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Emerging Issues in Neurovirology: New Viruses, Diagnostic Tools, and Therapeutics

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“The middle of the 20th century can be viewed as the end of one of the most important social revolutions—the eradication of infectious diseases as a significant element in social life”.

—Sir Macfarlane Burnet, 1962

In the mid-twentieth century, the discovery of antibiotics, the control of syphilis, the development of poliovirus and measles vaccines, and the anticipation of world eradication of smallpox led many to predict an end of infectious diseases as a serious public health problem. Burnet’s false prophecy was not alone; Robert Petersdorf [1] and others wrote obituaries for the specialty of infectious diseases and left the field.

Unforeseen was the emergence of new players, that is, new infections to replace and even dwarf the old. In the 1960s and 1970s, this emergence was foreshadowed by the appearance of new recombinant (duck-human) influenza viruses, legionnaires’ disease, toxic shock syndrome, Lyme disease, and the neurovirulent La Crosse strains of California encephalitis virus. The crushing realization that emerging infections could pose an enormous global threat came in the 1980s with the emergence of HIV infections and AIDS [2].

Over the past 2 decades, significant outbreaks of disease due to “new” agents have occurred almost yearly (Box 1). Some have been due to the evolution of more virulent agents (eg, enterovirus 71, chikungunya virus, and drug-resistant microbes), some to geographic relocation of agents (eg, Dengue type 3 in Sri Lanka and West Nile virus in North America), and some to

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Box 1. Emerging infections 1981–2003^a

- 1981, **HIV** (worldwide)
 1982, **Lyme borreliosis** identified (northeastern United States)
 1984, Cryptosporidiosis (Texas)
 1985, **Bovine spongiform encephalopathy** (United Kingdom)
 1987, Multidrug-resistant tuberculosis (prisons in United States and Russia)
 1989, Dengue 3, subtype III, virus (Sri Lanka)
 1991, **Guanarito virus** (Venezuela)
 1992, *Vibrio cholerae* 0139 (India)
 1993, *Escherichia coli* 0157:H7 (western United States);
 Hantavirus pulmonary syndrome (southwestern United States)
 1994, **Variant Creutzfeldt-Jacob disease** (United Kingdom);
 Hendra virus (Australia)
 1995, Largest Ebola outbreak (Congo)
 1996, **Reversion poliovirus vaccine virus** (Dominican Republic)
 1997, Influenza virus (Hong Kong); vancomycin-resistant
Staphylococcus (United States)
 1998, **Enterovirus 71 fatal rhomboencephalitis** (Asia); **Nipah virus encephalitis** (Malaysia and Singapore)
 1999, **West Nile virus encephalitis** (New York)
 2000, Rift Valley fever (Saudi Arabia)
 2002, SARS (Asia and Canada)
 2003, Monkeypox (midwestern United States)
 2006, **St. Louis encephalitis** (Argentina)

^a Agents with a major impact on the central nervous system are in bold type.

contact with animals and crossing of species barriers (eg, bovine spongiform encephalopathy, variant Creutzfeldt-Jakob disease, Nipah virus, and the severe acute respiratory syndrome [SARS] virus). The majority of these agents have been viruses.

Added to this threat over the same time has been the growing concern over the weaponization and intentional release of infectious agents, culminating in the anthrax attacks in 2001. Millions have died and will die from the spontaneously emerging infections. In the three US terrorist acts, that is, the contamination of salad bars by a religious group in Oregon in 1984, the malicious contamination of donuts and muffins probably by a disgruntled worker in a Texas laboratory in 1996, and the anthrax mail contamination by an unknown assailant in 2001, only the latter resulted in deaths. The fear of far greater devastation from bioterrorism is real and likely to happen. The spontaneous emergence of new lethal agents is a certainty.

Origins of emerging infections

Several dramatic examples of new outbreaks of viral encephalitis have appeared in the past decade. The three examples summarized herein represent new clinical syndromes due to viruses appearing after evolutionary changes, geographic relocation, and zoonotic infection transmitted to humans.

Enterovirus 71

This enteric picornavirus, closely related to Coxsackie A 16, was originally recovered in California in 1969 and was associated with hand-foot-mouth syndrome, herpangina, occasional cases of viral meningitis, and a single case of fatal encephalitis [3]. Surprisingly, the virus appeared in Bulgaria and Hungary between 1975 and 1978 associated with a poliomyelitis-like syndrome, and isolates from these outbreaks caused flaccid paralysis after inoculation into monkeys [4]. The virus had evolved to simulate poliovirus disease. After another interval, the virus appeared in Southeast Asia in 1997 as the cause of fatal rhomboencephalitis with cardiopulmonary collapse in children under 5 years of age [5,6]. This brainstem localization of clinical findings, imaging studies, and neuropathologic findings indicate a further evolution of neurovirulence to cause a novel and highly lethal illness.

