Emerging Viruses: The Evolution of Viruses and Viral Diseases

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Challenged by the sudden appearance of AIDS as a major public health crisis, the National Institute of Allergy and Infectious Diseases and the Fogarty International Center of the National Institutes of Health (NIH), together with The Rockefeller University, jointly sponsored the conference “Emerging Viruses: The Evolution of Viruses and Viral Diseases” held 1–3 May 1989 in Washington, DC. It was convened to consider the mechanisms of viral emergence [1] and possible strategies for anticipating, detecting, and preventing the emergence of new viral diseases in the future. To provide a broad perspective, participants comprised virologists, infectious disease specialists, theoretical biologists, historians, epidemiologists, ecologists, and molecular biologists (see below for program and participants).

Theoretical Considerations

The enormity of the problem was outlined in the keynote address by Lederberg, who said that humankind’s only real competitors for dominion of the planet are viruses, which can serve as both parasites and genetic elements in their hosts. Not only do they have considerable genetic plasticity, enabling them to evolve in new directions, but their genetic and metabolic entanglements with cells uniquely position them to mediate subtle, cumulative evolutionary changes in their hosts as well. Their effects are not always so subtle, however; viruses also can decimate a population. The fact that natural selection in the long run favors mutualism offers only limited encouragement to the human race, as too many people might suffer as the result of viral mutation before an equilibrium could be reached. [2]

According to May and Anderson [3, 4], coevolution of virus and host can follow several possible lines, and pathogens may not always evolve towards lower virulence. In a model developed by Levin (described in [3]), for example, a virus strain that kills much faster will not be favored over a less-virulent strain if it has a modest transmission advantage, but it will prevail if it is much more readily transmitted than the less-virulent strain. Examples such as myxomatosis in Australia support the notion that in the tradeoff between transmissibility and virulence, many viruses evolve toward a middle course, favoring transmissibility but allowing them to retain some virulence. Viruses that are transmissible over a long time (e.g., human immunodeficiency virus [HIV]) have a selective advantage even when their effective rates of transmission are relatively low [5].

Although mathematical approaches offer useful insights, May and Haase caution that models will often prove inadequate in predicting outcomes because viral emergence and host interactions are complex, being dependent on both genetics of the host and external conditions. For example, as discussed by Lovejoy, there are many examples of disease emergence precipitated by environmental change, but it is impossible at present to predict or model accurately how global warming or other possible environmental changes will affect viral disease emergence.

Historical Lessons on Disease Emergence

According to McNeill [6], the most striking examples of emergence of infectious diseases arose from new patterns of human movement, leading to new contacts across what had previously been geographic boundaries that contained a disease. Examples are the introduction of smallpox into the Americas and of syphilis into Europe.

Disease emergence resulting from expanded geographic boundaries of viruses or their vectors was a recurring theme discussed in talks by Johnson, Monath, Evans, Shape, and others. Yellow fever probably emerged in the New World as a result of the African slave trade, which brought Aedes aegypti in water containers of ships. Similarly, the rise of dengue hemorrhagic fever in Southeast Asia in the late 1940s is attributed to rapid migration to cities with open water storage, which favored proliferation of the mosquito or other suitable
vectors. Of current concern in the USA is the fact that *Aedes albopictus*, an aggressive and competent dengue virus vector, was brought to Houston in used Asian tires and has established itself in at least 17 states. Krause noted that with dengue hemorrhagic fever “lapping at our shores,” we have a potentially serious public health problem in the making.

Recent Examples of Emerging and Potentially Emergent Viruses

Table 1 lists some viruses that have been associated with “emergent” disease. Disease emergence often follows ecologic changes caused by human activities, such as agriculture or agricultural change, migration, urbanization, deforestation, or dam building. For example, Argentine hemorrhagic fever increased as agricultural changes favored the rodent that carries this virus.

