

Roadblocks to translational challenges on viral pathogenesis

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Distinct roadblocks prevent translating basic findings in viral pathogenesis into therapies and implementing potential solutions in the clinic. An ongoing partnership between the Volkswagen Foundation and *Nature Medicine* resulted in an interactive meeting in 2012, as part of the “Herrenhausen Symposia” series. Current challenges for various fields of viral research were recognized and discussed with a goal in mind—to identify solutions and propose an agenda to address the translational barriers. Here, some of the researchers who participated at the meeting provide a concise outlook at the most pressing unmet research and clinical needs, identifying these key obstacles is a necessary step towards the prevention and cure of human viral diseases.

Moving ahead on HIV prevention and cure

One of the most dramatic therapeutic advances in the history of medicine was the development and clinical implementation of antiretroviral therapy (ART) for HIV infection, a singular achievement that converted an almost universally fatal infection into a manageable chronic condition and provided a powerful intervention to reduce the rate of transmission¹. This advance originated with basic understanding of the molecular mechanisms of HIV replication and, by any standard, must be considered the quintessential paradigm of modern bench-to-bedside research translation. Despite this accomplishment, the HIV epidemic continues to grow with the number of people in need of

therapy increasing at a faster rate than the number of people with access to care. Moreover, even in resource-rich regions, the majority of HIV-infected people are not diagnosed, in care and/or on effective therapy². Many factors account for this failure, including the expense of therapy, the side effects of therapy, and the difficulty in adhering to any regimen for years to decades. To tackle this epidemic and perhaps end AIDS in our time, two advances must be made—a vaccine that effectively and durably protects those at risk for HIV acquisition and a therapy that will eradicate the virus in those already infected.

The need for an effective HIV vaccine has been appreciated since the identification of HIV in the early 1980s, and the possibility of cure has been considered since the advent of effective ART more than a decade ago. Why, then, has progress on these most compelling needs been so slow? One obvious general difference between the successful development of ART and the lack of an effective vaccine or cure strategy is that solutions for the latter two problems cannot be found by the study of HIV or HIV-infected cells alone; instead, they must be formulated by analysis of the complex *in vivo* interactions between the virus and the host.

For vaccine development, there are two crucial needs: first, to identify immunologic vulnerabilities of HIV (not an easy task, considering that HIV and its simian immuno-

deficiency virus precursors have evolved to efficiently evade immunity and therefore cause chronic active infection), and second, to develop a vaccine approach that safely and durably exploits such vulnerabilities³. For cure, the issues are even more complex, requiring both understanding of the nature and homeostasis of the HIV reservoir in ART-suppressed subjects and development of approaches to activate latent virus and destroy all cells harboring replicating or potentially replicating HIV^{4,5}. Whereas some aspects of this pathophysiology can be investigated in test tubes, attaining the level of understanding necessary to rationally design prophylactic vaccines or clinical interventions for cure requires *in vivo* analysis. This is a daunting challenge, given that HIV is a human-specific virus that resides largely in difficult-to-reach tissues and that infects and destroys the immune system, leading to host-virus interactions that are complex and difficult, if not impossible, to ‘untangle’.

Despite this complexity, human studies have led to crucial advances in understanding HIV transmission and persistence. For example, most recently, the Rv144 vaccine trial has provided evidence that HIV transmission might be vulnerable to vaccine-elicited immune responses⁶, and the increasing number of monoclonal antibodies isolated from HIV-infected individuals has unequivocally showed that the humoral immune system can generate

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a potent, broadly HIV neutralizing antibody response⁷. Moreover, both the apparent cure of the 'Berlin patient' and the identification of post-treatment HIV controllers⁵ have showed that the HIV reservoir is potentially vulnerable to post-ART control and perhaps clearance. However, capitalizing on these observations will continue to be limited by inherent characteristics of the human 'model', including a restricted ability to determine cause and effect by active *in vivo* intervention (any treatment must have both therapeutic potential and an acceptable safety profile), the fact that every human infection is with a different virus, and the fact that researchers often have limited access to human tissues other than blood. In addition, for transmission analysis, natural HIV exposure is variable and uncertain and can only be determined very generally in retrospect.