West Nile virus

West Nile virus was originally recovered from the blood of a febrile woman in Uganda in 1937 [7]. Subsequently, the virus was associated with a dengue-like illness in African children and later with occasional cases of aseptic meningitis in the Middle East. Ironically, the first documented cases of encephalitis due to West Nile virus were in New York City in 1954 after 95 patients with advanced cancer were inoculated with the virus on the premise that the “harmless” virus might selectively destroy tumor cells. Nine patients developed encephalitis, and virus was recovered from the cerebrospinal fluid of three patients [8]. Naturally occurring cases of encephalitis began to appear in the Mediterranean basin in the 1990s. A radical change in the epidemiologic pattern was then seen, with large epidemics of encephalitis with high mortality rates. The first epidemics occurred in Bucharest in 1996 and then in Volgograd in 1999. Clearly, a more virulent strain of the virus had evolved in this region [9].

In the summer of 1999, a cluster of patients with encephalitis and severe weakness were reported in the New York borough of Queens. They ranged in age from 58 to 87 years, had no common exposure, reported no family illnesses, and spent their evening hours out of doors where investigators found many mosquito larvae in stagnant water. The disease was correctly assumed to be arthropod borne but tentatively diagnosed as St. Louis encephalitis. A subsequent virus isolate from a bird dying with encephalitis

in the neighboring Bronx was identified as West Nile virus, and reassessment of the human encephalitis cases showed them to be caused by West Nile virus [10]. Sequence analysis of the New York virus showed striking similarity to an isolate from a goose in Israel in the previous year [11].

Similar to recent European outbreaks, encephalitis was a common complication, and in 10% of patients there was flaccid paralysis indicating involvement of anterior horn cells of the spinal cord [12]. The virus may have been transported by a mosquito aboard an international flight or by a smuggled bird or viremic animal. Whatever the courier, the virus found a compatible habitat, successfully overwintered, and spread across the United States and Canada over the next 3 years [13]. The disease took on major public health importance in 2002 when it spread coast to coast, causing over 3000 human illnesses and 153 deaths. In 2003, 9862 cases of disease were recorded in humans; 2860 patients had meningitis or encephalitis, with over 200 deaths.

In 1999, many had predicted that the virus would not survive the winter or that future summers would present small geographically defined outbreaks similar to those caused by other arthropod-borne viruses in North America. The annual outbreaks and the spread from coast to coast, across Canada, and into Latin America have occurred, but cases also continue to occur annually in Eastern states. The wide range of transmission-competent avian and mosquito species portend a serious future health threat from this neurovirulent strain of West Nile virus now firmly established in its new habitat [14].

Nipah virus

In September of 1998, cases of encephalitis were reported among pig farmers and their families in Malaysia. Initially the disease was thought to be Japanese encephalitis, an endemic arthropod-borne disease in the region; however, the new disease affected primarily adults, occurred in household clusters, was associated with contacts with pigs that were ill, and affected some persons who had been immunized against Japanese encephalitis virus. Each of these factors suggested an alternative cause. In March of 1999, a paramyxovirus named Nipah virus was recovered and related to the disease [15]. An outbreak in a single abattoir in Singapore gave insight into the epidemiology of the infection, since only workers transporting or slaughtering pigs became infected. No family member or medical caregiver had evidence of infection, indicating a lack of human-to-human spread [16]. The slaughter of tens of thousands of pigs in Malaysia brought an end to the outbreak, but only after over 100 patients had died.

The clinical disease was unique, with multifocal neurologic signs such as cerebellar ataxia, brainstem signs, and segmental myoclonus. Imaging studies showed multiple small lesions scattered through the white matter. Pathologic studies showed widespread vasculitis with giant cell formation and

occlusion of small vessels as well as neuronal infection [17]. Follow-up studies have shown that over 7% of survivors relapse [18].

The virus is related to the Hendra virus, a paramyxovirus associated with encephalitis in horses and a few handlers in Australia. The natural hosts of Hendra virus are fruit bats. Nipah virus has now been recovered from the saliva and urine of bats, including bats that hung in fruit trees overhanging the pig yards in Malaysia [19].