Surprisingly, most emergent viruses are zoonotic, with natural animal reservoirs a more frequent source of new viruses than is the sudden evolution of a new entity. The most frequent factor in emergence is human behavior that increases the probability of transfer of viruses from their endogenous animal hosts to man. Rodents and arthropods are most commonly involved in direct transfer, and changes in agricultural practices or urban conditions that promote rodent or vector multiplication favor increased incidence of human disease.

Other animals, especially primates, are important reservoirs for transfer by arthropods.

According to Monath, ~100 of the >520 known arthropod-borne viruses (arboviruses) cause human disease. At least 20 of these might fulfill the criteria for emerging viruses, appearing in epidemic form at generally unpredictable intervals. Most arboviruses have enzootic cycles involving a vector (e.g., mosquitoes, ticks, or biting flies) and wild vertebrate hosts, most of which rarely manifest overt disease. Many arboviruses can also be transmitted vertically within their vectors, assuring survival over winters or dry seasons. Periodic amplification occurs when vector and susceptible host population densities and other factors favor rapid virus transmission, and it may be followed by epidemics in humans or domestic animals. In such situations, arboviruses may enter into a second transmission cycle involving one or more arthropod species different from the enzootic vector species and humans or domestic animals as viremic hosts. This makes surveillance difficult. Eldridge described how knowledge of mosquito evolutionary relationships could help to predict new mosquito vectors.

Another virus of current interest in the USA, Seoul virus, was identified ~10 years ago in Korea as a Hantaan-like virus whose natural host is the urban rat. Serologic surveys detect it worldwide, including seroprevalence rates of 12% in urban rats in Philadelphia and ~64% in Baltimore rats, as
reported by LeDuc et al. [7]. Although acute hemorrhagic fever was not identified in inner-city Baltimore, 1.3% of 1148 local residents were antibody-positive and the possibility of viral association with chronic renal disease is under study.

Intensive surveillance in Africa during the smallpox eradication program identified a human case of monkeypox in Zaire in 1970. Since then, 404 cases have been reported, virtually all among children living in villages in tropical rain forests [8]. The virus appears unable to be sustained by naturally occurring person-to-person spread; both monkeys and humans are probably infected incidentally by contact with infected squirrels. Fenner commented that it was difficult to understand why monkeypox virus spread so poorly; if it spread readily from person to person by the respiratory route it would constitute a risk similar to that of smallpox. In fact, however, conversion of forest to agricultural land appears to be reducing its transmission from wildlife and its incidence in countries of West Africa.

Because little is known about interspecies transfer to humans at the molecular level, two recent examples in animals, canine parvovirus and seal plague, were presented by Parrish and Mahy, respectively. Canine parvovirus 2 may have descended from the feline parvovirus [9]; ability to infect the new species may have been conferred by a mutation in the capsid gene [10]. Seal plague, a newly recognized paramyxovirus, is related to, but distinct from, measles and canine distemper viruses [11]. The virus may have been transferred directly from another species of seal [12]; possibly an outbreak of canine distemper might have contributed to its transfer [13], although this is not clear.

**Influenza Virus as a Model for Viral Emergence**

Data relating to emergence of pandemic strains and genetic evolution are most extensive for influenza virus. The probability of interspecies transfer can be increased not only by increased contact between humans and an animal reservoir but also by increased opportunity for viral genetic reassortment or recombination within animal or insect hosts. Because influenza virus has an eight-segmented genome, it has considerable freedom for genetic reassortment. While small epidemics may arise from mutation (antigenic drift), all known human pandemic strains have been the result of reassortment, mostly involving the hemagglutinin (H) gene. This mechanism was probed in depth by Webster, who espoused the idea that influenza virus maintained in shore and migrating birds infects ducks raised on farms and rearsorts in pigs, from which new strains emerge to infect humans [14]. Recently Scholtissek and Naylor [15] also proposed this agricultural origin for pandemic strains.

