Emergence of New Epidemic Viruses through Host Switching

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NIH SPONSORED WORKSHOP: EMERGENCE OF NEW EPIDEMIC VIRUSES THROUGH HOST SWITCHING

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Steering Committee:

Colin Parish, Chairman

Linda Saif, Charlie Calisher, Robert Webster, Donald Burke, Peter Daszak, David Morens, Karen Lacourciere, Eun-Chung Park, Cristina Cassetti, Patricia Repik

Introduction

Workshop focus and organization: The over-all goal of the workshop is to consider the specific topic of viruses that could emerge and become widespread in the human population through host switching, and the special issues that are raised by that type of viral emergence. One objective is to consider how such emergences have occurred in the past, and the principles that can be learned from those examples. That information will be used to consider how such events might occur in human populations in the future, how those might be prevented, or if they do occur, how they might be controlled. Control strategies to be considered include those implemented early in the outbreak or others that would minimize the harm done if a virus does get away.

These are clearly big topics and it would be possible to have a busy 3 day meeting about each topic alone. The idea of this meeting is not to delve into the fine details of each system being discussed, but to define the broad principles of these topics in a way that would help the virological and public health community to understand these types of emergence and to us the information to make practical plans for the future.

We expect the discussions and presentations would range from very basic to the very applied, as any prediction or control strategies for host switched viruses requires information from many different areas. Topics to be discussed include the underlying virus-host molecular interactions, the evolutionary trajectory followed by the virus, the epidemiology of the virus at different stages of the outbreak, immunologic and other host responses that relate to the virus-host interaction and to vaccines, rapid detection methods for early identification of the agents, as well as other control strategies.

The assignments to workshop areas are mainly suggestions based on our understanding of people's areas of expertise. We appreciate that some people might not feel completely comfortable with the topic they are assigned. However, we hope that people can look at the questions that are posed under the topic and see if they can address some or all of these. If there are areas where you do not know or cannot find the answers please also bring those to the attention of the group – perhaps someone else knows the answer, or those may be areas where there are important gaps in our knowledge.

Outcomes, goals, and publication strategies: Identification of common and underlying themes, identification of gaps in our understanding, recommendations for future research directions and planning.

A review manuscript that reports the findings and conclusions of the workshop will be written for publication, most likely in the Journal of Infectious Diseases where the editors have agreed to consider such a report. That would be in the form of a review of the material covered – summarizing both the identified knowledge and the apparent gaps in that knowledge. We would use the information provided at the meeting, as well as additional details that may be requested from individual presenters, and we hope that can be approved by and published under the names of the workshop participants. This would also be used as the report to the NIH of the outcome of the meeting.

That review may be supplemented with a shorter summary in either Science or Nature. Those journals have been contacted, and although there appears to be some general interest, we do have any assurance that they would consider such a report.

Meeting organization: Several sub-topics have been identified, and we have framed a series of questions that relate to those areas. For each topic we now have a group of participants who we believe are experts and who can collectively identify themes in that area as well as any connections to the other areas covered in the workshop. Each invited presenter should prepare and circulate, in advance, a 1-2 page summary, so that people can come to the meeting prepared to ask questions and to make comments so as to enhance connections.

The summary and any recommendations from the meeting will be designed to identify the current state of our knowledge, and would include (1) things we know about host switching to form new epidemic viruses, (2) things that seem likely but for which there is less supporting data, and (3) things that we know little about but which should be investigated further.

Presentations: Should be 15 minutes in length, and could be in Powerpoint (we have a PC and Mac) or any other format that you think is appropriate. They should consider the major questions of the meeting and the specific subtopic, perhaps in broad strokes, rather than be detailed technical research presentations. We have tried to leave enough time for a reasonable discussion at the end of each set of presentations. We have identified a Chair and Co-Chair for each session who will help lead the discussions. We expect that we would have a group of presenters speak in succession, and then have panel-led discussions of the broad themes identified.

Wrap up session: This is on Thursday afternoon. We are asking the session Chair and Co-Chair to prepare, preferably on a overhead sheets or in a small number of Powerpoint slides, a summary or outline of the materials presented in their session (the Thursday morning sessions may be a little difficult to do this for). Those should address the main points of the session (e.g. the questions outlined in the program below) as well as any other issues identified below. Attempts will be made to connect the different sessions to make the connections between the different areas being considered.

Agenda

The schedule and timing of each session is not a reflection of their relative importance, but of the number people who we were able to identify who could help us address the issue(s) and attend, and in some cases people's schedules.

Tuesday, September 6, 2005

5:30 p.m. – 7:00 p.m. **Buffet Dinner**

7:00 p.m. – 7:30 p.m. **Welcome and Overview of Meeting Goals**
Carole Heilman
Director, DMID, NIAID, NIH, DHHS
Colin Parrish
Chair, Steering Committee

Introduction to the meeting; summary of the objectives; plan for the presentations and discussions.

7:30 p.m. - 9:30 p.m. **Viral reservoirs in animals - the effects of human behavior and interaction with other animals or effects on the environment that can potentially result in new viral emergences.**

Viral emergence and zoonosis
Mark Woolhouse*
University of Edinburgh
UK.

SARS coronavirus and masked palm civets
Zhihong, Hu
Wuhan Institute of Virology
Chinese Academy of Sciences
China

Analyzing the causes of zoonotic disease emergence
Peter Daszak**
Consortium for Conservation Medicine

Patrolling the Borders: Viral Emergence, Planning and Preparedness at the Animal-Human Interface
Stephen Morse
Columbia University

**** and *: Chair and co-Chair of the session.**

Wednesday, September 7, 2005

7:30 a.m. – 8:30 a.m. **Continental Breakfast**

8:30 p.m. – 11:00 a.m. **Introduction**
Catherine Laughlin
Chief, Virology Branch, DMID, NIAID, NIH, DHHS

Introduction to the meeting objectives—NIH perspective

Host-virus interactions at cellular, molecular and/or receptor levels and the control of host range.

SARS coronavirus and HIV receptors
Michael Farzan
Harvard University Medical School

Protein and carbohydrate determinants of the species specificity of coronavirus infection
Kathryn Holmes*
University of Colorado Health Sciences Center

Molecular Mechanisms of Coronavirus Cross Species Transmission
Ralph Baric
University of North Carolina-Chapel Hill

Hepatitis E Viruses: Cross-species Infection and Zoonosis
X.J. Meng
Virginia Polytechnic Institute and State University

The emergence and variation of canine parvovirus as a new virus in dogs – a series of evolutionary steps allowed a feline virus to adapt to an alternative host receptor
Colin Parrish**
Cornell University

Molecular changes associated to the internal proteins of influenza virus during interspecies transmission
Daniel Perez
University of Maryland

11:00 a.m. - 11:30 a.m. **Break**

11:30 a.m. - 1:30 p.m. **Adaptation to and pathogenesis in the new host; innate and adaptive host responses to viruses.**

Biologic aspects of the interspecies transmission of coronaviruses and other enteric RNA viruses
Linda Saif**
The Ohio University

Host ranges and variation of viruses, including influenza and SARS
Ab Osterhaus
Erasmus Medical Centre
The Netherlands

Poxvirus host interactions: keys to host tropism
Grant McFadden*
Robarts Research Institute, University of Western Ontario
Canada

Heterologous Immunity and CD8 T cell crossreactivity during viral infections
Liisa Selin
University of Massachusetts Medical School

The biology and pathogenesis of rotavirus and host responses to viruses
John Patton
NIH, DHHS

1:30 p.m. - 2:30 p.m. **Buffet Lunch**

2:30 p.m. - 4:00 p.m. **Viral evolution and the process of transfer and adaptation to new hosts.**

Evolution and Population Genetics of Viruses
Edward Holmes**
Pennsylvania State University

Evolution of viral-host relationships
Luis Villarreal*
University of California-Irvine

Virus evolution and variation
Esteban Domingo
Universidad Autonoma de Madrid
Spain

4:00 p.m. – 4:30 p.m. **Break**

4:30 p.m. – 7:00 p.m. **Acquiring high transmissibility among new hosts by viruses, and epidemic spread; also insect vector adaptation of arboviruses.**

Mathematical modeling of disease outbreaks
Neil Ferguson*
Imperial College London
UK

Epidemiology of Infection Transmission
James Koopman
University of Michigan School of Public Health

The population dynamics and coevolution of parasites and their hosts
Andrew Dobson and Juliet Pulliam
Princeton University

High-Throughput Laboratory Network Against Influenza and Emerging Diseases
Scott Layne
UCLA School of Public Health

The remarkable arboviruses: host-switching as a way of life
Charles Calisher**
Colorado State University

Thursday, September 8, 2005

7:30 a.m. – 8:30 a.m. **Continental Breakfast**

8:30 p.m. – 10:30 a.m. **The process of post-transfer adaptation – how do viruses become optimized for replication in and transmission between the new host?**

Virus evolution and adaptation strategies, poliovirus eradication
Mark Pallansch*
CDC, DHHS

Emergence of influenza (H1N1) and host adaptation
Jeff Taubenberger**
Armed Forces Institute of Pathology

Influenza virus cell interactions
Wendy Barclay
University of Reading, UK

Viral emergence and epidemiology
Don Burke*
Johns Hopkins Bloomberg School of Public Health

Influenza variation and host adaptation
Ruben Donis
CDC, DHHS

10:30 a.m. - 11:00 a.m. **Break**

11:00 a.m. - 1:00 p.m. **The epidemiology of new pathogens at different stages of the adaptation process. Infectious disease prevention and control strategies and their use in the cases of emerging epidemic viruses.**

Animal models of SARS and control strategies for influenza
Kanta Subbarao
NIH, DHHS

Viral outbreaks, epidemiology and public health
C. J. Peters**
University of Texas Medical branch

Emerging diseases and veterinary medicine
Lonnie King*
Michigan State University & CDC

Viruses and Viral Diseases
Marc van Rengemortel
Biotechnology School of the University of Strasbourg
France

Summary and conclusions
Chairs and Co-Chairs of the Sessions

1:00 p.m. - 2:00 p.m. **Buffet Lunch**

Adjourn

Abstract

Influenza virus:cell interactions

Wendy Barclay
University of Reading, UK

Reassortment between an avian and a human influenza virus could be considered the ultimate genetic mechanism for post-transfer adaptation in the human host. There is increasing evidence that reassortment could occur in the human respiratory tract, as well as in the pig as previously described. A limited degree of avian viral replication can be detected in the ciliated cells of cultured human airways. Human influenza viruses infect largely non-ciliated cells, which bear sialic acid presented in a different linkage to that found on ciliated cells. However, human influenza antigens are also detected in some ciliated cells, demonstrating the potential for reassortment to occur in those cells. By this mechanism viral genes already well adapted for promoting efficient replication in humans can be acquired in one foul swoop. But which genes must be acquired? And why? And are there any limitations to the combinations of avian and human viral gene segments that can create a well-adapted human pathogen?

Clearly the **PB2 component of the viral polymerase** commands a degree of host specificity. In both the reassortment events that generated the 1957 and 1968 pandemic viruses, the PB2 gene segment was acquired from a human virus. Viral polymerase complexes containing PB2 genes from an avian source function poorly in mammalian cells, but activity is greatly enhanced by a number of different mutations particularly in the carboxyl half of the protein (Yongxiu et al. 2001).
Replication of avian polymerase and productive infection by avian influenza viruses can also be 'rescued' in mammalian cells by introduction of portions of chicken genome. In a series of radiation hybrid cells in which **chicken chromosome fragments** were inserted into hamster cells, we identified positive cell lines that shared a common fragment of chicken genome which was not present in any of the hybrid cell lines unable to support avian viral polymerase function. One or several of these chicken genes may interact with avian virus PB2, or another avian virus gene, to allow replication. Presumably the mammalian homologue of this gene cannot be utilized by the avian influenza virus components.

The **NS1** gene shows an evolutionary rate in human viruses which might indicate that it is adapting to enhance viral replication. One of the best described functions of NS1 is as an interferon antagonist. It is possible that avian virus NS1 genes do not function in this role efficiently in the mammalian host, perhaps due to inherent differences in innate immunity between birds and mammals. Indeed we have noted that mammalian cells deficient in the interferon response become permissive to avian influenza strains. We assembled a panel of avian and human influenza viruses and tested the extent to which they induced an interferon response. In general the avian viruses induced more interferon than the human viruses supporting the idea that an inability to counter interferon induction could limit avian virus replication. However, the trait did not map genetically to the NS1 protein. Moreover, we identified several human viruses which also induced high levels of interferon. Some of those viruses were able to suppress the expression of newly transcribed mRNAs, so that the interferon induced genes would not be expressed. In

contrast some of the avian strains were less able to suppress expression from the interferon stimulated response element (ISRE). It may be that productive viral infection is facilitated only when a temporal balance between virus and host cell is achieved in which a sufficient level of viral gene expression is accumulated before host innate immunity 'kicks in'.

We also noted that infection of human cells with some avian viruses resulted in a rapid cytopathic effect, and this trait did map to RNA segment 8 which encodes the NS1 gene. If avian viruses are unable to prevent cell death triggered during infection, this might limit their ability to complete the replication cycle before disintegration of the human cell.

One thing that has become obvious is that influenza viruses vary remarkably in their interaction with the host innate immune system. It is interesting to speculate about the effects of one virus upon another during the coinfection process required for the reassortment event. Some viruses induce high levels of interferon during infection which might then render the infected cell refractory to co-infection with a different parental virus. In addition other viruses are reported to be somewhat resistant to interferon, and these might be very good partners at facilitating productive co-infection of cells during reassortment.

One gene which will certainly be acquired from an avian viral source during the next pandemic is the **HA**. If the HA contains a multibasic cleavage site and has a relatively high pH for fusion, certain viral M2 ion channel genes might not be compatible to generate viable reassortants (Harvey et al. 2004). Moreover, it is extremely likely that the avian HA gene will acquire some 'humanizing' mutations before it can facilitate the efficient transmission of virions between people. This has been noted during the early evolution of H2 and H3 HA genes. We previously found two mutations which together allowed enhanced binding of H5 HA protein to the human form of sialic acid receptor (Harvey et al. 2004). It is likely that such possibilities exist for all strains within the different avian HA subtypes, and that such mutations will facilitate a change in tropism of the virus within the human respiratory tract. Moreover we have also described some interesting trends in the evolution of human influenza HA proteins of viruses currently circulating in man (Thompson et al. 2004). A combination of antigenic drift and possibly evasion of other innate immune system such as NK cells is driving evolution of HA to result in loss of sialic acid affinity. Whether the periodic near-extinction of a human influenza subtype paves the way for the introduction of an avian virus is an interesting possibility.