No further major epidemic has been reported, but scattered small outbreaks of Nipah encephalitis have been reported in Bengal and Bangladesh. These cases have not been related to exposures to sick pigs; therefore, some other modes of transmission from fruit bats to humans occur.

Viral infections of the nervous system on the horizon

Hepatitis C virus

The flavivirus hepatitis C virus (HCV) represents one of the leading chronic viral epidemics globally, with over 130 million infected individuals [20]. In many areas of the world, HCV infection parallels HIV-1 infection, with injection drug use or blood transfusion being the chief modes of transmission [21]. Like HIV-1, HCV is defined by multiple subtypes and immense molecular heterogeneity within subtypes with varying disease severity, largely evident as inflammatory liver disease, which progresses to a cirrhotic state. HCV-infected persons also report neurologic symptomatology, including neurocognitive symptoms (fatigue, mental slowing, poor concentration, forgetfulness, apathy) in the absence of liver disease and exhibit neurologic signs including low-grade encephalopathy (impaired attention and learning, psychomotor slowing) with an overall prevalence of 13% [22] and peripheral neuropathy (10%) [23] with or without cryoglobulinemia. Indeed, polymyositis, demyelinating polyneuropathies, and acute demyelinating disseminated encephalomyelitis have also been associated with HCV infection.

The underlying pathogenesis of HCV-related disease appears to be largely cellular immune-mediated liver damage in response to chronically infected hepatocytes. Multiple studies indicate that leukocytes are chronically infected, together with a persistent viremia. More recently, several studies suggest that HCV-encoded genome (both positive and negative strand RNA) and proteins are detectable in the cerebrospinal fluid and brain, often in the setting of HIV-1 coinfection. Both monocytoïd (macrophages and microglia) and astrocytes can express selective viral proteins. The extent to which accompanying cellular (and humoral) immunity contributes to neural tissue injury as a bystander cytotoxic effect, or whether virus-encoded proteins are secreted and subsequently injure proximate cells (as proposed for HIV-1) remains uncertain to date. Nonetheless, quantitative neuropsychologic studies and neuroimaging including MRI and positron emission

tomography (PET) disclose abnormalities in HCV-infected patients, which are compounded by HIV-1 coinfection. Although pegylated interferon-alpha is an effective therapy for some HCV subtypes, its impact on HCV-related neurologic disease remains uncertain. As this epidemic grows, the increased risk of neurologic disease requires vigilance and intervention as indicated.

Factors promoting emergent agents

The emergence of increasing numbers of new agents appears to be accelerating. This increase may be, in part, an artifact of better surveillance and reporting, but contemporary society has made changes that encourage the evolution of organisms (Box 2).

Widespread use and misuse of antibiotics and antiviral drugs has caused natural selection of drug-resistant agents. Increased preschooling and nursery care magnifies the spread of enteroviruses. Clearing forests for agriculture has led to encounters with new zoonotic agents. The building of dams has created ecological changes that alter the habitats of infectious agents. Contemporary animal husbandry with massive feedlots facilitates

Box 2. Societal changes that enhance the evolution and spread of neurotropic agents

Providing an adequate pool of susceptibles

Increasing global population

Increasing human contacts (travel)

Altering forms of human or animal contact

Societal mores

Increased sexual contact

Day care with early exposure

Altering environment for urban expansion and recreation

Agricultural clearing or irrigation

Global movement of animals and animal products

Medical practices

Blood transfusions

Immunosuppressive therapy

Organ transplants (infected donor)

Antimicrobial drugs (encourage resistance)

Data from Johnson RT. The Soriano Award Lecture. Emerging infections of the nervous system. *J Neurol Sci* 1994;124:3-14.

the spread of agents. Even medical advances in the administration of blood products and transplantation have led to the emergence of infections.

The two most important factors are the rapidly growing human population and its movement both in speed and volume [24,25]. In 1850, the world population was under 1 billion, and around-the-world travel under sail required over a year for the very few who ventured out. Now the world population has burgeoned to over 6 billion. The world can be circumnavigated by air in 24 hours. Furthermore, each year, over 500 million persons cross international borders on commercial airplane flights, 70 million work in other countries, and 50 million are refugees or internationally displaced persons [26]. The population can maintain agents that spread human-to-human, and the speed of travel allows any known infectious agent worldwide to be in a community within one incubation period.