Virulent strains of influenza virus also can arise from a single mutation, even if pandemic strains have not generally arisen this way. For example, in 1983 a single mutation in a relatively avirulent strain gave rise to an H5N2 strain that caused a fatal epidemic in chickens in Pennsylvania [16]. The point mutation in the H gene changed thr to lys, exposing a previously glycosylated site. Similarly, if pigs are infected experimentally with an avirulent mutant [17], the swine virulent parental phenotype emerges within a few days, indicating rapid evolution and emergence in vivo of the virulent form [18].

For viruses with nonsegmented genomes, recombination provides another genetic avenue for emergent diseases, according to Strauss. For instance, viral genetic sequence analysis revealed that western equine encephalomyelitis virus, an alphavirus, arose from a recombination event that seems to have involved a Sindbis-like virus and eastern equine encephalomyelitis virus, probably occurring 100–200 years ago [19]. Monath suggested that Rocio encephalitis virus may have arisen similarly. Genetic recombination also seems to have occurred between the envelope protein genes of human T lymphotropic virus (HTLV)-I and HTLV-II [20].

**Mutation Frequency**

The mutation rate of any genome is inversely proportional to its size, so theoretically any virus can mutate rapidly, although according to Holland, RNA viruses usually have higher mutation rates than do DNA viruses of the same genome size. This is generally ascribed to the lack of error-correcting mechanisms in RNA synthesis. Palese reported high mutation rates for influenza virus genes. The changes occurred in the nonstructural protein (NS) gene of influenza A virus during a single cycle of replication in tissue culture. A mutation rate of ~10⁻³ changes per nucleotide site per replication cycle was observed [21]. Similar tissue culture experiments revealed mutation rates ~10-fold lower (~10⁻⁶ mutations/site/cycle) for poliovirus [21] and 10-fold higher (~10⁻⁴ mutations/site/cycle) for Rous sarcoma virus [22].

In addition, Palese reported on the evolution rate of influenza viruses. In contrast to the mutation rate, this rate describes the changes observed when the viruses are passaged in humans. The evolution rate of the influenza A virus NS gene is 1.95 × 10⁻³ changes/site/year, several orders of magnitude greater than that of eukaryotic genes [23]. Also, influenza A viruses generally follow an evolutionary pattern in which a single lineage dominates. In contrast, coexisting lineages occur in the case of influenza C viruses [24], and their evolution rate is much slower than that of the influenza A viruses. Influenza B viruses [24] also appear to have coexisting lineages, and their evolution rate appears to be slower than that of influenza A viruses but distinctly faster than that of influenza C viruses.

Doolittle and colleagues [20, 25] looked at the evolutionary rates of change of ten genes from retroviruses. Overall, the reverse transcriptase showed the slowest rate of change and the outer portion of the envelope protein the most rapid, evolving three times faster. The core portion of the gag pro-
tein changed ~1.6 times as fast as the transcriptase, the proteinase 1.8 times as fast, and the 140 amino acids at the amino terminal of gag 2.5 times as fast. The viral proteinase is pepsin-like, and that from human immunodeficiency virus (HIV) is as similar to that of visna virus as human pepsin is to its fungal homologue. The proteinases of HIV and HTLV-I differ from one another even more than human pepsin does from the fungal proteinase. The retroviruses thus are changing extraordinarily rapidly.

The term viral genetic sequence can be misleading because it implies a single sequence. Most species of RNA viruses actually consist of a population of genomes showing considerable variation around a master sequence [26, 27]. The population concept is important. In experimental systems, defective members of the genomic population can play a significant role in viral expression [28].

Selective Pressures and Constraints

What, if any, limits are placed on virus variation? Despite high mutation rates and opportunities for genetic reassortment, numerous factors act to minimize emergence of new influenza A epidemics, according to Murphy. Even though avian and human influenza viruses are widespread (in humans, an estimated 100 million infections yearly), pandemic influenza viruses emerge infrequently (every 10–40 years). Powerful constraints appear to be at work since pandemic human influenza strains vary in their H gene, with or without a concomitant change in the neuraminidase, whereas the NS gene and most other genes are conserved.