Y. Yongxiu, L.J. Mingay, J.W. McCauley and W.S. Barclay. Sequences in influenza A virus PB2 protein that determine productive infection for an avian influenza virus in mouse and human cell lines. 2001. Journal of Virology 75 5410-5415.

R. Harvey, A. C.R. Martin, M. Zambon and W. S. Barclay. 2004**.** Restrictions to the adaptation of influenza A virus H5 haemagglutinin to the human host. Journal of Virology. 78: 502-507.

C.I. Thompson, W.S. Barclay and M.C. Zambon. 2004. Changes in In Vitro Sensitivity of Influenza A H3N2 viruses to a Neuraminidase Inhibitor Drug during Evolution in the Human Host. Journal of Antimicrobial Chemotherapy. 53: 759-765

Molecular Mechanisms of Coronavirus Cross Species Transmission:

Ralph S. Baric, PhD
Department of Epidemiology, Department of Microbiology and Immunology
University of North Carolina at Chapel Hill

Many new emerging virus infections in humans are animal viruses that have crossed the species barrier and colonized the human host. Coronaviruses include many important human and animal pathogens and these viruses are capable of rapid evolution by mutation and RNA recombination. During the past 25 years, several newly emerged coronaviruses have been identified, mostly in domesticated animals that have resulted in global pandemics of disease, especially within the swine, poultry and cattle industries. The emergence of the highly pathogenic human coronavirus, SARS-CoV, firmly established zoonotic coronaviruses as likely sources of future human and animal emerging diseases. Our laboratory has studied the molecular mechanisms governing coronavirus cross species transmission and host shifting, using mouse hepatitis virus as a model.

We have developed two models for studying the molecular mechanisms of coronavirus cross species transmission and persistence. In the first model, MHV infection in mixed cultures of murine and hamster cell lines, reminiscent of conditions which might exist in human xenograph recipients, rapidly selected for host range mutants that replicated efficiently in hamster cells. The host range mutants MHV-H2 also replicated in normally nonpermissive human cell lines, indicating that coronavirus host shift may select for mutations that result in the emergence of "generalists" that replicate efficiently in multiple host species. Further passage in human cell lines, resulted in the emergence of a variant, C4 that replicated efficiently in many human cell lines. At this time, our data support a model system of virus cross species transmission occurring by recognition of orthologs of the normal receptor that is used for virus docking and entry, rather than via recognition of new receptor molecules for entry. Other changes in the genome likely contribute to replication efficiency in the new host background. Genetic changes were mostly confined within the S glycoprotein of the MHV-H2 and C4 host shifted viruses, a major surface glycoprotein which gives the virus its unique appearance in the electron microscope and interacts with surface host cell receptors, carcinoembryonic antigens (CEACAM1a) that are essential for MHV docking and entry into human cells.

Coronaviruses rapidly establish persistent infections in culture and in animals. In the second model, persistent MHV infection was established in culture. In this model, MHV persistence is mediated by virus selection for host cells that express little CEACAM1a receptor and these cells become resistant to wildtype virus infection. However, the emergence of resistant cells selects for co-evolving variant MHV viruses (V51) that recognize other murine CEACAM related receptors for docking and entry into mouse cells and replicate efficiently in the newly emerged "nonsusceptible" host cells. Expanding receptor usage in murine persistent cultures is accompanied by expanding recognition of human orthologue CEACAM receptors, allowing for V51 replication in some human cells. Our data support a generalized model of coronavirus host shifting that

is primarily mediated by changes in the S glycoprotein gene that increased generalized recognition of parlogues or orthologues of the normal receptor needed for docking and entry in vitro.

Genetic changes in the S glycoprotein dominate in the MHV host range mutants that were selected in both model systems, but mutational profiles are completely unique and also differ from mutational profiles found in other MHV host range mutants. Genetic analysis of chimeras containing portions of the host shifted viral S glycoprotein genes fused into the parental S glycoprotein gene backbone have clearly demonstrated that the S glycoprotein is a critical genetic determinant of host shifting and that only a few mutations are essential for mediating this phenotype in vitro. Consequently, multiple genetic pathways in S can derive host shifted coronaviruses that replicate efficiently in human cells. However, other genetic changes in the replicase gene likely contribute to replication efficiency in the new host species. The nature and function of these alleles in host shifting is under study.

The basic principles that we can derive from these studies is that persistence, RNA recombination and mutational processes drive coronavirus evolution and cross species transmission. Importantly, only a few amino acid changes are necessary to promote host shifting and multiple genetic pathways in S can mediate host shifting. Our data also supports that hypothesis that coronavirus host shifting selects for “generalists” that can replicate efficiently in multiple new host species and that this is initially mediated by changes within the S glycoprotein gene. While our data support the evolution of S glycoprotein alterations that allow for recognition of orthologues of the normal receptor for docking and entry in new host species, it is likely that other pathways likely exist as well. Finally, our data indicate the replicase gene of coronaviruses promote replication efficiency in alternative host species, although the exact nature of this phenomena remains to be discovered.

The remarkable arboviruses: host-switching as a way of life

Charles H. Calisher, Ph.D. [Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523]

Whereas under natural conditions most other viruses are capable of infecting one host, two closely related hosts, or two hosts that are distantly related evolutionarily, the arthropod-borne viruses (arboviruses) are able to replicate in arthropod hosts of various species and in vertebrate hosts of many species. That these viruses can replicate in cells of extremely dissimilar life forms has been a subject of interest for many decades. The mechanism by which the diverse arboviruses attach to and enter cells varies diversely but a key question is "How do viruses with such diverse genetic and phenotypic differences manage to switch between diverse hosts?"

Clearly, answers to this question are key to our understanding of virus infectivity, host susceptibility, and host-switching. Certain arboviruses may be used as model systems for such studies because they not only can switch hosts but they switch hosts as a matter of course, as part of their life cycles. Indeed, many arboviruses not only can switch back and forth from mosquitoes to vertebrates to mosquitoes but from mosquitoes to vertebrates to ticks to vertebrates and so on. Obviously, there is a combination of virion properties and cell properties that mediate binding, attachment and entry into the host cell. Surely the cell plays a principal role in all this and cells of mosquitoes, ticks, humans, deer mice and kangaroos likely do not share all possible viral receptors. It is not unreasonable to accept that closely related viruses, such as the four dengue viruses, St. Louis encephalitis virus and West Nile virus, Yellow fever virus and Wesselsbron virus, all flaviviruses, might have properties in common. However, it is counterintuitive to assume that their many arthropod and vertebrate hosts, which differ widely, share all their receptors. More likely, these and other arboviruses have a built in flexibility, such that they can utilize alternative receptors to attach to cells of vertebrates belonging to dissimilar taxa.

A confounding exception to the host specificity of a rodent-borne virus may provide us with some insights. Within the virus family *Bunyaviridae* are nearly 400 viruses, placed in five genera or not assigned to a genus. Viruses of four of these genera are transmitted by arthropods, blood sucking and otherwise. The exception, the hantaviruses, use as their natural cycle transmission from rodent-to-rodent. Each hantavirus has a principal rodent host, such as Sin Nombre virus in deer mice, *Peromyscus maniculatus*. Other rodents have been shown to be infected with Sin Nombre virus, but the highest prevalence of antibody to this virus is in deer mice. However, at a site in southeastern Colorado where we have identified 21 species of rodents belonging to 13 genera, including deer mice, pinyon mice and three other *Peromyscus* species mice, only deer mice and pinyon mice appear to be infected with Sin Nombre virus. Indeed, rodent host specificity is a hallmark of hantaviruses, such that "one host-one virus" now is a mantra among hantavirologists. This has been reasonably confirmed by virus and rodent sequencing data, which show a parallel between hantaviruses and their principal rodent hosts. So, if

Sin Nombre virus rarely if ever infects conspecific rodents, how could it infect and cause serious illness in humans? The answer to this question may be simple, but we do not yet have that answer.

In contrast to arboviruses, therefore, the hantaviruses, and arenaviruses also, appear to use receptors that did not evolve for their betterment. For example, Mackow, Gavrilovskaya (1) and their associates have been studying the relation between hantaviruses and a platelet membrane glycoprotein IIIa, called beta-3 integrin. The integrins are receptor proteins of importance in the way cells bind to and respond to other cells and to the extracellular matrix. In other words, they have their own responsibilities in normal cellular mechanisms. Functional integrins consist of two transmembrane glycoprotein subunits, alpha and beta. These workers have found that pathogenic and non-pathogenic hantaviruses use beta-3 and beta-1 integrins, respectively, to enter endothelial cells. Beta-3 receptors have been shown to bind receptors regulating vascular permeability, and the interaction of beta-3 integrin receptors and hantaviruses may provide a clue as to the pathogenesis of certain hantaviruses. They found that hantaviruses that are pathogenic for humans blocked endothelial cell migration on beta-3, but not beta-1 integrins and, moreover, that only pathogenic hantaviruses, which use beta-3 integrins, dysregulate endothelial cell migration; beta-1 integrins, which adenoviruses and coxsackie viruses use to enter and affect cells, are ignored by the pathogenic hantaviruses. Thus, an adaptive or coincidental use of a receptor has been key to the pathogenesis of some hantaviruses, but not others; this seems to be functional for them. SARS coronavirus, HIV, and other viruses that appear to have jumped species by chance may be other examples of occurrences that have been fortuitous for viruses.

At the same time, species-jumping is what arboviruses do, what they have always done, how they are defined. Either arboviruses are viruses of arthropods which have adapted to replicate in either vertebrate or arthropod cells, or they are vertebrate viruses which have adapted to replicate in either vertebrates or arthropods. The importance of arboviral replication in arthropods is obvious: it gives them the opportunity to be amplified, to avoid the immune mechanisms of the vertebrate host, and to persist under certain deleterious environmental conditions, such as winter, using mechanisms including transovarial or transstadial transmission. The importance of arboviral replication in vertebrates also is obvious: it gives them the opportunity to move long distances, to be exposed to other arthropod species, and to allow their progeny to be selected as the winners by the vertebrate host immune system.

Gavrilovskaya IN, Peresleni T, Geimonen E, Mackow ER (2002) Pathogenic hantaviruses selectively inhibit beta3 integrin directed endothelial cell migration. Archives of Virology 147:1913-1931.

Analyzing the causes of zoonotic disease emergence

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There is a great deal of interest in emerging zoonotic diseases, particularly those from wildlife reservoirs, and recent analysis suggests that around 75% of human EIDs are zoonotic (Taylor et al., 2001). There are also clear links between increased contact with wildlife and domestic animals and the emergence of SARS, Nipah virus, Ebola virus and other high profile EIDs. The underlying causes of zoonotic disease emergence usually involve broad environmental changes (e.g. encroachment into wildlife habitat, landuse changes), changes to human behavior (e.g. wildlife trade, medical technology) or changes in human demography (e.g. urbanization). However, despite the importance of these underlying drivers, very little analytical work has been published on how they cause diseases to emerge.

In this talk, I will attempt to re-assess how we can study this process of disease emergence at such a broad scale. I will draw examples from the emergence of Nipah and Hendra virus, from the role of wildlife trade in disease spread and from a rise in EIDs of wildlife themselves to provide an overview of the factors underlying disease emergence. In these studies, collaboration between wildlife biologists, veterinarians, ecologists has been important to identify wildlife reservoirs, then to pinpoint the causes of emergence. The collaborative approach involves understanding how changes to environment, demography and behavior act on contact rates between wildlife, domestic animals and ultimately humans to allow diseases to emerge.

We have begun to analyze broad patterns in the underlying drivers of disease emergence using a large EID database. Our preliminary analyses show that we can identify areas where human activities within a background of high wildlife diversity result in high-risk 'hotspots' for disease emergence. This approach may ultimately allow some measure of predictive capacity to understand and deal with the potential for diseases to emerge.

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Evolution and Population Genetics of Viruses

Edward C. Holmes
Department of Biology, The Pennsylvania State University

I will review the major mechanisms of RNA virus evolution and what impact they may have on the likelihood of emergence. The most important general observation is that despite the extremely high mutation rates observed in most RNA viruses there are also major constraints to adaptability, some a direct outcome of high mutation rates, and that these have a fundamental impact on emergence.

Mutation. Mutation is the fuel of evolutionary change in RNA viruses. Although mutation rates are often difficult to measure directly, estimates of the rate of nucleotide substitution are in the region of 10^{-3} to 10^{-4} per site, per year (although some notable outliers exist; Jenkins *et al*. 2002). Not only are these rates many orders of magnitude higher than those seen in eukaryotes but they imply that RNA virus genomes pick up errors on nearly every replication. However, high rates of mutation do not guarantee rapid adaptive evolution and hence easy emergence as there is growing evidence that the vast majority of mutations within RNA virus populations are deleterious (or slightly so). In short, RNA viruses carry a heavy burden of deleterious mutations that will also affect their adaptability and hence potential for emergence.

Recombination. Because recombination may increases fitness by creating advantageous genotypes and removing deleterious mutations it might also be expected to assist in emergence. However, other than in the retroviruses, recombination is not a particularly common process in RNA viruses and there is no reason to suppose that it is any more than a mechanistic by-product. For example, recombination appears to be extremely rare in negative-sense RNA viruses, most likely because their RNA is always encapsidated thereby preventing template-switching (Chare *et al*. 2003). As a number of emerging viruses have negative-sense RNA genomes, this argues against recombination as a general process in viral emergence. Similarly, although recombination is more common in positive-sense RNA viruses, in most cases it appears to be a sporadic event that does not occur at a high enough frequency to make it a key evolutionary strategy (although rare events like recombination may sometimes kick-start the process of viral emergence). Overall, as the rate of recombination, per base, will be very much lower than that of mutation for most RNA viruses we can conclude that recombination is not a key requirement for emergence, but rather a happy coincidence.

