
Meeting Summaries

The Hot Zone—1997: Conference on Emerging Infectious Diseases

On June 27-28, 1997, the University of Kentucky College of Medicine, University of Cincinnati College of Medicine, and Kentucky AIDS Consortium held a conference for clinicians and researchers in Lexington, Kentucky. Participants presented the latest findings from worldwide epidemiologic studies, basic science, and clinical research on emerging infectious diseases. The findings indicate that the war against infectious diseases is far from over. In the United States, between 1980 and 1992, deaths from infectious diseases increased by 58%, making serious infections the third leading cause of death; HIV infection is the leading cause of death among 25- to 44-year-olds; and antibiotic resistance costs the health-care system an estimated \$100 million to \$30 billion each year.

With the global population growing from 2.5 to 5.8 billion over the last 25 years, large urban centers throughout the developing world are overcrowded and have inadequate sanitation, ideal for the emergence of infectious diseases. By 2025, the global population will reach 8.6 billion. In developing countries, this represents an 84% increase, which will intensify overcrowding in these areas. In industrialized countries, an aging population base, the advent of immunosuppressive medications, and the emergence of HIV are combining to increase the risk for opportunistic infection. Moreover, with increased travel, clinicians see increasing numbers of patients with exotic diseases acquired abroad. Recent migration of epidemic diphtheria from the former Soviet Union to Europe and the emergence of multidrug-resistant tuberculosis (TB) in the United States and elsewhere are but two examples of infections resulting from international travel; in addition, nearly 70% of the fruits and vegetables consumed in the United States originate in developing countries; disease outbreaks related to imported food frequently go unreported.

Extensive cross-species contact among humans and certain domestic animals can dictate antigenic shifts in influenza viruses. The likelihood of the emergence of a new influenza virus in the near future increases with the growth of the hog population in China. The emergence of

new viruses, such as HIV and filoviruses, indicates the virtually unlimited capacity of pathogenic organisms to mutate and rapidly adapt to environmental changes and selective pressures.

HIV/AIDS Research

Recent data from long-term survivors support the concept that HIV replication occurs when the number of CD4+ cells drops below the minimum level required to maintain CD8+ cell control of HIV. CD4+ cell production of IL-2 is needed for strong cell-mediated immunity. Without CD8+ cell responses, a more virulent, highly cytotoxic viral strain emerges, killing greater numbers of CD4+ cells and leading to AIDS. Because CD8+ cell loss appears to be related to a shift from a TH-1- to a TH-2-type cytokine response, therapeutic approaches that maintain TH-1 cell response, or enhance CD8+ cell anti-HIV activity through factors yet to be fully defined, are being actively investigated. Vaccines relying exclusively on antibody responses will almost certainly prove to be of limited value, while those using CD8+ cell antiviral activities hold substantially greater promise.

A basic science research forum described the use of a murine model of immunodeficiency induced by a type C retrovirus. Because models of this type reproduce a number of the clinical pathophysiologic manifestations associated with human AIDS, they can significantly enhance our understanding of retrovirus-induced immunodeficiency.

The results of recent research involving host immune responses to *Pneumocystis carinii* infection indicated that, compared with healthy controls, HIV patients with less than 200 CD4+ cells have similar IL-4 levels but significantly lower peripheral blood mononuclear cell proliferative responses and IFN- γ levels to *P. carinii* major surface class glycoprotein (MSG). Centers for Disease Control and Prevention (CDC) Class 3 patients with previous *P. carinii* pneumonia (PcP) have significantly higher IL-4 (but not IFN- γ) levels than Class 3 patients with no history of PcP. HIV+ patients who have recovered from PcP have sufficient memory cells to recognize MSG, but demonstrate a shift from a TH-1- to a TH-2-type antigen recall response.