Constraints on viral evolution are not surprising when one considers the selective pressures imposed by the host at each stage of the virus life cycle [29]. Tissue tropism determinants, discussed by Fields and Shenk, include site of entry, viral attachment proteins, host cell receptors, tissue-specific genetic elements (e.g., promoters), host cell enzymes (e.g., proteinases), host transcription factors, and host resistance factors such as age, nutrition, and immunity. Host factors contribute significantly: Sequences such as hormonally responsive promoter elements and transcriptional regulatory factors can link viral expression to cell state.

The interaction of virus and host is thus complex but ordered, and can be altered by changing a variety of conditions. Unlike bacterial virulence, which is largely mediated by bacterial toxins and virulence factors, viral virulence often depends on host factors, such as cellular enzymes that cleave key viral molecules. Because virulence is multigenic, defects in almost any viral gene may attenuate a virus [30]. For example, some reassortants of avian influenza viruses are less virulent in primates than are either parental strain, indicating that virulence is multigenic [31].

Viral and host populations can exist in equilibrium until changes in environmental conditions shift the equilibrium and favor rapid evolution [32]. It seems reasonable to expect that new viruses will emerge occasionally, but the stochastic and multifactorial nature of viral evolution makes it difficult to predict such events.

According to Doolittle, retrovirus evolution is sporadic, with retroviruses evolving at different rates in different situations. For instance, the human endogenous retroviral element is shared with chimpanzees, indicating no change in >8 million years, whereas strains of HIV have diverged in mere decades. Endogenous retroviruses carried in the germ line evolve slowly compared with infective retroviruses. Retroviruses probably arose from transposable elements and became infectious relatively recently, certainly after the emergence of vertebrates. Doolittle hypothesizes that exogenous retroviruses arose from endogenous retroviruses that escaped one species and infected another by horizontal transmission. They then may integrate into germ line cells as new endogenous retroviruses. Most endogenous retroviruses eventually become degenerate sequences accumulating in the germ line [20].

Generation of new viral pathogens is rare, Temin concluded, and usually possible only because of high mutation rates that permit many neutral mutations to accumulate before selective pressure forces a change. Unlike conventional selection-driven evolution, which is stepwise, this mutation-driven process allows new and probably unpredictable viral pathogens to develop. Hence, recognition of new pathogens undoubtedly must await their emergence, making the time required to note HIV unexceptional in this regard, particularly because of the special biologic challenges posed by all retroviruses, especially lentiviruses, which can evade the immune system and become latent [33, 34].

Broader Surveillance Strategies

New viruses may therefore best be contained by specialized surveillance conducted by a rapid response team, a task that could most effectively be carried out, according to Holland, by a single laboratory with a worldwide focus. Although it may not be practicable to conduct rigorous surveillance worldwide, it would be worthwhile in countries with tropical rain forests or dense populations where the likelihood of disease emergence is high. Henderson suggested developing a network of internationally supported health and research centers based in periurban areas of major tropical cities near rain forests. Each would combine clinical, diagnostic, and epidemiologic research and training units. Several participants noted an increasing, and potentially critical, shortage of trained researchers and field-workers in all areas of viral and vector biology.

Approaches to Virus Detection

Constant surveillance is essential to uncover new diseases, especially in cases of large outbreaks with no dramatic manifestations or very small outbreaks with serious conse-
quences but no visibility. For identifying viruses that are increasing their range, seroepidemiology is invaluable with surveys of sentinel or high-risk populations, as discussed by Shope and Evans. Examples include tracking HTLV-1 [35] and La Crosse (California encephalitis group) virus.