Natural selection. RNA virus populations contain some of the best examples of molecular adaptation described to date. Indeed, their large population sizes are predicted to allow selection to work with relatively high efficiency, in turn facilitating the adaptation to new hosts. However, there are a number of reasons why RNA viruses may not be as adaptable as usually thought. First, although intra-host population sizes are large, there may also be major bottlenecks at inter-host transmission (their magnitude in part depending on transmission mode) which will strongly affect the structure of genetic diversity. At present the long-term effects of population bottlenecks on RNA viruses in

nature remain uncertain. Second, the small genomes of RNA viruses mean that complex fitness trade-offs are expected to be commonplace (as is epistasis – see below). Both experimental and computational studies are now revealing more evidence for these intricacies, which act to constrain viral evolution. For example, escape from cytotoxic T-cell recognition often appears to be subject to strong structural constraints (Berkhoff et al. 2005). More generally, vector-borne RNA viruses seem more constrained than other RNA viruses, most likely caused by an antagonistic pleiotropy that stems from replicating in very different cellular environments (Woelk & Holmes 2002). This in turn may inhibit their ability to develop sustained transmission cycles in new hosts.

Epistasis. Understanding the complex fitness interactions among mutations is one of the goals of modern evolutionary genetics. Such epistasis is predicted to be especially strong in RNA viruses due to their small genome sizes, use of overlapping reading frames, and complex secondary structures. Accordingly, recent experimental studies have found that positive epistasis is an important phenomenon in some RNA viruses (Bonhoeffer et al. 2004; Sanjuan et al. 2004). Large-scale comparative studies have also revealed wide-spread positive epistasis among RNA viruses, particularly within localized sequence regions (< 30 amino acids), thereby suggesting a strong influence of secondary structure. This, in turn, has implications for the evolution of mutational robustness and recombination and will also in part determine the dynamics of adaptation.

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Protein and carbohydrate determinants of the species specificity of coronavirus infection

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Coronaviruses cause epidemics of respiratory or enteric diseases in many species of animals and birds, and 5 respiratory coronaviruses cause disease in humans. There are 3 distinct phylogenetic groups of coronaviruses: Group 3 coronaviruses infect avian species, while Groups 1 and 2 infect mammals. I will discuss only the mammalian coronaviruses. The large spike glycoprotein (S) of coronaviruses is a type 1 viral fusion protein that binds to specific receptor glycoproteins and mediates fusion of the viral envelope with host cell membranes to initiate infection. Glycans on cell surface moieties or on viral glycoproteins also play important roles in the specificity of coronavirus interactions with their host cells.

For some coronaviruses, there is strong species specificity at the level of coronavirus spike/receptor interactions. For example, although all strains of mouse hepatitis virus (MHV, a group 2 virus), use murine carcinoembryonic antigen cell adhesion molecule 1a (CEACAM1a) as their receptor, the closely related rat coronavirus (RCoV, group 2) cannot use either mouse or rat CEACAM1 as a receptor (2, 3). In contrast, bovine coronavirus (BCoV, group 2) has a broad host range and can cause transmissible disease not only in cattle, but also in turkeys (16). In addition to S, most group 2 coronaviruses bind to cells by a hemagglutinin esterase glycoprotein (HE) on the viral envelope. HE recognizes carbohydrate moieties and may be a virulence factor *in vivo*, although it is not essential for replication in cell culture (12). Some experiments with bovine coronavirus (group 2) suggest that coronavirus HE is not sufficient for infection, but facilitates infection by binding virions tightly to cells where the viral S protein can interact with a receptor glycoprotein (9). Because different host species express different carbohydrate moieties on cell membranes, the carbohydrate specificity of viral HE binding may help to determine viral species specificity. Extended host range variants of MHV selected in cell cultures can be associated with mutations in S that mediate binding to carbohydrate moieties on cell membranes (14).

Carbohydrates are also involved in coronavirus/receptor interactions in other ways. Coronaviruses mature by budding into membranes of the endoplasmic reticulum/Golgi intermediate compartment (ERGIC), followed by exocytosis of virions from living cells. Glycoproteins in the ERGIC are incompletely glycosylated, having many endogycosidase H-susceptible, high mannose N-linked glycans. These endoH-sensitive glycans, and virions that contain them, can be recognized by DC-SIGN or L-SIGN, C-type lectins expressed on dendritic cells or endothelial cells and liver, respectively. These lectins bind many types of enveloped viruses including HIV, dengue, Marburg, Ebola, and SARS coronavirus and either deliver the infectious virions to susceptible cells that express the appropriate receptor glycoprotein or mediate entry and inefficient infection of cells that do not express the receptor glycoprotein (1, 5, 8, 11, 13).

Group 1 coronaviruses include porcine enteric TGEV and respiratory PRCoV, canine and feline coronaviruses and human respiratory coronaviruses 229E. All of these viruses use aminopeptidase N of the appropriate host as their receptors. Although 229E cannot use porcine APN, and TGEV cannot use human APN, all of these group 1 viruses can use feline APN as receptors in cell culture, suggesting that the viruses may have descended from a feline coronavirus ancestor (15). However, the porcine and human viruses do not cause disease or transmissible infection in cats. Other factor(s) in addition to receptor activity is required for disease and transmission *in vivo*. APN is highly conserved in mammals, but some surface residues differ, causing antigenic differences and coronavirus receptor specificity. A single N-linked glycan at position 291 of porcine APN but absent in human APN is the basis for lack of 229E receptor activity of porcine APN (17). S glycoproteins of several group 1 coronaviruses including TGEV bind to carbohydrate moieties as well as to APN, and this carbohydrate binding is associated with alterations in viral tissue tropism and virulence (10).

SARS coronavirus uses as its principal receptor angiotensin converting enzyme 2 (ACE2)(7). The interactions of SARS-CoV with ACE2 proteins of different species are important determinants of viral virulence and host range. An N-linked glycan found on rat, but not human, ACE2 is one reason why rats are resistant to SARS-CoV infection. Surprisingly, the newly discovered human coronavirus NL63 (group 1), like SARS-CoV (group 2), uses human ACE2 as a receptor (4). The interactions of these two viruses with ACE2 are likely to be very different, because the S glycoproteins of these two viruses are very different.

Host factors other than receptors also are important for the species specificity of coronavirus disease. In an attempt to create a small animal model for human coronavirus 229E, mice transgenic for human APN were created (6, 18). However, although cells from these animals were susceptible to 229E infection, the mice resisted infection and disease. Only when the hAPN transgenic mice were bred with STAT1 knockout mice did the animals become susceptible to infection (6). Thus, in addition to receptors, differences in the innate immune response to different coronaviruses can determine whether infection and transmission can occur *in vivo*.

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SARS coronavirus and masked palm civets

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Severe acute respiratory syndrome (SARS) is a novel infectious disease in the new millennium. It has been ascertained that a new coronavirus, SARS-CoV, is the etiological agent of SARS. While the extraordinarily rapid isolation and full genome sequencing of SARS-CoV constituted a remarkable scientific achievement, identification of actual animal reservoir of SARS-CoV remained unresolved. To date, evidences indicate that the masked palm civet (*Paguma larvata)* is the primary suspect of the animal origin of SARS (Guan et al., 2003, Song et al., 2005). But whether masked palm civet is a reservoir or an intermediate host remains unclear. This presentation summarizes the studies on SARS-CoV like viruses in masked palm civets and tried to address some of the common questions about animal reservoir.

The masked palm civet is naturally arboreal, nocturnal and largely solitary. The farming of masked palm civets in China started in the 1950s mainly for their fur but breeding became popular in the late 1980s when demands for them as a culinary delicacy had increased. By 2003, about 40,000 masked palm civets were being raised in about 600 farms all over China. These animals were mean to sold to markets and then restaurants in South China. It was reported that anti-SARS-CoV antibodies in the market animals (78%) was much higher than the overall prevalence in farm animals (10%) (Tu et al., 2004).

We investigated the existence of SARS-CoV like viruses in farmed masked palm civets. Immunohistochemistry studies have indicated that the virus was present in the respiratory and digestive systems of masked palm civets. The prevalence of viral RNA in oral and anal swabs was around 40-80%. Results of antibody prevalence varied using different analyzing kits suggesting diversity of antigens among different strains. No neutralizing antibody against SARS-CoV was found from these farmed civets. Yang et al. (2005) recently reported that pseudovirus with spike from masked palm civets were difficult to be neutralized and it was suggested that masked palm civet SARS-CoVs have evolved to resist antibody neutralization.

So far 20 spike sequences were obtained from masked palm civet SARS-CoV like viruses. The amino acid identity among these sequences were above 98.6%. Comparative analysis of the sequences indicated that there was no identifiable conserved mutation in the spike protein of viruses from humans SARS-CoV in comparison to those from civets. This may signify that a diverse gene pool of spike proteins already existed in viruses of civets that either directly or by recombination event would result in a virus that infectious to humans. There are, however, a few amino acids were conserved in more than 80% of the spike sequences from masked palm civets and are different from those in SARS-CoVs which caused 2003 epidemic. These mutations may be important for the host switch from masked palm civet to human. It is reported recently that two amino acids mutations (R/K479 to N479, and S487 to T487) might be responsible for the transmission

of SARS-CoV like virus from palm civet into humans (Li et al., 2005, Qu et al., 2005). It worth pointing out that N497 mutation already existed in eight of the sequenced civet viruses and the double mutation was identified in one of the viruses.

The understanding of the relationship of masked palm civets and SARS-CoV is important for future prevention of SARS. The culling of masked palm civets in Guangdong Province in 2004 is believed to be helpful for preventing re-emerging of SARS. However whether this is efficient or necessary, need to be further proved. Currently, efficient diagnosis kit for animal surveillance and wider investigation in other animals are needed for getting a whole picture of SARS-CoV and its animal reservoir(s).

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Epidemiology of Infection Transmission

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New study designs, data collection tools, and analysis methods are needed so that the transmission characteristics of an emerging infection can be quickly established and good decisions made about control. The time frame for making control decisions and instituting control actions could be narrow. We must learn quickly how much transmission is occurring under what conditions via what modes from symptomatic and asymptomatic individuals. We must determine times of contagiousness with regard to illness in the source case as well as how long agents remain a threat in the environment. Then we need to establish geographic and social containment strategies involving isolation and quarantine as well as amelioration of transmission strategies involving contact reduction, hygiene, sanitation, and decontamination activities. Both over reacting and under reacting could have devastating consequences. So we need to get our responses right.

Severe emerging infections will result in hospitalizations early in the epidemic. Health officials need to learn about transmission from these cases not only to stem transmission in hospital settings, but in the community as well. Community level studies of transmission will always be more difficult, time consuming, and expensive than hospital level studies. Thus inferences about community control actions will be made from hospital level data. Information on the role of droplets, hands, feces, surfaces, air, water, and food in transmission could be obtained from hospital studies. But to apply this to community control action decisions, background knowledge on how different modes of transmission affect control decisions needs to be established well before threatening infections emerge. Obtaining such background data will have many uses. Evolutionary adaptation to utilize different transmission mediators is, after all, a key step in generating emerging infection threats. It is not enough to say that some mediator can carry transmission. We need the capacity to determine the role that different mediators play at the transmission system level. Control decisions might be quite distorted by merely identifying mediators or modes of transmission that can act.

Adequate methods to make initial transmission determinations in the hospital setting are currently lacking – as are background data needed to project what those determinations imply about community level control actions. One reason for these deficiencies is that the roles of different transmission mechanisms have been treated too cavalierly in population models of infection transmission. Another reason is that technologies for data collection, data analysis, and model based projections are under developed.

The technological base needed already exists and should be extended in important ways in the near future. Technologies for infectious agent detection in the environment are especially useful. The science of analyzing infection transmission systems is poised for important growth that will help overcome current deficiencies. Methods to estimate

transmission system parameters from diverse sources of data can now be readily developed within the context of Markov Chain Monte Carlo (MCMC) procedures. Two things, however, are still lacking: (1) coherent priority setting and coordination of research activities and technological developments, and (2) adequate investment in both the infrastructure needed for this science and the studies needed to realize our potential to understand control action effects.

Studies that integrate environmental measurements of microbial contamination with estimation of transmission model parameters could provide a strong basis for developing the science needed to meet these challenges. Environmental measurements have been used mainly to help establish modes of transmission or identify risky settings where control actions should be undertaken. Currently there are efforts to use environmental microbial measurements in surveillance as well.

There could be far greater value to using environmental microbe identifications in a completely different way that meshes with infection transmission system models. Used in this way the determination of viability of the identified microbes is not crucially important, though such identification could be helpful. The models relating to such data might incorporate transmission via droplets, hands, feces, surfaces, air, water, and food or they might only incorporate the temporal-spatial dimensions of transmission that relate to transmission involving these different mediators. The collection of environmental samples needs to be focused on those venues that are key to disseminating and controlling transmission. The value of the environmental samples would be greatly extended if the genetic relatedness of microbes detected in different venues could be determined.

To develop the background data on transmission systems that will help control emerging infections, common viral and bacterial agents that are endemically sustained should be studied. Also models like those developed by Barrett and Eubank under the MIDAS program should be extended to more specifically capture the space-time dimensions related to different transmission mediators. Developing estimation methods for parameters in such models should be a high priority. Those estimation methods should incorporate every possible data source and established theory including human infection identification, human movement patterns, human hygiene behaviors, environmental microbe identifications, genetic distance determinations, knowledge of microbial dilution or viability decay in different environments, dose-response infection risks, excretion rates from different body surfaces or fluids, and all aspects of the natural history of infection.

In summary, to adequately prepare for emerging infection control decisions, we need to advance the science of infection transmission system analysis to incorporate environmental microbial identifications and sequencings. Then we can establish base models defining how different modes of transmission relate to population transmission dynamics and make estimations early in an epidemic that allow us to apply this base knowledge to control decisions.

High-Throughput Laboratory Network Against Influenza and Emerging Diseases

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In August 2004, the United States Department of Health and Human Services released a draft of its Pandemic Influenza Response and Preparedness Plan. The Plan's surveillance annex offered six specific recommendations for system enhancements and next steps. Many of these enhancements could be achieved by developing a high-throughput laboratory network that expands capabilities of the existing centers on influenza. With such enhancements, centers would be able to collect samples from people with febrile respiratory illnesses, record epidemiologic observations, and ship samples directly to the high-throughput network. At each site, high-throughput automated systems for would work together and, within days, epidemiologic observations and test results would appear in the laboratory's web-enabled database for analysis. Internet-based capabilities would allow centers to examine their own data and improve surveillance in an iterative manner. In tracking significant changes in epidemic strains, the new system would facilitate non-biased proportional sampling of people with febrile respiratory illnesses. In detecting the emergence of novel stains with pandemic potential, the new system would facilitate the use of rapid and more sensitive methods.