The evolution of new agents, the relocation of viruses, and the crossing of species barriers are not new. The measles virus has only one host, humans, but to maintain the virus an interactive population of about 200,000 is required; otherwise, the virus disappears as it does on sparsely populated islands. The virus could not have persisted in early Neolithic or nomadic cultures. Indeed, the typical exanthem of measles was not described by Greek and Roman physicians but was first recorded by Rhazes of Baghdad. He dated its arrival in the Arab world to the sixth century. Contemporary sequence analysis indicates that the measles virus was probably derived from rinderpest virus of African or Asian cattle, suggesting an early zoonotic spread across species barriers. The appearance of measles in France and Northern Europe has been dated to the eighth century after the Moorish invasion. Apparently, it was confined below the Pyrenees for centuries until an army crossed the mountains, maintaining the virus by soldier-to-soldier spread. Today, a similar virus could cross this geographic barrier in hours.

New diagnostic tools

With the advent of new high-throughput technologies including polymerase chain reaction (PCR) arrays, virus-specific cDNA and DNA microarrays, and the capacity to perform large-scale DNA sequencing [27], diagnostic tools for viral infections are rapidly advancing in complexity and in the spectrum of agents that can be detected or newly discovered. Indeed, multiple molecular tools have been used over the past decade to characterize West Nile virus, the SARS coronavirus, and Kaposi's sarcoma-associated herpesvirus (HHV-8) [28]. The recent development of this combined diagnostic approach was particularly rewarding because it led to the discovery of a new human arena virus causing fever and encephalitis. Both PCR and oligonucleotide microarray studies were unremarkable, but subsequent high-throughput DNA pyrosequencing of

patient-derived cDNA, together with bioinformatics analyses of protein sequences to exclude human sequences, resulted in the identification of a new neurovirulent arena virus. This metagenomic pyrosequencing approach, albeit, cumbersome at a bioinformatics level, is unbiased in terms of identifying novel sequences and will be invaluable for the discovery and detection of new infectious agents of the nervous system [29].

In the past 5 years, new protein technologies, including matrix-assisted laser desorption ionization (MALDI) and stable isotope labeling with amino acids in cell culture (SILAC), have also advanced, permitting the detection of new proteins in body fluids including cerebrospinal fluid and the sequencing of individual proteins [30]. These technologies have immediate value as biomarker detection tools, facilitating the diagnosis of syndromic entities and the evaluation of response to specific treatments. Eventually, using nanotechnologies including microfluidic approaches coupled with conventional methods such as antibody detection or PCR, large-scale viral detection will be feasible in the field far from high-technology laboratories, permitting expedited diagnosis and intervention.

Responses to emergent neurologic infections

Little can be done to slow the emergence of new microbial threats. The challenge is to anticipate them, detect them early, and respond in effective ways that will contain them (Box 3). First, surveillance must be global, and international cooperation is crucial. It is easier to contain Ebola virus with improved infection control in African hospitals than to counter it at international airports. The SARS virus epidemic might have been restricted by quarantines more promptly had information and efforts been shared internationally. New methods of rapid identification and characterization of agents are being developed, but the capacity to perform these studies should be put in place worldwide with major strengthening of public health infrastructure. We cannot prevent the emergence of new microbes and diseases, but we can improve our knowledge of the ecology and molecular biology of these agents. We also can anticipate that every change we make in the environment may impact on the evolution, new encounters, or habitats of these agents.

Box 3. Control of emerging infections

Global surveillance and cooperation
Rapid identification methods
Characterization to determine origin
Development and deployment of vaccines and drugs
Strengthen the public health infrastructure worldwide

New therapeutic approaches are rapidly evolving for chronic and acute viral infections. Aside from new antiviral drugs that are being developed for a range of viruses such as tenofovir and others, strategies involving the use of therapeutic vaccines, the delivery of monoclonal antibodies, or the use of therapeutically primed dendritic cells represent highly novel approaches. The use of these agents has not yet reached neurovirology; however, other technologies, including the use of nanotechnology-derived liposomes for drug delivery to the brain, are now reality. Perhaps the greatest promise lies in the use of small molecules to prevent bystander-mediate injury of the brain. For example, the recent use of minocycline, beta-lactam antibiotics, and free radical scavengers as neuroprotective therapies raises exciting possibilities, albeit, with some caution given the variable outcomes from different studies.

In the current era of escalating globalization with rapid transport, changing climate, and an ever growing human population with associated changes in lifestyle, poverty, and war, the emergence of new neurologic infections is highly plausible. Understanding their origins using epidemiologic and molecular tools will contribute to improved control of agent spread throughout vulnerable populations. Although few interventions are effective in acute epidemics, the prompt identification of new infectious agents and the roll-out of vaccines together with new antiviral and neuroprotective drugs are promising for the management of future epidemics.

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