HIV Therapy

The current state of CD4 and viral load testing and the 11 drugs available for HIV treatment were reviewed. Although combination

drug therapy has consistently proved more effective than monotherapy in maintaining reduced viral load, the results of a recently completed follow-up study confirmed the effectiveness of zidovudine (AZT) in preventing neonatal HIV transmission; in a large cohort of pregnant women, AZT plus high titer immunoglobulin G was no more effective than AZT plus placebo, with both regimens producing results comparable with those achieved with AZT alone. A number of issues in HIV treatment remain unresolved, including when treatment should begin, how to manage inadequate response to therapy, whether to use HIV resistance genotyping to direct therapy, and how to best deal with prophylaxis for opportunistic infections in patients showing dramatic reductions in viral load.

Dengue and Dengue Hemorrhagic Fever (DHF)

Tens of millions of cases of dengue and hundreds of thousands of cases of DHF are reported annually, and more than 2.5 billion people are at risk for infection. Factors contributing to the emergence of dengue include unplanned and uncontrolled population growth associated with urbanization in tropical regions, lack of effective mosquito control, deteriorating water systems that increase densities of *Aedes aegypti*, and viral migration among tropical urban centers due to increasing international air travel. More than 50% of all air travel from the United States is to tropical destinations, and from 1977 to 1994, 2,248 suspected cases of imported dengue were reported in the United States. Because their clinical symptoms are initially nonspecific, dengue and other arboviral infections can be difficult to distinguish from other viral, bacterial, and parasitic infections. Correct diagnosis requires a detailed clinical summary, thorough epidemiologic information (including recent travel history), and a diagnostic laboratory test.

The severe hemorrhagic form, DHF/dengue shock syndrome (DSS), has an average incubation period of 4 to 6 days before sudden onset of fever and nonspecific signs and symptoms. Because the major pathophysiologic abnormality observed in DHF/DSS is increased vascular permeability and leakage of plasma from the vascular compartment, early fluid replacement is effective. The geographic distribution of DHF/DSS has been expanding and now can be found in tropical areas of Asia, the Pacific, and the Americas, including Central America and

Mexico. This expansion is associated with increased movement of dengue viruses by airplane travelers and the development of hyperendemicity in the Pacific Region and the Americas. A similar scenario, generated largely from human encroachment into new environments, may also emerge for other *Aedes*-transmitted illnesses such as yellow fever. The most cost-effective approach to control dengue and DHF is larval source reduction in disease-endemic areas. Programs should use both government and community resources to integrate environmental sanitation with the use of insecticides and biologic controls, targeted to breeding grounds such as tire dumps.

Other Viral Diseases

The exponential increase in ecologic change, both environmental and behavioral, was cited as the major driving force for the increasing human risk for viral infection. Microbial variability can play a causal role in disease emergence, but it more often enables viruses to adapt to new circumstances. Travel of infected humans and international transport of microbes and vectors help provide the maximum possible microbial evolutionary opportunities in the minimum amount of time.

Viruses have emerged in the past, with measles providing a good example of the worrisome potential for future emerging RNA viruses. The emergence of cities in the Mesopotamian basin, resulting largely from the advent of irrigated agriculture, provided a populated substrate for interhuman transmission of short-incubation, nonlatent viruses. Domestication of livestock likely brought measles progenitors into close proximity with humans; a precursor of rinderpest/peste de petit ruminants then made an interspecies leap; much later measles spread to Europe, and, in the post-Columbian interchange, to the Americas.