The plan integrates available biological, engineering, and informatic technologies into a networked capability and makes them available via the Internet. Influenza is well suited to this approach because of its obvious public health importance but also because there already exists a well-established infrastructure that includes global surveillance, standardized laboratory methods, surveillance-based recommendations, and targeted vaccines. Key elements would be as follows.

Current high-throughput automated laboratory systems are capable of operating 24 hours per day. At each site, epidemiologic questionnaires and instructions would arrive by the Internet and bar coded samples would arrive by air freight. Larger sites could operate systems for genotyping, phenotyping, replicating, and archiving influenza viruses. Smaller sites could operate systems for genotyping and archiving viruses. In serving as resources, each site would provide reagents and supplies for analyzing all influenza subtypes. They would also perform control assays on a daily basis and maintain a quality assurance program, the documentation of which would be stored in the database. Automated laboratory methods would build upon manual methods currently in use and, because they can reduce working (liquid) volumes by at least five to ten-fold, they would enable economies of scale.

The high-throughput laboratory network would give rise to three domains of associated data from surveillance. *Epidemiologic* pertaining to dates, locations, hosts, outcomes, histories, and exposures. *Genotypic* pertaining to the exact sequence of nucleotides in all eight viral RNA segments. *Phenotypic* pertaining to immunologic pedigrees (HI titers) and antiviral drug sensitivities (resistance conferring SNPs, IC-50s) of sample strains.

Some practical public health and scientific uses of such organized data are outlined below.

Vaccine strain selection. The high-throughput laboratory network would help in two ways. It would provide faster information for vaccine strain selection, potentially saving one to two months in vaccine delivery. It would also continuously monitor for the emergence of escaping influenza strains and thereby guide critical decisions to update pandemic vaccines or use them in combination with limited supplies of antiviral drugs. Researchers and drug companies are developing modern methods (based on reverse genetics and cell cultures, for example) to manufacture influenza vaccines that could cut delivery times in half. Within the next few years, these new methods in combination with a high-throughput network could save additional vaccine delivery time and in so doing save lives.

Phenotypic and genotypic associations. Although sequencing influenza viruses is useful for understanding viral mixing and evolution, it cannot delineate how immunologic (i.e., drift and shift) variants relate to one another at the amino acid and RNA coding levels. To develop such understanding, a large base of phenotypic data must be associated with its corresponding genotypic data. For each receptor subtype, phenotypic data would consist of HI titers and genotypic data would consist of RNA sequences from the same virus. Building a rough association matrix would be the first step understanding how various variants relate to one another at the amino acid and RNA levels. Subsequently, a more complete association matrix would be used to develop models that can predict whether viral strains are immunologically related from sequences alone. Such efforts could help develop influenza vaccines that protect against a wider range of variants and establish a more fundamental molecular basis for influenza surveillance.

Outbreak control. Researchers have proposed using antiviral drugs, such as oseltamivir, to halt an avian influenza outbreak in humans. The strategy could require administering millions of doses to people in the epicenter and surrounding geographic zone within weeks. Immediate recognition of the outbreak and rapid surveillance to determine its size would be essential. Drug resistant avian influenza viruses would likely emerge at some point, representing a potential threat to emergency control efforts, and health authorities would need real-time information on their location and number. Such emergency interventions would generate thousands of samples for laboratory analysis within days. Given current laboratory surge capacity, a high-throughput laboratory network may be the only feasible means to meet the challenge.

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POXVIRUS HOST INTERACTIONS: KEYS TO HOST TROPISM

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Myxoma virus (MV), a member of the poxvirus family, causes lethal infection only in rabbits, but the mechanism underlying the strict MV species barrier is not known. Like all poxviruses, myxoma virus expresses a wide array of immunomodulatory proteins (Seet et al, Ann Rev Immunology 21, 377-423, 2003), but relatively few of these are actually rabbit-specific when tested in vitro. In fact, at least one such species-nonspecific immunomodulatory protein derived from myxoma virus, SERP-1, is currently in human clinical trials as an anti-inflammatory drug. The reason why myxoma virus exhibits strict specificity for the rabbit in vivo has been largely a mystery until recently. Our lab has now shown that MV infection of nonpermissive primary mouse embryo fibroblasts (pMEFs) evokes extracellular signal-regulated kinase (Erk) signaling which is integrated to interferon (IFN) regulatory factor-3 (IRF3) activation and type I IFN induction (Wang, et al Nat. Immunology, 5: 1266-1274, 2004). We have discovered that ERK1/2 inactivation or disruption of signal transducer and activator of transcription 1 (STAT1)-mediated IFN signaling breaks the cellular blockade to MV multiplication in non-rabbit cells. Moreover, STAT1 deficiency renders mice highly susceptible to lethal MV infection. Thus, ERK1/2-IFN-STAT1 signaling cascade induced by MV infection in nonpermissive pMEFs mediates an innate cellular barrier to poxvirus infection of mammalian cells outside of the rabbit species. This work has prompted us to investigate the use of myxoma virus as an oncolytic virus to treat human cancers that exhibit defective interferon responses (Sypula et al, Gene Ther Mol Biol 8: 103-114, 2004). The study of host tropism by poxviruses thus offers the potential for development of novel platforms for replication-restricted vaccine vectors and oncolytic viruses, but it also likely to produce novel insights into how and why poxviruses can occasionally leap from a long-term evolutionary host species to cause zoonotic infections in humans. In fact, there is a real need to better understand the dynamics of how “emerging” viruses in general can occasionally leap into non-evolutionary hosts to cause novel disease.

Hepatitis E Viruses: Cross-species Infection and Zoonosis

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Hepatitis E virus (HEV), the causative agent of human hepatitis E, is an important public health problem in many developing countries and is also endemic in industrialized countries including the United States. The mortality rate associated with HEV infection is generally less than 1% but it can reach up to 28% in pregnant women. HEV was classified in the *Caliciviridae* family but was recently declassified and placed in a new family *Hepeviridae*. Due to the lack of a cell culture system or a practical animal model, HEV is an extremely understudied pathogen. A vaccine against HEV is not yet available.

The recent discoveries of animal strains of HEV, swine HEV from pigs and avian HEV from chickens, have changed the way we used to think about hepatitis E and opened new avenues for HEV research. Hepatitis E is now considered as a zoonosis and swine (and possibly other animals) are reservoirs. Since its first identification in 1997 from a pig in the United States, swine HEV has now been detected from pigs in more than a dozen countries. Swine HEV is enzootic in pig herds worldwide (up to 80-100% seroprevalence rate in some farms), and active infection generally occurs in pigs of 2 to 4 month of ages. Swine HEV is genetically and antigenically closely related to human HEV, and shares significant sequence identity with, and in some cases has identical sequence to, strains of human HEV. Swine HEV isolates identified thus far all belong to genotype 3 or 4. It is believed that genotypes 3 and 4 strains, but not genotypes 1 and 2 strains, have the ability to cross species barriers and cause zoonotic infection. Cross-species infections of HEV have been documented: swine HEV infected non-human primates and genotypes 3 and 4 human HEV infected pigs. Pig handlers were found to be at a higher risk of zoonotic HEV infection. Sporadic cases of acute hepatitis E were definitively linked to the consumption of raw or undercooked pig and deer meats. More recently, another animal strain of HEV, avian HEV, was identified from chickens with Hepatitis-Splenomegaly (HS) syndrome in the United States. Like swine HEV, avian HEV is also genetically and antigenically related to human HEV. Avian HEV genome is about 600 bp shorter than that of human and swine HEVs, and shares only about 50% sequence identity with human HEV. However, motifs in the ORF1 putative functional domains were relatively conserved between avian HEV and mammalian HEVs, supporting the conclusion that avian HEV is also a member of the genus *Hepevirus*. Like swine HEV, avian HEV infection is also enzootic in chicken flocks: about 17% young chickens and about 36% adult chickens in the United States were seropositive. Pathological lesions characteristic of HS syndrome have been reproduced in chickens experimentally infected via oral route of inoculation with avian HEV. Unlike swine HEV, however, avian HEV failed to infect rhesus monkeys, even though avian HEV has been shown to cross species barrier and infect turkeys. The discoveries of these animal strains of HEV pose concerns for zoonotic risks but also provide alternative animal model systems to study this important human pathogen. However, the mechanism of HEV cross-species infection is not yet known.

Patrolling the Borders: Viral Emergence, Planning and Preparedness at the Animal-Human Interface

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A number of infections have emerged suddenly in recent years (2, 5). We can define emerging infections as those that are rapidly increasing in incidence or geographic range (2). Among the many notable examples in the last decade are Nipah, SARS, and H5N1 influenza in humans. In many cases, these are infections whose precursors exist in other animal reservoirs and that get new opportunities to come into contact with humans. Often, these new opportunities originate from agriculture or food processing practices, and may be further amplified in health care settings (2). These may be density dependent events. Once contact occurs, a virus may evolve (as SARS probably did) to increase its ability to infect humans (2, 5).

These occurrences suggest that the interface between humans and other animals is of great importance in the process of disease emergence. Better methods for identifying and preventing transfers across this interface are therefore essential. Recent modeling work suggests that early intervention is especially critical for viruses, such as influenza, that can rapidly evolve and have the potential for rapid transmission (1).

Although much has been learned, many fundamental questions remain. For example, what are the best strategies for reducing this transfer across the "species barrier" (which is often not a very high barrier)? Such suggestions as reducing close contact of different species, especially at high density (e.g., live animal markets), and observing precautions to reduce transmission from food animals to humans, appear to be good beginnings. Another fundamental question is the biological basis for transmissibility, and how it can be better predicted.

Public health response has traditionally been, and remains one of the keys to containing infectious disease outbreaks. Early warning is the essential first step (3). Greater availability of new communications technologies, including the Internet, have made it possible for early warning networks such as ProMED-mail (www.promedmail.org) and WHO's GOARN (Global Outbreak Alert and Response Network) to reach increasingly remote areas where an infection may emerge for the first time, and to link animal and human disease surveillance (although there is still much to be done here). However, warning must be followed by appropriate public health response. For all emergency public health functions, preparedness remains essential to appropriate response (4). There is also a need to develop appropriate triggers for response; history suggests that, even for influenza pandemics, there may sometimes be advance warning of the impending threat, if we know how to interpret these signals (6). Ironically, even while public health agencies, as a result of bioterrorism response funding, have become increasingly adept at rapid distribution of prophylactic measures, vaccine capacity has

been seriously limited in recent years. Limited vaccine capacity, and the long lead times required to develop new interventions, remain a serious concern.

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The emergence and variation of canine parvovirus as a new virus in dogs – a series of evolutionary steps allowed a feline virus to adapt to an alternative host receptor.

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Although most viruses have defined host ranges, occasionally a virus makes the jump into a new host by acquisition of a group of changes that allow it to infect and spread in that host. While such host switching can create major outbreaks of disease in humans or other animals, the details surrounding such events are still poorly understood. An example of host switching is the emergence of canine parvovirus (CPV) in dogs by mutation of a closely related virus of another carnivore, and this presentation summarizes the main points that we know about this host jump.

CPV was first recognized throughout the world during 1978 as a new virus infecting dogs. CPV is closely related (>99% identical in sequence) to the previously known feline panleukopenia virus (FPV), and it is clear that all of the canine-adapted viruses were derived from a single ancestor that emerged in the early 1970s. The phylogenetic branch between the feline viruses and the canine virus ancestor suggested that several changes were required to give the virus the initial ability to infect and spread among dogs. Since emerging in dogs CPV has undergone further evolution through processes of selection and genetic drift, and the descendent viruses are adapted to the new canine host, and have re-gained the ability to infect cats. They are also antigenically variant at a number of neutralizing epitopes in the viral capsid.

Two differences between FPV and CPV that control canine are found within the capsid, and when those were introduced into FPV together they allow that virus to infect canine cells. The structures of the CPV and FPV capsids show that those changes are on the surface of a raised region of the capsid, but that there are no direct structural connections between them. A third region of the capsid had a more subtle effect on canine host range and adaptation, and that was ~30Å distant from the other residues that control host range.

Those 3 viral changes act together to control the interaction of the CPV capsid with the transferrin receptor (TfR) on canine cells. This indicates that several changes in the capsid were required together to allow the altered canine host range of the virus. Analysis of the receptor also showed that residues in 3 positions of the apical domain of the TfR act together to influence viral binding. One of the differences in the canine TfR was the addition of a glycosylation site, and that difference was important in controlling the specificity of receptor binding. The post-transfer variation of CPV also changed its receptor binding abilities in subtle ways, and those changes appear to make them better adapted to their new host.

As well as changes in host ranges and TfR binding, the natural variation in the capsids also changes the antigenic structure with alterations of at least 2 antigenic determinants, indicating that these changes may be under antibody selection. Surprisingly, there

appears to be complete overlap between the receptor and antibody binding sites, so that many mutations affect the interaction of the capsids with both ligands. The significance of that overlap is not understood.

The principles that we can derive from the events surrounding the emergence of CPV is that changing the host range of the virus to become a successful pathogen of a new species required a primary set of coordinated changes that altered both the ability of the virus to interact with a new receptor, as well as secondary changes that likely allowed further adaptation of the virus to the new host environment. Those changes initially reduced the fitness of the virus for the ancestral host, although that host range was subsequently recovered through a number of additional mutations. The circumstances that allowed the acquisition of groups of substitutions in the virus are not yet understood, but they likely involved very rare multiple mutants arising under conditions where the virus also had a chance to enter and infect the new host. The post-transfer adaptation of the virus was apparently due to the acquisition of single changes in the virus genome, but those sometimes were selected only many years after the first emergence of the new host-adapted viruses.

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Molecular changes associated to the internal proteins of influenza virus during interspecies transmission.

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The influenza A viruses are the quintessential prototype of disease agents. The three major influenza pandemics in the 20th century together killed more people than any other natural or man-made disaster including World War I and II, distinguishing influenza as probably the deadliest acute infectious disease in human history. At the dawn of the 21st century, we continue to battle this virus to prevent the almost inevitable emergence of a new influenza pandemic strain.

In recent years, it has become evident that domestic poultry play an important role in the generation of novel influenza strains with the capacity to cross the species barrier and infect and kill humans (2, 4, 29). Wild aquatic birds such as ducks and shorebirds are the major reservoir from which all pandemic influenza strains are thought to derive. The process leading to the emergence of pandemic influenza strains remains poorly understood, although it is widely accepted that certain changes in the viral genome are required to enable the virus to cross to humans.