Richman classified methods for virus detection as open-ended when they do not require foreknowledge of the type of virus being sought and probe-specific when the search is for specific components. The major open-ended approaches include virus isolation, long the "gold standard" of virology, and electron microscopy. Both have limitations. Besides cost and the need for expertise, virus isolation requires identification of a susceptible host cell and a suitable marker such as a cytopathic effect, immunocytochemical test, or assay for a viral enzyme. For viruses that can be grown in culture, isolation offers great sensitivity (via biologic amplification), is relatively specific, and provides material for further characterization.

Even though electron microscopy requires special equipment and expertise and is rather insensitive, it is rapid, can be used directly with clinical materials, and can detect unknown viruses. Immune electron microscopy increases sensitivity and specificity, although high titers of virus are still required. The hepatitis A virus and rotavirus were discovered this way.

Probe-specific methodologies include detection of antigens and antibodies, nucleic acids, and, less frequently, viral enzymes such as reverse transcriptase. For identification of new agents, antigen and antibody detection methods (enzyme immunoassay, immunofluorescence, and traditional serologic methods such as complement fixation) require good luck and large quantities of antigen (e.g., discovery of hepatitis B virus or parvovirus B19) or cross-reactivity with a known agent (identification of seal plague virus using cross-reactions with agents responsible for rinderpest and canine distemper). The applicability of antigen-antibody detection methods can be broadened by using immune or convalescent sera to detect the unknown antigens, as was done, in an important early application of indirect immunofluorescence, to identify Han-taan virus (and, later, Hantaan-related viruses) in tissues of infected rodents [36], hepatitis delta virus in liver tissues of patients, and hepatitis C virus.

Nucleic acid isolation and cloning methods for viral identification, still under development, have great potential. Unlimited amounts of standardized reagents can be prepared by molecular cloning and oligonucleotide synthesis. When nucleic acid sequence data are available for a virus, probes of any desired specificity can be prepared and relatedness to other viruses can be determined. As discussed by Houghton, this technology was successfully applied by his group to hepatitis C virus [37, 38], as well as to the hepatitis delta agent, which is the first human pathogen of a unique type consisting of a small RNA [39].

There are currently three basic approaches to using nucleic acid probes for virus detection. In situ hybridization of nucleic acids in theory can detect a single cell containing the target sequence, according to Ward. The approach has been more valuable for studies of pathogenesis and tissue tropism than for primary detection. Filter and solution hybridization assays are insensitive, requiring 100,000–500,000 target molecules in a sample without amplification. Amplification of the target by the polymerase chain reaction (PCR) reduces that requirement to ≤10–100 molecules. Here specificity is the issue. To rule out cross-contamination, it is essential to use proper controls, such as amplifying a cellular gene (e.g., globin) at the same time.

Exciting advanced technologies include optical imaging techniques that permit visualization of a single integrated viral genome on a chromosome. Although powerful, many such techniques require specialized equipment and are limited to research use. More accessible to investigators in clinical laboratories are chemiluminescence technologies using compounds based on dioxetane chemistries. This methodology can increase sensitivity of any probe tagged with peroxidase, including nucleic acid probes or enzyme immunoassay conjugates, to ~0.1 pg (400 molecules) [40, 41].

Characterization of Emergent Viruses

Rapid identification of emergent disease-causing agents is an essential feature of a responsive control program. Classic virologic methods are most widely applied to characterization of new viral agents. Western blotting and nucleic acid hybridization are regularly used to look for viral relatedness, while gene sequencing permits more refined comparisons. PCR is clearly becoming important.

Computer programs to construct phylogenetic trees help in determining relatedness of viruses by meticulous comparison of homologous genetic sequences [42]. By this analysis, Myers concludes that known simian immunodeficiency virus (SIV) strains appear more closely related to HIV-2 than to HIV-1. Doolittle instead analyzes amino acid sequences of the viral proteins. Although many of his results are similar to those of Myers, Doolittle believes that HIV-1 and SIVsim have a common ancestor closer than HIV-2. Because the two prominent methods for determining evolutionary relationships from tree analysis depend on proper alignment of homologous sequences, disputes revolve around interpretation of alignments. Computer analysis of genetic relatedness is expected to continue to provide important insights about viral evolution, aided by the ability of powerful new techniques such as PCR to generate additional sequences for comparison. Knowledge of viral evolution is clearly still in its infancy.