Viral hemorrhagic fevers include those caused by Ebola filovirus and hantaviruses. Ebola hemorrhagic fever is characterized by extensive and disseminated infection and necrosis in major organs, and lymphoid depletion. Aerosol transmission of Ebola virus has occurred between nonhuman primates and guinea pigs, but no evidence exists for interhuman transmission by airborne infection. Barrier nursing precautions generally prevent the spread to humans, but in areas having

inadequate medical care facilities, the virus can amplify in humans and cause epidemics. Hantaviruses belong to a single genus in the family Bunyaviridae, and each virus infects a limited or unique rodent species with no apparent disease. Although hemorrhagic fever with renal syndrome has rarely been diagnosed in the Americas, hantaviruses from sigmodontine rodents cause hantavirus pulmonary syndrome, characterized by large bilateral pleural effusions and heavy, edematous lungs, interstitial pneumonitis, and extensive infection of endothelial cells in the pulmonary microvasculature. The first documented interhuman transmission of a hantavirus was an outbreak of 20 cases in Patagonia, with evidence overwhelmingly indicating spread between patients and physicians. The reasons for, and mechanism of, the spread are unknown, but the registry of U.S. cases was revised to ensure that this phenomenon was adequately monitored. Although early diagnosis and supportive care are potentially lifesaving in cases of hantavirus pulmonary syndrome, such efforts are of limited value in Ebola hemorrhagic fever.

Prion Illnesses

In prion illnesses such as Creutzfeldt-Jakob disease (CJD), risk factors (family history of CJD or dementia, history of poliomyelitis, exposure to sheep or cows) and iatrogenic factors (dura or corneal grafts and exposure to pooled human growth hormone) are important. At onset, CJD is commonly misdiagnosed as psychotic illness, Alzheimer's dementia, paraneoplastic syndrome, vascular brain disease, parkinsonism, or even drug-induced delirium. A variant of CJD, caused by a prion with an altered protein configuration, is bovine spongiform encephalopathy (BSE or mad cow disease). Although the precise mechanism of infection originally reported in U.K. cattle is unclear, BSE has been exported to other countries by feeding cattle inadequately processed bone meal. The potential for the emergence of BSE in the United States exists because similar reservoirs of infection are present. Casual handling of beef and beef products in processing plants and uncontrolled disposal of sick cattle may increase that risk. Several cases of CJD have been reported in Kentucky patients who consumed squirrel brains; however, a causal link has not been established. The most recently identified prion illness is a new variant of CJD reported in

England that, unlike sporadic CJD, occurs in much younger patients (16 to 50 years of age), can last longer than 1 year, and is characterized by the presence of psychiatric and sensory symptoms and the absence of kuru plaques and electroencephalogram periodic complexes. The illness is thought to be linked to BSE. Mathematical modeling suggests that 75,000 to 80,000 cases will occur in the foreseeable future.

Tick-Borne Diseases

As with many other emerging infectious diseases, concern about tick-borne zoonotic diseases in the United States has increased because of environmental changes brought on by human settlement and socioeconomic factors that place humans at greater risk for tick exposure, such as the development of suburban housing in disturbed natural settings. Current conditions appear favorable for continued increases in vector tick populations and their geographic expansion and for increasing interaction between ticks and humans. In the United States, the prevalence of Rocky Mountain spotted fever may continue to increase as the urbanization of the western and southern regions expands opportunities for human exposure to tick-borne pathogens.

The epidemiology of Lyme disease and tularemia was reviewed, and the concept of a southern (U.S.) tick-associated rash illness (STARI), the epidemiology of which is still being defined, was introduced. STARI is characterized by an expanding erythematous rash resembling that of Lyme disease, mild or absent constitutional symptoms, and no well-described sequelae. The rash responds well to antibiotic treatment. STARI is seen in the range of the human-biting Lone Star tick (*Amblyomma americanum*) and is frequently associated with a Lone Star tick bite. Studies indicate that this infection is not caused by *Borrelia burgdorferi* or other known tick-borne agents. Amplified segments of the genome of this spirochete have recently been described, and some investigators have proposed that it represents a new species, *B. lonestari*. It has been suggested that STARI may be closely related or identical to a commensal spirochete of deer, *B. theileri*.