Potential pandemic strains arise as a consequence of the introduction of a virus with a new HA subtype into the human population, which would obviously be immunologically naïve to this antigen. The new HA gene results in altered viral antigenicity, and therefore, the process known as antigenic shift. The segmented genome of influenza viruses contributes to the diversity of these viruses in nature. When a single cell is infected with two influenza strains at the same time, there is an opportunity for the exchange of gene segments and the generation of a novel strain. This process of genomic exchange is known as reassortment, and the new virus strain constitutes a reassortant. Viruses with various combinations of HA and NA have been isolated in nature, suggesting that reassortment can occur freely and often although it may not be a ramdom event (17, 24, 25, 34). The Asian flu pandemic of 1957 and the Hong Kong flu pandemic of 1968 were caused by reassortants. Phylogenetic studies revealed that the 1957 pandemic H2N2 virus was derived from the circulating H1N1, which acquired both the H2, N2, and PB1 genes from the avian influenza pool. Similarly, the 1968 pandemic H3N2 virus inherited the H3 and PB1 genes from the avian influenza reservoir, while the rest of the genes came from the donor human H2N2 influenza virus (16).

Mutations in the internal genes of influenza viruses (PB2, PB1, PA, NP, M, and NS) have been observed to affect their host range, virulence and the viruses' ability to transmit among members of different animal species. Phylogenetic sequence analyses cluster the internal proteins into host-specific lineages (10, 11, 19). The correlation of these lineages with virulence and host range has been analyzed in vitro and in vivo. Early studies showed that reassortants carrying a combination of genes from human and avian influenza viruses displayed restricted growth in mammalian tissue culture cells, ferrets or

squirrel monkeys (6, 26, 28, 31). However, the internal genes of some avian influenza viruses can evolve into strains with the capacity to efficiently replicate in mammalian cells (22, 33). Thus, an appropriate constellation of genes is necessary to interact with the host's environment and result in productive infections. Certain combinations of internal genes may give rise to mutator mutants, strains with the capacity to change more rapidly and adapt faster to the new environment (27). It is not clearly understood whether the incorporation of the PB1 of avian origin in the 1957, and 1968 pandemic strains contributed to their rapid adaptation to humans. Similarly, it is not well understood whether the incorporation of internal genes of human, avian, and swine origins in the recent swine North American H3N2 isolates have contributed to their perpetuation in swine and their recent transmission to turkeys (14, 30, 32). The continuous reassortment of avian influenza viruses in Asia has perhaps contributed to the increased host range of H5N1 viruses (5). Molecular markers of adaptation have been inferred in several internal genes, although only one of those changes has been shown to consistently correspond to virulence in mammals and adaptation to humans: Amino acid position 627 in PB2 contains a glutamic acid in avian influenza viruses and a lysine in human influenza viruses (12, 15). Lysine is also found in some of H5N1 influenza viruses isolated from humans, suggesting a rapid adaptation of these viruses to the human host. It is also apparent that the impact of position 627 in PB2 might be influenced by other amino acid changes; perhaps within PB2 or other internal genes, as highly virulent H5N1 viruses have been isolated from humans that contain the typical avian glutamic acid in that position.

The interaction domains within the viral ribonucleoprotein polymerase complex (vRNP) have been partially defined (1, 3, 7-9, 13, 18, 20, 21, 23). Interactions domains for PA, PB2, cRNA, and vRNA have been identified in the PB1 subunit. Regions of interaction to PB1 have been partially elucidated on PA and PB2 and also the regions of interaction between PB2 to NP. No direct interaction is apparent between PA and PB2. Interactions between the vRNPs and other viral internal components are known to exist but are less defined. Similarly, interactions between components of the vRNPs and host factors have been identified; however their implications for host range and interspecies transmission need to be better defined.

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Biologic aspects of the interspecies transmission of coronaviruses and other enteric RNA viruses

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A number of respiratory and enteric RNA viruses have diverse host ranges, but the mechanisms related to altered host range or tissue tropisms are poorly understood. Here we review evidence for the interspecies transmission of coronaviruses, caliciviruses and rotaviruses and the biologic factors that may promote or enhance interspecies transmission followed by adaptation to the new host and serial transmission in the alternate host.

Besides initial virus binding to host cells, events at each stage of the virus replication cycle (enty, replication, release) may influence host susceptibility to virus infections. For coronaviruses (CoV), although originally thought to have restricted host ranges, two group II CoVs, bovine CoV and SARS CoV can infect diverse host species, including wildlife (3,6,7,8), with transmission of bovine CoV even to non-mammalian species (turkeys) (4). Reasons for this broad host range are unclear, but for bovine CoV, the presence of an influenza C-like hemagglutinin that binds sialic acids may play a role in the initial binding of the virus to diverse host cell types.

Following binding, viral entry and replication in host cells may also rely on or be restricted by host cell signaling pathways. An example is the unique requirement of a porcine sapovirus (enteric calicivirus) for host-specific intestinal contents for replication in vitro in pig kidney cells (5) and likely also in vivo in the target duodenunal epithelial cells. Recent studies have revealed that host-specific bile acids present in the pig intestinal contents can upregulate a PKA signaling pathway and down-regulate STAT1 (IFN pathway) suggesting that host restriction to a virus infection may be mediated by a cell signaling pathway and down-regulation of innate immunity (2).

Finally as exemplified by a dual rotavirus infection of a bovine host, release of a non-cytolytic heterologous rotavirus (pig-like group C rotavirus) and fecal shedding by the infected animal may be enhanced by dual infection with a cytolytic homologous group A bovine rotavirus (1). This dual infection which effectively mediated enhanced fecal shedding of the heterologous group C rotavirus could potentially promote interspecies viral transmission and more efficient host-to-host spread of the heterologous virus.

Upon infection of a new host species, various biologic factors may also influence the efficacy of intraspecies viral transmission and spread. The SARS outbreak was characterized by several major “superspreading” events by unknown mechanisms. Studies of animal CoV infections have highlighted factors that influence both the quantity and duration of virus shedding, thereby creating “superspreaders” and increasing the exposure dose and window for intraspecies virus transmission. These include high exposure doses, aerosols, respiratory co-infections (viruses, bacterial LPS) and treatments

with corticosteroids. Thus the host biology and ecology of the enteric and respiratory tracts, although less studied in the context of interspecies viral infections, may greatly influence host susceptibility to viruses and inter- and intra-species viral transmission.

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Heterologous Immunity and CD8 T cell crossreactivity during viral infections.

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A comprehensive understanding of the mechanisms associated with the generation and modulation of immunological T cell memory will lead to a better understanding of how the immune system controls viral infections but also causes immune-mediated pathology. Our studies with viruses in murine systems have focused on virus-specific memory T cell populations, which demonstrate plasticity in antigen recognition and in their ability to accommodate new memory T cell populations. Memory T cells laid down as a consequence of one infection can influence protective immunity and immunopathology associated with a second unrelated virus. We have referred to this phenomenon as T cell-dependent heterologous immunity and immunopathology. The focus of our work is to develop a better understanding of the mechanisms associated with the induction of heterologous immunity, specifically the role cross-reactive memory T cell responses and cytokines play in decreasing or augmenting viral replication and altering immunopathology. We have identified a matrix of cross-reactive epitopes between viruses, and developed both systemic and respiratory infection model systems. We use several virus systems, but focus on lymphocytic chorimeningitis (LCMV) and Pichinde (PV) viruses, distantly related arenaviruses whose T cell responses are well defined, and on the poxvirus vaccinia (VV), which is used as a vaccine for smallpox and as a recombinant vaccine and vector for many antigens.

Our studies in human viral infections on heterologous immunity and cross-reactive T cell responses during Epstein Barr virus (EBV) infection only begin to scratch the surface of the prevalence and potential impact of cross-reactive T cell responses on both vaccine development and immunopathology. There is very little understanding of the structural and functional interaction of one TCR with two different ligands. We have identified directly ex vivo and in bulk T cell cultures HLA-A2-restricted cross-reactive CD8 T cell responses that recognize both EBV BMLF-1 and influenza A M1 HLA-A2 restricted epitopes. These cross-reactive T cells were found to participate in acute infectious mononucleosis (IM). Five of 8 young adult HLA-A2+ patients experiencing IM had an increased number of influenza virus (FLU)-$M1_{58\text{-}66}$ specific CD8+ T cells in their peripheral blood as compared to healthy donors. Two of 5 IM patients with augmented FLU-M1 responses had high levels of tetramer-defined cross-reactive cells as measured directly ex vivo in their peripheral blood. EBV likely activates multiple populations of cross-reactive memory cells involved in the development of IM, and we have been able to provide evidence that those specific to FLU-M1 can contribute to this phenomenon. In order to better understand how cross-reactive CD8 T cells may be modulating disease outcome by enhancing viral clearance or inducing immunopathology, such as that seen in IM, we continue to characterize the cross-reactive TCR, both functionally and structurally, and examine how cross-reactivity influences the evolution of antigen-specific TCR repertoire development and disease outcome in both mice and humans during viral infections.

Emergence of H1N1 influenza and Host adaptation

Jeffery K. Taubenberger

Influenza A viruses are negative strand RNA viruses. They continually circulate in humans in yearly epidemics (mainly in the winter in temperate climates) and antigenically novel strains emerge sporadically as pandemic viruses. In the United States, influenza is estimated to kill 30,000 people in an average year. Occasionally, and unpredictably, influenza sweeps the world, infecting 20% to 40% of the population in a single year. In these pandemic years, the numbers of deaths can be dramatically above average. In 1957-1958, a pandemic was estimated to cause 66,000 excess deaths in the United States. In 1918, the worst pandemic in recorded history was associated with approximately 675,000 total deaths in the United States, and killed an estimated 50 million people worldwide.

Studying the extent to which the 1918 influenza was like other pandemics may help us to understand how pandemic influenzas emerge and cause disease in general. On the other hand, if we determine what made the 1918 influenza different from other pandemics, we may use the lessons of 1918 to predict the magnitude of public health risks a new pandemic virus might pose.

The predominant natural reservoir of influenza viruses is thought to be wild waterfowl. Periodically, genetic material from avian strains is transferred to strains infectious to humans by a process called reassortment. Human influenza strains with recently acquired avian surface and internal protein-encoding RNA segments were responsible for the pandemic influenza outbreaks in 1957 and 1968. The change in the hemagglutinin subtype or the hemagglutinin and the neuraminidase subtype is referred to as antigenic shift. Since pigs can be infected with both avian and human strains, and various reassortants have been isolated from pigs, they have been proposed as an intermediary in this process. Until recently there was only limited evidence that a wholly avian influenza virus could directly infect humans, but in 1997 eighteen people were infected with avian H5N1 influenza viruses in Hong Kong and six died of complications after infection. Although these viruses were very poorly or non-transmissible, their isolation from infected patients indicates that humans can be infected with wholly avian influenza strains. In 2003-2005, H5N1 outbreaks in poultry have become widespread in Asia, and at least 50 people have died of complications of infection predominantly in Vietnam and Thailand. In 2003, a highly pathogenic H7N7 outbreak occurred in poultry farms in the Netherlands. This virus caused infections (predominantly conjunctivitis) in 86 poultry handlers and in 3 secondary contacts. One of the infected individuals died of pneumonia. In 2004 an H7N3 influenza outbreak in poultry in Canada also resulted in the infection of a single individual. Therefore, it may not be necessary to invoke swine as the intermediary in the formation of a pandemic strain since reassortment between an avian and a human influenza virus could take place directly in humans.

While reassortment involving genes encoding surface proteins appears to be a critical event for the production of a pandemic virus, a significant amount of data exists to suggest that influenza viruses must also acquire specific adaptations to spread and replicate efficiently in a new host. Among other features, there must be functional HA receptor binding and interaction between viral and host proteins. Defining the minimal

adaptive changes needed to allow a reassortant virus to function in humans is essential to understanding how pandemic viruses emerge.

Once a new strain has acquired the changes that allow it to spread in humans, virulence is affected by the presence of novel surface protein(s) which allow the virus to infect an immunologically naïve population. This was the case in 1957 and 1968 and was almost certainly the case in 1918. While immunological novelty may explain much of the virulence of the 1918 influenza, it is likely that additional genetic features contributed to its exceptional lethality. Unfortunately not enough is known about how genetic features of influenza viruses affect virulence. The degree of illness caused by a particular strain, or virulence, is complex and involves host factors like immune status, and viral factors like host adaptation, transmissibility, tissue tropism, or viral replication efficiency. The genetic basis for each of these features is not yet fully characterized, but is most likely polygenic in nature.

The 1957 pandemic resulted from the emergence of a reassortant influenza virus in which both HA and NA had been replaced by gene segment closely related to those in avian strains. The 1968 pandemic followed with the emergence of a strain in which the H2 subtype HA gene was exchanged with an avian-derived H3 HA RNA segment, while retaining the N2 gene derived in 1957. More recently it has been shown that the PB1 gene was replaced in both the 1957 and the 1968 pandemic strains, also with a likely avian derivation in both cases. The remaining five RNA segments encoding the PA, PB2, nucleoprotein, matrix and non-structural proteins, all were preserved from the H1N1 strains circulating before 1957. These segments were likely the direct descendants of the genes present in the 1918 virus. Sequence analysis of the 1918 influenza virus allows us potentially to address the genetic basis of virulence and human adaptation. Sequence and phylogenetic analysis of the completed 1918 influenza virus genes shows them to be the most avian-like among the mammalian-adapted viruses. This finding supports the hypothesis that (1) the pandemic virus contained genes derived from avian-like influenza virus strain and that (2) the 1918 virus is the common ancestor of human and classical swine H1N1 influenza viruses.

VIRUSES AND VIRAL DISEASES

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1 The nature of viruses

Viruses are molecular genetic parasites that use cellular systems for their own replication (12). Although viruses have a genome and are able to adapt to particular hosts and biotic habitats, they are not living microorganisms. The simplest biological system that can be said to be alive is a cell. Macromolecules and organelles found in cells are not themselves alive. Viruses lack essential attributes of living systems such as the ability to capture and store free energy and lack the resulting characteristic autonomy that arises from the presence of a set of integrated metabolic activities.

