Alcohol is advocated as a means of sterilization, but bacillus spores can survive in alcohol for a very, very long period of time.

I would be more content if we had a clear understanding of just what the biochemical targets are for different disinfectants, and what the likelihood of breakthrough would be, based on extrapolations from model situations to how the disinfectant swipe is acting. There was a very intriguing report from David Taylor on incinerated prions retaining some degree of activity. That work bears repetition and confirmation. I don't know whether it's going to end up being true or not, but I wouldn't reject it out of hand just on theoretical principles. But I am certain that no protein survives 600°C, so if there is infection-inducing or prion-inducing material in that ash, I'm quite confident it's not prion protein, per se. But who is to say what else could provoke the catalytic change from the normal cellular prion protein to the PrP scrapie. We have to be careful not to be too taken with the viral model. The prion is not a self-replicating particle. You don't have regeneration of a copy with a new sequence based on that as a template. The prion modifies existing normal prion protein to where it behaves like the infectious PrP scrapie and there may be other catalysts that aid in this transformation. Until we've had empirical data on what kinds of things do and do not provoke that change, it's really quite an open kind of territory. Since the change from one prion state to another is merely a conformational one, we have to be very careful in our handling of prion protein-containing materials that we do not inadvertently generate the infectious form in the very process of trying to remove the infectivity from the target material. None of what I've just said has been demonstrated experimentally, but it's an open possibility that we ought to keep in mind.

In the area of nosocomial infection control, I didn't hear any discussion about environmental monitoring to determine how clean the environments are. In this context, environment also includes healthcare workers. We probably don't want to know the extent to which nosocomial infection is borne by the surgeon, by healthcare workers, and nurses. Without interfering in the already difficult management of hospital care, sufficiently reliable, relatively low-tech, low-expense monitoring systems should be sought. We might then know

about the air quality in, for example, infection control units. In this room, for example, 30 or 40% of us are carrying some quite serious pathogens in our noses that administered in the wrong places could cause quite serious disease. How do we maintain that equilibrium with those pathogens until they get out of hand or get into the wrong place? Nobody ever studies that. One reason this may come to a head is the shadow of enormous financial liability that hospitals have when lawyers get the idea that nosocomial infection might be preventable.

That's the short list of the open questions that I am left with; I'd had so many of them that have actually been closed by the speakers. I feel enormously enriched by the experience of this Conference and I want to thank not just the speakers but the organizing committee for making this symposium possible.