The need for early diagnosis and treatment of Rocky Mountain spotted fever was stressed by presenting data from 94 infected patients in North Carolina. Death rates were significantly lower (6.5% vs. 22.9%, $p < 0.03$) in patients receiving appropriate treatment within 5 days of

onset of illness than in patients who received delayed or no treatment. Predictors of death included renal failure; elevated serum creatinine, aspartate transaminase, and bilirubin; decreased serum sodium; thrombocytopenia; neurologic involvement; and male gender.

Parasitic Diseases

The role of the cell surface glycoconjugate lipophosphoglycan (LPG) in the survival of *Leishmania* parasites whose life cycle alternates between intracellular parasitism and extracellular life in sand fly vectors was explored. Data indicating that LPG is a multifactorial molecule may lead to a biochemical rationale for LPG-targeted chemotherapeutic regimens.

Isolation of a full-length genomic clone of the acid α -mannosidase from an epimastigote genomic library of *Trypanosoma cruzi* was reported. Sequence analysis showed a single open reading frame encoding the α -mannosidase gene. Because the size of this frame is consistent with that of lysosomal mannosidases in humans, these results could lead to the exploration of new chemotherapeutic options in Chagas disease.

A bank of 5,200 insertion mutants has been used to characterize the parasitic mechanisms used by *Legionella pneumophila*. Results from transmission electron microscopy indicate that *L. pneumophila* has acquired genetic loci specific for survival and replication within mammalian cells, allowing evolution from a protozoan parasite into its present disease-causing agent. Alternatively, ecologic coevolution of *L. pneumophila* to parasite protozoa has led to the development of multiple redundant mechanisms, some of which do not function within mammalian macrophages.

TB

Poverty, changing immigration patterns, and the emergence of HIV disease were cited as factors contributing to an attenuated rate of decrease of TB incidence in the United States since the late 1980s and its increase as a major global problem. Poorly managed TB control programs, suboptimal access to health care, an inadequate physician knowledge base, and poor patient compliance have combined to increase the incidence of TB and, especially, multidrug-resistant TB; sensitivity testing is critical in the management of resistant TB. Updated CDC guidelines for interpreting the purified protein derivative skin test call for responses to be

considered positive if induration is greater than 5 mm in those with HIV or who have recent TB contact, and greater than 10 mm in foreign-born patients, intravenous drug users, the homeless, and immunocompromised patients. Because of the "boosting" phenomenon, two-step purified protein derivative skin testing is now recommended for health-care workers and nursing home patients who are retested periodically. Treatment of active TB should use multiple drugs, avoid adding single agents, and include compliance monitoring, preferably directly observed therapy. Workplace prevention measures should be driven by a consistently high index of suspicion and should include appropriate isolation of suspected cases, work-ups following exposure, rigorous reporting to health departments, and locating recalcitrant patients. Effective control of TB will require social, political, and cultural changes, as well as medical innovation.

Plague

The epidemiology, pathophysiology, and treatment of plague, as well as the improvement of diagnostic techniques for infections caused by *Yersinia pestis*, were reviewed. Such new tests will permit the public health laboratory to quickly identify a plague outbreak and apply the appropriate control measures to limit its spread.

The pathogenesis of plague was explored, and two separate, but essential, iron transport systems were identified in *Y. pestis*. The first, the yersinabactin (Ybt) system, enables the organism to proliferate from the site of an open wound or bite, while the second, identified as Yfe, is used by Ybt mutants to obtain iron during infection of internal organs. The Ybt iron transport system appears to be essential for growth in the early stages of bubonic plague infection, while the Yfe system functions to allow growth during, or after, infection of internal organs. Both systems are required for *Y. pestis* to be fully virulent.