During its replication cycle in a host cell, a virus takes on various forms. The virion stage can be fully described by intrinsic biochemical and structural properties of the particle (mass, size, chemical composition etc). Virions are not the same as viruses since only the latter possess various relational or emergent properties that are actualized only during transmission, infection and replication. These relational properties exist only by virtue of a relation with other entities (host, vector, environment) and they are present only in the system as a whole. Confusing « virus » with « virion » is akin to confusing the entity « insect », which comprises several different life stages, with a single one of these stages such as a pupa, caterpillar or butterfly (10).
Acute viral infection associated with active replication and production of virions (presence of disease) is very different from the persistent, latent and asymptomatic type of specific virus- host relationship (absence of disease) . In the latter case the virus genome is maintained in the host but no virions are produced for long periods of time and no antiviral host immune response is elicited. Small amounts of virions are produced episodically which is sufficient for transmission of the virus to new hosts. Host switching involves changing over from an absence of disease in the latent host to the reappearance of disease by reactivation in a different type of host. This is the event that sometimes gives rise to an emergent viral disease(13).

2 Fitness

Fitness is the property of an organism that ensures its survival and reproductive capacity in an environment that is unstable and unpredictable. Since viruses are not alive, the fitness of a virus is usually measured in terms of virus progeny, i.e. number of virions produced during an acute virus infection. In the case of a latent, persistent viral infection, fitness has no equivalent quantitative definition. Whereas for acute virus infections, fitness is measured at the expense of the host (infected cells die) in the case of latent infections, fitness of the virus merges with fitness of the host and the concept loses its usefulness for describing a differential ability of the molecular parasite.
Natural selection has been defined as the process whereby the organisms of a given variety outnumber those of other varieties, due to their greater fertility and adaptedness, and thus prevail in the long run (4). The selection in fact amounts only to differential survival and since « fitness » is defined as anything that promotes the chance of survival, survival of the fittest and natural selection amount to no more than survival of the survivors. In this sense, fitness is an abstraction and does not exist. It has been called a phantom construct of the human mind (7) and it certainly lacks causal efficacy.

Virus evolution is a long sequence of singularities and unique unpredictable events and it seems illusory to try to « explain » it by an underlying set of natural selection principles that would replace the earlier pre-Darwinian reliance on design as causal explanation (7).

3 Molecular diversity and pairwise sequence analysis of viruses

Virus species and genera can be demarcated by pairwise sequence analysis of viral genomes and proteins (2,5,9,11) . PASC distributions display multiple peaks that correspond to different levels of molecular diversity (strains, species and genera) within virus families. For instance, pairwise sequence identity scores of around 80% often correspond to separate species and this agrees with distinctions based on biological criteria (10,11). However, the basis for the appearance of peaks at specific percentage values in pairwise comparisons is not at all understood, which underlines our ignorance of patterns of virus adaptation and evolution. It is remarkable that sequence identity scores for viruses belonging to different genera in the same family are around 25%, ie the same as random, in spite of similar overall 3D structure and biological properties.

4 Control and prevention of emerging virus diseases

a) Develop international surveillance and genome sequencing of newly emerging viruses to track down genetic reassortments and viral adaptations. In the first six months of 2005, a total of 140 sequences of H3N2 influenza isolates, 100 sequences of H5N1 and 30 sequences of H9N2 isolates have been deposited in GenBank (6)

b) The greatest need is to revitalize the ailing vaccine industry. It is essential to turn the manufacture of vaccines again into an attractive business. This requires financial incentives, i.e. sensible and fair prices as well as the guaranteed purchase of vaccine supplies needed to combat possible future epidemics or pandemics (1,6). Combatting viruses evokes metaphors of battles and wars and it is appropriate to point out the similarities between the vaccine and armament industries. Both are required to protect the nation against future attack and both represent forms of social protection and insurance that justify considerable government spending. It has been counterproductive for the state in the past to endanger the profitability of the vaccine industry by refusing to provide liability protection for the rare adverse effects of vaccines not imputable to faulty manufacture. It would equally make little sense to make the armament industry liable to claims arising from injury derived from the use of weapons.

c) Collaboration between government agencies, academic institutions and private industry must be stepped up as well as research on live attenuated influenza vaccines, on cell culture methods to replace the use of embryonated eggs, on new adjuvants, on nasal immunization etc

d) Modify regulatory processes for vaccine licensure to cope with the threat posed by pandemics. Acceptable risk-benefit ratios are not the same in different epidemiological environments. Recent examples of this situation are the polio and Rotashield vaccines.

Polio: Paralysis cases (1 in 10^6 doses) are linked to excretion of reverted mutants in live attenuated vaccines. Fortunately this risk was not appreciated at the time of licensure and the subsequent use of OPV allowed nearly complete eradication of polio in developing countries. IPV is now replacing OPV in some countries. Why polio type 2 was fully eradicated in 1999 using OPV and not types 1 and 3 is not understood.

Rotavirus: The Rotashield vaccine (Wyeth) licensed in 1998 was withdrawn in 1999 following 15 cases of intussusception (bowel obstruction) in the US. This amounts approximately to one case in 10 000 children vaccinated and was felt to be unacceptable in a country where only 20 - 40 children die each year from complications of rotavirus infection. There are, however, between 1300 - 2000 cases of intussusception in the US every year (8).

Subsequent reappraisal of the Rotashield vaccine by NIH found no overall excess risk of intussusception when infants were given the first dose of vaccine at younger than 60 days of age

(3,8). At present new rotavirus vaccines (Rotarix, GSK and Rotateq, Merck) are tested in developing countries which total over 600,000 deaths a year from rotavirus infection. If Rotashield trials had taken place in developing countries instead of in the US, the vaccine would not have been withdrawn since the benefits of reduced death rates would have been found to outweigh by far the extremely small risk of intussusception. Many thousands of lives would then have been saved in developing countries.
The assessment of risk-benefits ratios for vaccines directed at emerging pandemics may have to be based on the type of situation that currently prevails for many infectious diseases in developing countries.

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Dr. Luis Villarreal

Previously, I have proposed that two distinct life strategies can defined to exist for most virus families. These life strategies were called acute and persistent infections. They differ from each other in several fundamental characteristics, especially with respect to virus and host evolution. Persistent viruses are highly host and tissue specific, phylogenetically congruent with their host and stable in an evolutionary timeframe. These two life strategies must also differ in their basic fitness definitions. The majority of emerging viral diseases involve the transition of a host specific persistent infection to an acute life strategy in a new host. Historically this stable persistent source of emergent disease has been called a reservoir host. In this presentation, I will argue that this term is misleading and has obstructed understanding as it infers a similar relationship between virus and host evolution for the two life strategies. Because of this, we seldom study the molecular determinants of persistence thus misunderstand the changes associated with emergence. Specific examples of this situation will be presented.

Viral emergence and zoonoses

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A total of 1407 different species of human pathogen are currently recognised. Of these, 206 are viruses, mostly (83%) RNA viruses. Viruses are greatly over-represented among the emerging or re-emerging pathogens, with 77 species regarded as belonging in these categories. There are, however, no marked differences in the probability of being emerging/re-emerging between the major virus families or according to genome type (e.g. RNA viruses vs DNA viruses).

There is a weak tendency for viruses which are known to be zoonotic to be emerging/re-emerging (relative risk, RR = 1.2). The non-human reservoirs for emerging/re-emerging viruses are varied, ungulates and rodents being numerically the most important categories: reflecting reservoirs for zoonotic viruses generally. However, a (statistically significant) larger fraction of emerging/re-emerging viruses are associated with a broader host range. The determinants of virus host range are incompletely understood, but an important factor appears to be cell receptor usage: viruses using phylogenetically conserved host receptors are more likely to have a broad host range.

Emerging/re-emerging viruses exploit a wide range of transmission routes, which largely reflect transmission routes for viruses in general. The numerically most important route is vector-borne, especially transmission by dipteran vectors (viruses transmitted by acarid vectors are under-represented in the emerging/re-emerging category). Sexually-transmitted viruses, although much rarer overall, appear over-represented in the emerging/re-emerging category.

The drivers of virus emergence/re-emergence are many and varied, but the dominant drivers are related to changes in agricultural and land-use. Factors such as changes to human demography and society (e.g. urbanization) and poor population health are also implicated.

Most emerging and re-emerging pathogens are not completely new. In total 38 new species of human pathogen associated with emerging disease problems (as opposed to newly recognised agents of established diseases) have been reported since 1980. Viruses are massively over-represented among this category, especially RNA viruses (which account for two-thirds of the total, equating to an average of 1 per yr, noting that this excludes sub-specific variants, e.g. H5N1 influenza A).

Many (though not all) novel viruses are believed to have zoonotic origins, implying that they have recently (i.e. in ecological rather than evolutionary time) made a host species jump (e.g. HIV1 from non-human primates). Several requirements must be fulfilled for a pathogen to jump successfully between host species, including the ability of the pathogen to adapt to the new host (here, ‘adapt’ refers specifically to becoming sufficiently transmissible to cause sustained outbreaks). It has been proposed that RNA viruses are

more able to jump between hosts because their genomes are more labile and so adaptation can occur more rapidly.

So far, this discussion has addressed the question of whether or not a virus is associated with an emerging or re-emerging disease problem and not the magnitude of that problem. In practice, the majority of viruses which enter the human population from a non-human source are not highly transmissible between humans (whether directly or via a vector). A small minority are sufficiently transmissible to cause major outbreaks (e.g. SARS coronavirus). The remainder (perhaps ~ 20%) are transmissible between humans but, so far, have not 'taken off' in the human population or, more formally, their basic reproduction number is not greater than 1 (e.g. Ebola). It is clearly important to understand the factors that affect the transmissibility of this group since small increases in the basic reproduction number could lead to much larger increases in the incidence of infection. One relevant factor is transmission route: for example, arboviruses are relatively unlikely (RR = 0.23) to be transmissible (via the vector) between humans, i.e. most or all infections are acquired (via the vector) from a non-human reservoir.

Finally, there is the question of whether or not viral emergence is in any sense predictable and, if so, whether or not the public health consequences are predictable. On the one hand, perhaps the most striking feature of emerging and re-emerging viruses (and other pathogens) is their diversity: taxonomic diversity; diversity in breadth of host range and type of reservoir hosts; diversity of transmission routes; diversity of factors driving an increase in incidence; and so on. On the other hand, it is encouraging that even a fairly cursory analysis reveals some characteristics that appear to be associated with aspects of emergence or re-emergence.

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Biosketch

Dr Wendy Barclay graduated in Natural Sciences from Cambridge University, UK in 1985. She studied for her PhD under the joint supervision of Dr David Tyrrell FRS and Dr Fred Brown FRS based mainly at the Common Cold Unit, Harvard Hospital, Salisbury but also spending time at the Wellcome laboratories. The subject of the thesis was The Immune response to Rhinovirus. Dr Barclay learned molecular virology in the laboratory of Professor Jeff Almond at Reading University during a postdoctoral fellowship from 1988 to 1991, during which time she developed replicons for the study of poliovirus replication and packaging.

In 1992 Dr Barclay was recruited by Dr Peter Palese and travelled to Mount Sinai Medical Center, New York where she acquired the technique of reverse genetics for influenza viruses. In 1995 she returned to Reading University, UK to become a group leader and assume a junior lectureship. Since then Dr Barclay has collaborated widely with the UK influenza community and has maintained a particular interest in the molecular and genetic determinants that restrict the host range of avian influenza. She has served on the Virus Group committee of the Society for General Microbiology, organized influenza workshops, and spoken widely, both to scientists and to the public, about influenza viruses. She is currently Reader in Virology at the School of Biological Sciences, Reading University.

The current focus areas of the Barclay group are The role of interferons in host range and pathogenesis of influenza The role of the newly discovered PB1-F2 influenza gene in avian influenza pathogenesis and emergence of pandemic strains The sequence changes in the H5 HA protein that might enhance entry into human cells and transmission amongst people The evolution of the H3 subtype in humans.

Dr. Ralph Baric received his BS degree from North Carolina State University in 1977. He obtained his PhD from the Department of Microbiology at North Carolina State University in 1982, studying alphavirus-host interaction and pathogenesis under the direction of Dr. Robert E. Johnston. Postdoctoral work focused on coronavirus replication and pathogenesis under the direction of Dr. Michael M.C. Lai at the University of Southern California. In 1986, Dr. Baric was hired as an assistant professor in the Department of Parasitology and Laboratory Practice and is currently a professor in the Departments of Epidemiology, and Microbiology and Immunology at the University of North Carolina at Chapel Hill. During his early training, Dr. Baric was a Harvey Weaver Scholar for the National Multiple Sclerosis Society and an Established Investigator for the American Heart Association, awards associated with his studies focusing on coronavirus replication, cross species transmission, persistence, evolution and pathogenesis. He is currently a member of the editorial board of Journal of Virology, has served as a reviewer in many NIH study sections, has been a consultant for WHO, CDC and NIH, and has served on various institutional recombinant DNA review committees. Dr. Baric has published over 76 peer-reviewed manuscripts including some in high profile journals and his research efforts are supported by several research grants from the National Institutes of Health.
Dr. Baric studies coronavirus and norovirus (human calicivirus) replication, pathogenesis, virus-receptor interactions and vaccine development. Noroviruses are important causes

of severe epidemic gastroenteritis in infants, children and adults, worldwide and coronaviruses are emerging pathogens that cause severe lower respiratory tract disease in humans. Both are positive strand RNA viruses that cause significant human morbidity and mortality. Dr. Barics' laboratory has established reverse genetic systems for several coronaviruses including the highly pathogenic severe acute respiratory syndrome coronavirus (SARS-CoV) and is using these reagents to study the genetics of host shifting and pathogenesis. Using noroviruses as models, Dr. Baric has also demonstrated that ABH histo-blood group antigens likely function as receptors for Norwalk virus docking and entry. More importantly, his group has demonstrated that human polymorphic genes, like the fucosyltransferase gene (Fut 2) and other A, B and O blood group genes, that regulate ABH expression function as susceptibility alleles for Norwalk virus infection.