Role of Basic Research

Biotechnology is a dynamic field, with advances providing a continuous succession of improvements. However, successful
application of sophisticated technologies to control viruses is restricted by our limited knowledge of many key aspects of viral pathogenesis, including factors responsible for efficient person-to-person spread, and of viral immunology and immunogenetics. Many speakers also emphasized their concern for availability of adequate personnel and resources in the future. Only by fully understanding how viruses interact with their hosts can we hope to rationally devise effective preventive and therapeutic strategies.

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Conference Program

The Planning and Organizing Committee consisted of William P. Allen (NIAID), Richard M. Krause (Fogarty International Center, NIH), John R. La Montagne (NIAID), Thomas P. Monath (US Army Medical Research Institute of Infectious Diseases), Stephen S. Morse (Rockefeller University [Chairperson]), Neal Nathanson (University of Pennsylvania), Peter Palese (Mt. Sinai School of Medicine of City University of New York), Ann Schlueterberg (NIAID), and Robert E. Shope (Yale University).

After an introduction by La Montagne and consideration by Morse of some of the questions raised by viral emergence and viral evolutionary potential, Joshua Lederberg (President, Rockefeller University), the keynote speaker, was introduced by Krause. The program consisted of four sessions.

Historical Lessons on Disease Emergence was chaired by Frank Fenner (Australian National University) with speakers William H. McNeill (University of Chicago), patterns of disease emergence in history; Robert G. Webster (St. Jude Children’s Research Hospital), influenza; and Karl M. Johnson (formerly Centers for Disease Control and US Army Medical Research Institute of Infectious Diseases), viral hemorrhagic fevers.

Examples of Viruses in the Process of Emergence was chaired by Philip K. Russell (US Army Medical Research and Development Command) and Monath, with speakers Monath, dengue and other vector-borne viruses; James W. Le Duc (US Army Medical Research Institute of Infectious Diseases), Hantaan and related rodent zoonoses; Colin R. Parrish (Cornell University), canine parvovirus, a probable example of interspecies transfer; Brian W. J. Mahy (Centers for Disease Control), seal plague virus; Michael Houghton (Chiron Corporation), new hepatitis viruses; and Gerald Myers (Los Alamos National Laboratory), human retroviruses.

Viral Evolution was chaired by Howard M. Temin (University of Wisconsin-Madison) with speakers John J. Holland (University of California–San Diego), mutation and rapid evolution of RNA viruses; Palese, mutation rates and evolution in influenza and other RNA viruses; James H. Strauss (California Institute of Technology), recombination in RNA viral evolution; Russell F. Doolittle (University of California–San Diego), evolution of retroviruses; Brian Murphy (NIAID), factors restraining emergence of mutant influenza viruses; Bernard N. Fields (Harvard Medical School), viral virulence factors; Thomas E. Shenk (Princeton University), tissue tropism in DNA viruses; and Temin, mutation-driven evolution of viral pathogens.

Approaches for Assessing Factors in Viral Emergence was chaired by Shope with speakers Shope and Alfred S. Evans (Yale University), geographic factors and transport; Thomas E. Lovejoy (Smithsonian Institution), global environmental change; Bruce F. Eldridge (University of California–Davis), evolution of arthropod vectors; Robert M. May (Oxford University and Imperial College, London), ecology and evolution of host-virus associations; Douglas D. Richman (University of California–San Diego), detection systems for viruses; David C. Ward (Yale University), new technologies for detecting viruses; and Donald A. Henderson (Johns Hopkins University), surveillance systems and intergovernmental cooperation.

The conference closed with a panel discussion chaired by Krause and Edwin D. Kilbourne (Mt. Sinai School of Medicine) with additional discussants Ashley T. Haase (University of Minnesota) and Lederberg.

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