A novel mechanism by which a *Y. pestis* virulence protein is sequestered in, and transported within, host cells was described. When yersiniae contact a eukaryotic cell, a signaling event activates the expression and secretion of a set of four toxins (Yops) and causes their vectorial translocation into the cell cytoplasm. Three of the Yops derange cell signaling and cytoskeletal functions by kinase, tyrosine phosphatase, and actin depolymerization activities. Although no activity or intracellular target has

been identified for the fourth known translocated Yop, YopM, immunoblot analysis and laser scanning confocal microscopy demonstrated that most YopM is vectorially translocated into HeLa cells or the macrophagelike cell line J774 by adherent *Y. pestis* and travels to the eukaryotic cell nucleus. Because a growing number of important human pathogens (e.g., *Salmonella*, *Shigella*, and *Pseudomonas*) have similar, but less well-studied, secretion/translocation mechanisms and putative secreted toxins, these findings will facilitate studies that ultimately could lead to novel therapies for these agents.

Antibiotic Resistance

The current development of staphylococci resistant to methicillin or fluoroquinolones and gram-negative bacilli resistant to extended-spectrum beta-lactams are but the most recently recognized patterns of antibiotic resistance. General approaches for modifying these trends include 1) source control, particularly handwashing, and the need to wear gloves during contacts with all patients, 2) improved antibiotic use and control, 3) improved infection control devices, and 4) better use of pathophysiology and immunologic modulation.

The growing problem of vancomycin-resistant enterococci (VRE), with an incidence of 20% to 40% in some groups of U.S. hospitalized patients, necessitates maximal use of all these approaches. Skin proliferation of VRE produces extensive environmental contamination that may require universal use of gloves to control outbreaks or hyperendemic disease. In addition, vancomycin should be limited to treatment of beta-lactam resistant gram-positive bacteria (such as methicillin-resistant *Staphylococcus aureus* and gram-positive bacteria in beta-lactam allergic patients) and *Clostridium difficile* (only after metronidazole failure) and to endocarditis prophylaxis. Vancomycin should be avoided in routine surgical prophylaxis, empiric treatment of febrile neutropenia with negative cultures, and pneumonia prophylaxis in the intensive care unit. A number of experimental peptides and other agents (such as quinupristin-dalfopristin) under investigation as treatments for VRE infections were identified.

Future trends in resistance may include further spread of vancomycin-resistant staphylococci (already reported in Japan), quinolone-

carbapenem-resistant gram-negative bacilli, and treatment-resistant viruses. Seventeen isolates of methicillin-resistant *S. aureus* with unique genotypes were studied to determine rates of resistance to the fluoroquinolones ciprofloxacin and levofloxacin. The mean single-step resistance to 4 x MIC ciprofloxacin was 1.05×10^{-5} and to levofloxacin was 4.03×10^{-6} . When serially passaged in increasing antibiotic concentrations, the geometric mean MICs for ciprofloxacin and levofloxacin increased 3.0 ± 1.5 times and 1.8 ± 1.4 times, respectively ($p < 0.0005$). Only four strains became resistant to levofloxacin, but eight became resistant to ciprofloxacin, indicating that ciprofloxacin selects methicillin-resistant *S. aureus* more frequently than levofloxacin.

Other Topics

The laboratory evidence supporting the role of *Chlamydia pneumoniae* in the development of atherosclerosis was reviewed. Recent data indicate that infection of vascular endothelial cells with *C. pneumoniae* is associated with the production of chemokines and adhesion molecules that promote transendothelial migration of neutrophils and monocytes. These findings suggest that immunopathogenic responses to *C. pneumoniae* infection may contribute to the development of clogging deposits.

Data were presented demonstrating the proliferation of human CD4+ T cells from unexposed persons in response to in vitro exposure to *Toxoplasma gondii*. Further studies showed that this proliferative response depends on HLA-DR molecules and requires processing of *Tg* antigens. In contrast to typical exogenous superantigens, analysis of TCR V β expression after stimulation with *Tg* did not show a pattern of preferential increase of a specific TCR V β -bearing subpopulation. $\alpha\beta$ T cells secreted significant amounts of IFN- γ after incubation with *Tg*-infected monocytes. This process may play an important role in the early events of the immune response to *T. gondii*.

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