Dr. Donald S. Burke is Professor of International Health and Epidemiology at the Johns Hopkins Bloomberg School of Public Health, where he is also Associate Chair for the Disease Prevention and Control Program of the Department of International Health and Director of the Center for Immunization Research. He was born in 1946 in Cleveland, Ohio, received his B.A. from Western Reserve University in 1967, and his M.D. from Harvard Medical School in 1971. His post-graduate medical training was in Internal Medicine at the Boston City Hospital and the Massachusetts General Hospital, and in Infectious Diseases at the Walter Reed Army Medical Center. Professor Burke served on active duty in the US Army Medical Research and Development Command for 23 years. He held assignments at the US Army Medical Research Institute of Infectious Diseases in Frederick Maryland (1973-75) at the Armed Forces Research Institute of Infectious Diseases in Bangkok, Thailand (1978-84) and at the Walter Reed Army Institute of Research in Washington DC (1976-78 and 1984-97). Throughout his career he developed new methods to prevent and control viral infections of global importance, focusing on diseases of military importance and tropical infections such as dengue, Japanese encephalitis, and hepatitis, HIV/AIDS, and influenza. He has conducted basic laboratory research, epidemiological research, clinical vaccine studies, and field vaccine trials. In 1997 he retired from the Army and accepted an academic appointment at Johns Hopkins University, where he teaches graduate courses on infectious diseases, epidemiology, and vaccines. His current research focuses on the development of new strategies to predict and prevent epidemics, and on theoretical and computational approaches to infectious disease epidemiology. Professor Burke has served on numerous advisory boards and councils for the World Health Organization, Institute of Medicine, National Institutes of Health, Centers for Disease Control, Department of Defense, and other national and international agencies. He has published over 200 articles and chapters in medical and scientific journals. He is a Fellow of the American Association for the Advancement of Science, a Fellow of the American College of Physicians, a Fellow of the Infectious Diseases Society of America, and a past-President of the American Society of Tropical Medicine and Hygiene. He lives in Washington DC with his wife Jane. They have two grown daughters.

Dr. Charles Calisher received a B.S. degree (Bacteriology) in 1958 from the Philadelphia College of Pharmacy and Science, an M.S. degree in 1961 from the University of Notre Dame, and a Ph.D. degree from Georgetown University in 1964. From 1961 to 1965 he was employed by Microbiological Associates, Bethesda, Maryland, and then moved to the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta. In 1973 he was transferred to the CDC laboratory in Fort Collins where he became the Director of the W.H.O. Centre for Arbovirus Reference and Research. In 1992 Dr. Calisher retired from federal service and joined Colorado State University, where he has served as Professor in the Department of Microbiology, Immunology and Pathology in the College of Veterinary Medicine and Biomedical Sciences. Author of nearly 350 publications, Dr. Calisher has spent the past 11 years conducting longitudinal studies of hantaviruses in the rodent hosts in Colorado. Dr. Calisher has spent the past 40 years studying the epidemiology, evolution, and interrelationships among arboviruses and rodent-borne viruses and the mechanisms by which these viruses are amplified and cause human, livestock and wildlife diseases.

Dr. Peter Daszak received a BSc honors (Zoology) in 1986 and a Ph.D. in parasitology in 1994 from the University of East London, UK. He conducted postdoc research on the ecology of wildlife diseases. After moving to the US in 1998, he worked first at the CDC under Sherif Zaki (Pathology Activity) during the Nipah virus outbreak, then at the University of Georgia on wildlife disease ecology. In 2001 he became the Executive Director of the Consortium for Conservation Medicine, a formal partnership between Johns Hopkins School of Public Health, Tufts University College of Vet. Med, Harvard Med School, the USGS National Wildlife Health Center and Wildlife Trust. He directs research programs studying the emergence of Nipah and Hendra virus, the ecology of West Nile virus in the USA, the conservation impact of wildlife diseases and the environmental and ecological factors that drive disease emergence.

Dr. Esteban Domingo received a BsC. in Chemistry (1965) and a Ph. D. in Biochemistry (1969) both from the University of Barcelona (Spain). He did postdoctoral work in Molecular Biology and Virology at Univ. California Irvine (with R.C. Warner, 1969-1973) and at the Univ. of Zürich, Switzerland (with C. Weissmann, 1974-1977). He was visiting professor at Univ. California San Diego (1988-1989, 1996), His main interests are viral quasispecies and new antiviral strategies, topics on which he has published 200 articles. Currently he is Professor of Research at the Spanish Research Council (CSIC) at Centro de Biología Molecular “Severo Ochoa”.

Dr. Ruben Donis obtained his degree in veterinary medicine in 1978 from the National University of Buenos Aires, in Argentina, and his PhD in Virology at Cornell University in 1987. He received postdoctoral training in molecular virology at St. Jude Children’s Research Hospital before joining the faculty at the University of Nebraska-Lincoln in 1989. At the U. of Nebraska, he studied viral replication and host interactions in pestiviruses and influenza viruses and was promoted to Professor in 2001. Since 2003 he is Chief of the Section of Molecular Genetics int the Influenza Branch at the Centers for Disease Control and Prevention in Atlanta, GA, and Adjunct Professor of Microbiology and Immunology at Emory University, also in Atlanta. His current research interests

include molecular mechanisms of viral replication, and their role in the pathogenesis, interspecies transmission, and evolution of influenza viruses.

Dr. Michael Farzan received his A.B degree (Government) in 1984 from Harvard College, and his Ph.D. from Harvard Medical School (Immunology) in 1997. Dr. Farzan spent the years between college and graduate school as a computer programmer for two small computer graphics companies. His Ph.D. work focused on the role and biochemistry of HIV-1 and SIV coreceptors, and he continued these studies during his post-doctoral fellowship. Dr. Farzan was appointed Instructor of Pathology at Harvard Medical School in 1999, and Assistant Professor of Medicine in 2002. Dr. Farzan's discoveries include the presence of and critical role for tyrosine sulfation on the HIV-1 and SIV coreceptor CCR5, and on a class of human antibodies that mimic CCR5. His laboratory has also identified ACE2 as the receptor for the SARS coronavirus. His laboratory has moved to the New England Primate Research Center in 2005, and is currently an Assistant Professor of Microbiology and Molecular Genetics at Harvard Medical School. Current research interests include the receptors and entry processes of several diverse viruses, and the use of mass spectrometry as a tool for describing adaptive immune responses to HIV-1 and SIV.

Dr Neil Ferguson received the B.A. degree (Physics) in 1990 and the D.Phil. in 1994, both from the University of Oxford. He held a Wellcome Trust Biomathematics Train Fellowship and then a Royal Society University Research Fellowship at the Dept. of Zoology, University of Oxford. He was appointed to a readership at the University of Nottingham in 2000 before taking up a Chair in Mathematical Biology at the Dept. of Infectious Disease Epidemiology at Imperial College London in 2001. Dr Ferguson uses mathematical and statistical models to investigate the processes shaping infectious disease pathogenesis, evolution and transmission. He advises governments and agencies on disease control policies in public health, clinical and veterinary contexts. As well as basic theoretical work on evolutionary and epidemiological dynamics, Dr Ferguson has advised both governments and international agencies on planning for and controlling outbreaks of a range of pathogens. These include pandemic influenza, SARS, foot-and-mouth disease, BSE/vCJD, and potential bioterrorist agents. Recent work has focused on developing mathematic models to examine containment and mitigation strategies for pandemic influenza. He has also been extending earlier work on exploring how host-driven selection and pathogen transmission dynamics shape the evolutionary dynamics of influenza and other pathogens.

Dr. Kathryn Holmes received the A.B. degree (Biology) from Radcliffe College in 1962, and the Ph.D. degree (Virology/Cell Biology) from the Rockefeller University in 1968. Following postdoctoral research at the Harvard Biological Laboratories, she held faculty positions in the Depts. of Microbiology and Immunology of Georgetown University Schools of Medicine and Dentistry and the University of Texas Southwestern Medical School, and in the Dept. of Pathology at the Uniformed Services University of the Health Sciences School of Medicine. Since 1995 she has been Professor of Microbiology at the University of Colorado Health Sciences Center. Dr. Holmes has studied coronaviruses of many animal species, with particular emphasis on the specificity

of the interactions of coronavirus spike glycoproteins with their host cell receptors and the role of spike-receptor interactions in virus host range, tissue tropism and virulence.

Dr. Zhihong Hu received the B.S. degree (Virology and Molecular Biology) in 1986 from Wuhan University, China. She obtained the M.S. degree (Virology) in 1989 from Wuhan Institute of Virology, Chinese Academy of Sciences (CAS) and afterwards became a staff of the institute. In 1993, with a Marie Curie fellowship she went to Wageningen Agricultural University, the Netherlands, for virology research and later obtained a sandwich PhD fellowship from the university. She obtained the PhD. degree from Wageningen Agricultural University in 1998. From 1997 she is Professor at Wuhan Institute of Virology (CAS) and from 2000 she is the Director of the Institute. Her researches mainly focus on molecular biology of baculovirus. After the SARS outbreak in 2003, she becomes interested in the animal origin of SARS-CoV and is doing related research in masked palm civets.

Dr. James Koopman received both his B.S. degree M.D. degree in 1969 from the University of Michigan. He completed a pediatrics residency at Harbor General Hospital UCLA in 1972 and then spent two years as an EIS officer in the state of Washington where he was acting state epidemiologist at the end of his tenure. He worked in service epidemiology in Colombia South America, until 1978 when he took a position as assistant professor at the University of Michigan. At the University he focused on practical infectious disease epidemiology and surveillance, especially for enteric infections but including influenza. From 1984-86 while on leave from the University he initiated a field epidemiology program in Mexico which has continued to be highly successful. In 1986 he turned to developing basic theory for a science of infection transmission system analysis. Initially he focused on HIV and demonstrated the potential ongoing importance of the early stage of infection in sustaining endemic and generating epidemic levels of infection. He then turned to developing new theory and methods for vaccine evaluation with a focus on childhood bacterial infections such as non-typeable *Haemophilus influenzae*. He also developed new methods for addressing microbial risk assessment for enteric pathogens, new theory about how pathogenic processes generate patterns of joint effects between risk factors, and new theory about how immune response generates selective pressure driving the evolution of pathogens. His current work focuses on developing better methodologies to advance infection transmission system analysis. In particular he is developing approaches to advance both theory and infection control decisions through a process of inference robustness assessment that entails relaxing model assumptions by switching model forms in a way that provides information as to whether disease control or theory choice inferences might be changed by relaxing specified model assumptions.

Dr. Scott P. Layne received the B.A. degree (Chemistry) in 1976 from DePauw University and the M.D. degree in 1980 from Case Western Reserve University. He is Board Certified in Internal Medicine (1997) and Infectious Diseases (1998), with a fellowship in adult infectious diseases. From 1982-1986 he served as Postdoctral Fellow and Staff Member at the Los Alamos National Laboratory and from 1986-1992 as a Physicist at the Lawrence Livermore National Laboratory. After serving residency at the UCLA School of Medicine from 1992-1994, he joined the UCLA School of Public Health as an Associate Professor of Epidemiology. Dr. Layne is known for cross disciplinary work involving biology, physics, and policy related issues. He has authored over 45 publications, including three U.S. patents on methods to access and operate high-throughput laboratories. He is an editor of *Firepower in the Lab: Automation in the Fight Against Infectious Diseases and Bioterrorism* published by Joseph Henry Press in 2001 and also of *Jane's Chem-Bio Handbook, second edition.* In 1988, Dr. Layne organized the workshop *A National Effort to Model AIDS Epidemiology* for the Office of Science and Technology Policy (OSTP) and oversaw the publication of a White House report that helped to influence AIDS research priorities in the United States. In 1999, he also organized the meeting *Automation in Threat Reduction and Infectious Disease Research: Needs and New Direction* under the auspices of the Institute of Medicine and National Academy of Engineering. Dr. Layne teaches graduate level courses at UCLA on infectious diseases, mathematical modeling, and public health responses to bioterrorism. He is also an instructor on bioterrorism preparation and response for the U.S. Department of Homeland Security and lectures throughout America in this capacity.

Dr. Grant McFadden received the B.Sc. degree (Honours Biochemistry) in 1970 and the Ph.D degree (Biochemistry) in 1975, both from McGill University in Montreal, Canada. He has been a Canada Research Chair (Tier I) since 2001 and a recipient of a Howard Hughes Medical Institute International Scholarship (2005-2010). He became a Fellow of the Royal Society of Canada in 2004. He is a Professor in the Department of Microbiology and Immunology at the University of Western Ontario and a co-director of the BioTherapeutics Research Group at Robarts Research Institute where he has maintained a research laboratory since 1997. Dr. McFadden is recognized as a world leader in the field of virology. Although McFadden's interests have varied over the years and range from examining virus replication, DNA repair/recombination, and viral pathogenicity in poxviruses, his lab now studies how viruses that cause immunosuppression in infected animals interact with the immune system. It is becoming increasingly clear that viruses, which make their living within cells of higher-order vertebrates, must have evolved to specifically accommodate the workings of the host immune system. The McFadden lab pioneered the field of viral immune subversion, and he is credited with the discovery of viroceptors and their immunomodulatory properties. His lab has made many fundamental discoveries over the past 20 years and can claim success through the identification of diverse viral inhibitors of mammalian cytokines, identifying and outlining the anti-apoptotic pathways controlled by poxviruses within infected mammalian cells, and demonstrating that poxvirus tropism is intimately linked with the interferon response pathway of the host. Most recently McFadden has co-founded Viron Therapeutics, Inc. (with Dr. A. Lucas) in London to explore the use of viral proteins for therapeutic purposes.

Dr. X.J. Meng received the M.D. degree in 1985 from Binzhou Medical College (Binzhou, China) and the Ph.D. degree (Virology) in 1995 from Iowa State University (Ames, Iowa). He then joined the Laboratory of Infectious Diseases at the National Institute of Allergy and Infectious Diseases, NIH (Bethesda, Maryland) as a John E. Fogarty visiting scientist and later as a senior staff scientist where he studied the hepatitis E virus. In 1999, he joined the faculty at the College of Veterinary Medicine, Virginia Polytechnic Institute and State University (Blacksburg, Virginia). Over the years Dr. Meng has been studying emerging and re-emerging viral diseases of veterinary and human public health importance and zoonotic viral diseases including arterivirus, circovirus, coronavirus and hepatitis E virus, and has published extensively (over 120 peer-reviewed papers and book chapters) in the field. His most recent accomplishments include the discoveries of swine hepatitis E virus from pigs and avian hepatitis E virus from chickens, and the demonstration of their abilities to cause cross-species infections. Currently Dr. Meng is an Associate Professor of molecular virology at Virginia Polytechnic Institute and State University.

Dr. Ab Osterhaus is professor of Virology and Head of Department at Erasmus MC in Rotterdam since 1992 and professor of environmental virology at the University of Utrecht since 1990. He is the director of three WHO affiliated reference laboratories and co-founder of three commercial companies owned by Erasmus MC Holding.

After finishing his veterinary studies and his PhD in 1974 and 1978 respectively at the University of Utrecht, he fulfilled several positions at the National Institute of Health and the Environment in Bilthoven until 1992, the last of which was head of the laboratory of Immunobiology. He received several international awards, served as member and chairman on numerous international scientific committees. He is the director of three WHO reference centres, including the Dutch Influenza Centre. He is a member of The Dutch Health Council and of the Royal Dutch Academy of Sciences and is the chairman of the European Scientific Working group on Influenza (ESWI). In 2003 he was knighted to “Commander in the Order of The Dutch Lion”.

His research activities are mainly in the field of human and animal virus-host interactions, with a strong emphasis on antiviral immunity and the development of antiviral intervention strategies. With his group he discovered more than a dozen new viruses of humans and animals. He was the mentor of more than 35 PhD students, holds several key patents in his research area and published more than 600 peer reviewed scientific papers.

Dr. Mark A. Pallansch received the B.S. degree (Biochemistry) in 1976 from Virginia Tech and the Ph.D. degree (Biochemistry) in 1982 from the University of Wisconsin-Madison. He also received post-doctoral training in virology at Rockefeller University from 1982 to 1984. After joining the CDC staff in 1984, he has served as Chief, Enterovirus Section/Team and is currently a Distinguished Consultant in the Respiratory and Enteric Viruses Branch/DVRD/NCID. He is responsible for multiple areas of research and testing with poliovirus and the non-polio enteroviruses. Research areas

include studies of natural variation and recombination, molecular epidemiology, and association of enterovirus infection with neonatal infections and "chronic" diseases such as juvenile-onset diabetes and myocarditis. Dr. Pallansch is also responsible for enterovirus diagnostics, which includes laboratory support for epidemiologic studies, characterization of enterovirus isolates, characterization of new picornaviruses, identification and strain characterization of poliovirus isolates, and development of improved diagnostic techniques and reagents. He is directly involved in supporting design, technology and implementation of the poliovirus laboratory network as part of the global poliovirus eradication initiative. More recently, he has provided technical expertise and conceptual evaluation of issues for strategic planning within the eradication program, particularly related to surveillance and vaccination strategies for the post-eradication period.

Dr. Colin Parrish received a BSc (Hons) degree in Microbiology and Biochemistry in 1978 from Massey University in New Zealand. He then completed a Ph.D. in Virology in 1984 from Cornell University, studying the basis of host range control of parvoviruses, and continued at Cornell for two years of further post-doctoral studies. He then undertook further post-doctoral studies at Monash University in Melbourne Australia between 1986 and 1988, examining dengue and other flaviviruses. He joined the faculty of Cornell University in 1988, and has since been promoted to the rank of Professor. His major interests have been in defining the processes of viral infection and the control of host range, using a variety of approaches to reveal the natural history and molecular basis of the issues involved. He has been member of various NIH and other review panels and is currently the Councilor for Veterinary Virology with the American Society for Virology.

Dr. John Patton received both his B.S. (1976) and Ph.D. (1980) degrees from Virginia Tech. His graduate research was on the replication of autonomous parvoviruses under the direction of Dr. R.C. Bates. From 1980-1983, Dr. Patton was a postdoctoral fellow in the laboratory of Dr. G.W. Wertz where he studied the molecular biology of vesicular stomatitis virus. In 1983, he joined the faculty of the University of South Florida, and then in 1987, relocated to the University of Miami School of Medicine. Dr. Patton was recruited to the Epidemiology Section of the Laboratory of Infectious Diseases at the NIAID/NIH in 1996 where he currently holds the position of Senior Investigator. His research interests during the last 20 years have focused primarily on the biology of rotaviruses, members of the Reoviridae that represent the leading cause of morbidity and mortality in infants and young children due to severe acute dehydrating diarrhea. Dr. Patton’s laboratory has a long history of studies exploring the replication and packaging of the segmented dsRNA genome of rotaviruses, and the development of a reverse genetics system for these viruses. Most recently, his group has been investigating mechanisms by which rotaviruses subvert the innate immune response.

In 1989, **Dr. Daniel Perez** obtained his BSc/MS from the National University of Cordoba, Argentina. In 1995, he completed his PhD in the Department of Veterinary and Biomedical Sciences at the University of Nebraska, Lincoln. Dr. Perez' area of expertise includes virus-virus and virus-host protein interactions of influenza virus and bovine viral diarrhea virus. In March 2000, Dr. Perez joined the Department of Virology at St Jude Children's Research Hospital. Under the guidance of Dr. Robert Webster, Dr. Perez became more focused on understanding the interspecies transmission, pathogenesis, and evolution of avian influenza viruses and the role of cross-protective immunity in the spread of highly pathogenic avian influenza viruses to other birds and mammals. Since 2003, Dr. Perez is an Assistant Professor in the Department of Veterinary Medicine at the University of Maryland, College Park, where he continues to study the role of terrestrial birds in the emergence of influenza viruses with pandemic potential. Dr. Perez is currently program director of the program Prevention and Control of Avian Influenza in the US, a multi-institutional project. Dr. Perez coordinates the work of researchers in 17 states, funded by the largest grant ever made by the U.S. Department of Agriculture to study a single animal disease or health threat. Dr. Perez' studies of influenza in quail prompted Hong Kong officials to ban the sale of live quail in live bird markets as these birds can act as efficient silent carriers of avian influenza.

Dr. C. J. Peters received his B.A. degree in chemistry at Rice University, Houston, TX in 1962 and his M.D. degree from Johns Hopkins, Baltimore, M.D. in 1966. After being on the Parkland Hospital, Dallas, TX internal medicine house staff for two years he became a NIAID Research Associate at the Middle America Research Unit in the Canal Zone. He extended past his two year obligation for a total of 5 years and acquired an interest in tropical diseases, chronic virus infections, arenaviruses, and ecological determinants of disease transmission. When the "war on cancer" and a prematurely declared victory on infectious diseases resulted in the demise of the Middle America Research Unit as an intramural NIAID laboratory he went to the University of California in San Diego to finish his internal medicine training and become board certified. He then spent 3 years as an immunology fellow at the Scripps Clinic and Research Foundation. From 1977-1991 Dr. Peters was at the U.S.Army Medical Research Institute for Infectious Diseases where he began working as a research scientist and later became Division Director and Deputy Commander. While there he worked with biothreats and emerging diseases, particularly hemorrhagic fevers. In 1992 he moved to the Centers for Disease Control and Prevention where he was head of Special Pathogens Branch in the National Center of Infectious Diseases and was involved in epidemiological investigations of hemorrhagic fevers and other emerging infections. He moved to the University of Texas Medical Branch in 2001, where he is professor of Pathology and of Microbiology and Immunology as well as being Director for Biodefense of the Center for Biodefense and Emerging Infectious Diseases. Some of the emerging infectious diseases he has been closely involved with are Rift Valley fever virus, arenavirus hemorrhagic fevers, Ebola virus, Nipah virus, and hantavirus pulmonary syndrome. His current research interests are Rift Valley fever biology and vaccines, *Phlebovirus* pathogenesis, SARS coronavirus, and monoclonal antibodies for therapy.

Dr. Juliet Pulliam received the A.B. degree (Biological Sciences) in 2002 from Duke University and the M.A. degree (Ecology & Evolutionary Biology) in 2004 from Princeton University, where she is currently a Ph.D. candidate. Her dissertation, entitled "The Ecology and Molecular Biology of Emerging Viruses," focuses on predictive approaches to viral host-jumps, determinants of host range, and ecological and demographic approaches to prediction and control of emerging pathogens, including a case study on the dynamics of Nipah virus emergence in peninsular Malaysia.

Dr. Linda J. Saif, PhD OSU Distinguished University Professor, Food Animal Health Research Program, OARDC, Veterinary Preventive Medicine Dept, The Ohio State University; Member National Academy of Sciences. Dr Saif is a virologist and immunologist, whose research focuses on comparative aspects, including the zoonotic potential, of enteric and respiratory viruses (coronaviruses, rotaviruses and caliciviruses) of food animals and humans. A related focus is mucosal immunity to these viruses and vaccine development. Her lab discovered the gut-mammary axis of the common mucosal immune system and exploited this concept to design vaccines to prevent enteric viral infections of newborn animals. A continued focus is elucidating the immunologic interrelationships and memory responses among distinct mucosal tissues to devise new mucosal vaccines. Current research emphasizes bioengineered rotavirus-like particle vaccines with immunomodulators to prevent rotavirus diarrhea, a leading cause of mortality in both infants and young animals. Her lab discovered, characterized and developed novel cultivation methods and diagnostic assays for new enteric viruses including group C rotavirus and enteric caliciviruses. Besides comparative pathogenesis studies of human and animal enteric and respiratory viruses in gnotobiotic animals, her lab is also investigating the antigenic and genetic relationships of new animal viruses such as the enteric caliciviruses and SARS-like coronaviruses to their human counterparts to assess their zoonotic potential and to delineate mechanisms of interspecies transmission.

Dr. Liisa K. Selin received her B.Sc. degree (Biology and Psychology) in 1974 and M.D. degree in 1979, both from Dalhousie University, Halifax, Nova Scotia. She is Board Certified in Internal Medicine (1984, in Canada), with fellowship training in infectious diseases. She also completed her PhD training in microbiology and immunology at the University of Manitoba, Winnipeg, Manitoba (Canada) in 1993 followed by 2 years of postdoctoral training at the University of Massachusetts Medical School, Worcester, MA. She joined the faculty in the Dept. of Pathology at the University of Massachusetts Medical School as an Instructor in 1994 and was promoted to her present position Associate Professor in 2001. Dr. Selin has studied the role T cells in response to viral infections, specifically initially identifying and focusing on the phenomenon of heterologous immunity, whereby memory T cell responses to pathogens can influence the outcome to subsequent infection with unrelated pathogens. The effects of heterologous immunity are now known at least in part to be mediated by cross-reactive T cell responses and mediate both protective effects, immune enhancement and can induce immunopathology. In the past decade she has published and spoken on numerous aspects of the heterologous immunity and CD8 T cell crossreactivity.

Dr. Jeffery K. Taubenberger serves as Chief of the Department of Molecular Pathology at the Armed Forces Institute of Pathology in Washington, DC, a position he has held since 1994. He received his M.D. in 1986 and Ph.D. in 1987 from the Medical College of Virginia, and did a residency in Pathology at the National Cancer Institute. His clinical activities involve diagnostic molecular genetics. He holds dual board certifications in Anatomic Pathology and in Molecular Genetic Pathology from the American Board of Pathology and the American Board of Medical Genetics. His clinical interests are chiefly in the development and implementation of molecular diagnostic assays for neoplasia and infectious diseases. His research interests include 1) influenza virus biology and surveillance, including characterization of the 1918 influenza virus that killed 40 million people; 2) Biology of other RNA viruses including SARS and marine mammal morbilliviruses; and 3) gene expression during early lymphocyte differentiation. He is the recipient of numerous awards and is a frequent speaker at national and international meetings, including multiple keynote addresses. He has published over 80 papers in such journals as *Science* and the *Proceedings of the National Academy of Sciences*, and has written twelve book chapters. His work has been funded by grants from the National Institutes of Health, the Department of Veterans Affairs, the Environmental Protection Agency, and the American Registry of Pathology. He is currently the principal investigator on two NIH grants to characterize the 1918 influenza virus. His 1918 influenza work has generated national and international publicity since 1997.

Dr Marc Van Regenmortel is an Emeritus Director at the Biotechnology School of the University of Strasbourg, France. He was born and educated in Brussels, Belgium and received his PhD degree in 1961 from the Medical School of the University of Cape Town, South Africa .From 1965 to 1966 he was an International Fellow of the US Public Health Service at the Virus Laboratory in Berkeley, California, and subsequently he held positions as professor of Virology and Microbiology at several Universities in South Africa and France. In 1978, he became Director of the Immunochemistry Laboratory at the Molecular Biology Institute of the French National Center for Scientific Research in Strasbourg, a position he held for 22 years. Dr Van Regenmortel has published 15 books and over 375 papers and reviews in virology, viral taxonomy, immunochemistry and biosensor technology and has supervised the research work of 30 PhD students. He is currently Editor-in-Chief of *Archives of Virology* and *Journal of Molecular Recognition* and an Executive Editor of *Analytical Biochemistry*. He also serves on the editorial boards of *Advances in Virus Research, Biologicals, Journal of Immunological Methods*, *Methods* and *Expert Reviews of Proteomics*. Dr Van Regenmortel has served (1984-1990) as Vice-Chairman and Chairman of the Virology Division of the International Union of Microbiological Societies (IUMS) and was for nine years (1990-1999) Secretary General of IUMS. From 1996 to 2002, he was President of the International Committee on Taxonomy of Viruses (ICTV).

Dr. Luis P. Villarreal received the B.S. degree (Biochemistry) in 1971 from the California State University at Los Angeles. He went on to earn his Ph.D. (Biology) from the University of California, San Diego, in 1976 working under John Holland on negative strand RNA viruses and persistence. He completed his postdoctoral fellowship in Stanford working with Paul Berg on recombinant SV40 expression before going on to accept a position as assistant professor of Microbiology and Immunology at the University of Colorado, School of Medicine, from 1978-1985. There he initiated animal studies on polyomavirus tissue specificity. He came to the University of California, Irvine, in 1985, as a professor of Microbiology and Biochemistry. His career interests include virus evolution, viral gene expression, tissue specificity, gene therapy vectors and cancer virology. In the past decade he has published numerous articles and several books, including a recent book on virus and host evolution. He has also been the director of a BSL3 and recombinant DNA laboratory He is currently the Director of the Center for Virus Research, an organized research unit in the University of California. He also recently joined the Recombinant DNA Advisory Committee (RAC) for NIH.

Dr. Mark E.J. Woolhouse is Professor of Veterinary Public Health and Quantitative Epidemiology at the University of Edinburgh, Scotland. He trained as a population biologist with a BA from Oxford (UK), a MSc from York (UK) and a PhD from Queen's (Canada) before turning to epidemiology, holding research posts at the University of Zimbabwe, Imperial College London, the University of Oxford, and now Edinburgh. He heads a multi-disciplinary research group with interests in the epidemiology and pathogenesis of animal and human infectious diseases, covering a variety of infectious disease systems ranging from prion diseases to viruses, bacteria, protozoa and helminths. The common theme is the development of a formal, quantitative understanding of the dynamics of parasites and pathogens within their host populations with particular emphasis on informing the design of disease control programmes. Professor Woolhouse was a government advisor during the UK 2001 foot-and-mouth disease epidemic and is a Fellow of the Royal Society of Edinburgh.

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