Observational and interventional studies in humans can therefore take the field only so far—often far enough to raise questions and define desirable outcomes, but not sufficiently far to answer these questions or provide a clear path to therapeutically achieve these outcomes. Although iterative and adaptive clinical trials have been proposed as a solution to this conundrum⁸, the reality is that the questions are too many and the acceptable interventions too few to cover the necessary bases. Unless we are very lucky, identifying and optimizing an effective vaccine or cure strategy by human studies alone will take a very long time.

Thus, the first step in developing effective vaccine and cure strategies will invariably involve use of a relevant animal model. Although some aspects of HIV pathophysiology can be modeled in humanized mice^{9,10}, the most useful models for studies of HIV immuno- and pathobiology are based in nonhuman primates^{3,11,12}. Many nonhuman primate AIDS virus models exist, but they are not equivalent to each other, and not all models are appropriate for every scientific question. Indeed, misapplication of a model can lead the field seriously astray. For example, the ability

of responses elicited by adenovirus 5 vaccine vectors to control infection of a CXCR4-tropic SIV-HIV hybrid virus and protect against the acute disease caused by this virus^{11,12} led to the expectation that the HIV version of this vaccine would provide substantial clinical protection. But the human trial of the adenovirus 5-HIV vaccine famously failed, a result that would have been predicted by analysis of the inability of this vaccine to protect against CCR5-tropic, chronic aggressive SIVs that cause an infection that more closely resembles HIV infection^{11,12}. Hindsight suggests other mistakes that limited the usefulness of nonhuman primate models in past studies—notably, group sizes that were too small (leading to inconclusive studies) and the use of SIV challenge doses that were unrealistically high in vaccine studies, particularly via the intravenous route. In this regard, recent studies using repeated limited-dose mucosal challenge, which more closely reflects human sexual transmission, and larger group sizes have revealed immune vulnerabilities of highly pathogenic SIV that could not have been appreciated with previous standard approaches, including a vaccine-mediated protection against acquisition of highly pathogenic SIV infection that has clear similarities with the protection observed in the Rv144 human vaccine trial^{3,13}.

Nonhuman primates are not small humans and should not be considered a surrogate for humans or a 'gatekeeper' for human trials; however, analysis of appropriate nonhuman primate models can both define and answer crucial questions relevant to HIV vaccine development and cure, as well as explore the feasibility and efficacy of general approaches. Indeed, given the complexities of the relevant scientific issues and the myriad potential pathways to achieve therapeutic success, it can be argued that better exploitation of the nonhuman primate system (as well as further development of humanized mouse models) will be required. The clear need for coordination of nonhuman primate models and clinical research is not new, but obstacles to progress remain. Nonhuman primate research

is expensive, and thus experiments with appropriate statistical power invariably exceed the cost limits of common funding mechanisms. Facilities and expertise appropriate for these experiments, as well as the animals themselves, are quite limited, creating a bottleneck in which potentially key experiments are underpowered, delayed or not performed at all.

Translation of preclinical findings to the human system is also problematic, with clinical investigators facing a number of barriers to success. Gaining knowledge about HIV transmission and persistence during therapy will require the study of mucosal and lymphoid tissues, but very few groups can routinely access and process such tissues. Clinical studies increasingly require teams of clinical and basic scientists, leading to uncertain academic advancement pathways and sources of support. Translational studies of new concepts are difficult to get through peer review, as they are typically small pilot studies. Investigation in humans also invariably involves risk that deters many funders and that draws attention from multiple oversight committees, which can lead to overwhelming regulatory hurdles. Finally, human studies aimed at defining the biology of HIV infection and/or selecting therapies for future, more definitive clinical trials are expensive, requiring considerably more funds compared to laboratory- and small-animal model-based projects. As is increasingly recognized by the key funders of HIV vaccine and cure research, the traditional academic research model of independent laboratory- and animal model-based research groups funded by small project research grants will not, by itself, achieve the goals of the HIV vaccine and cure agendas. One of the key challenges of our field will be to adapt to the cooperative, multidisciplinary nonhuman primate model and clinical research-oriented science that will be necessary for success. —LP & SD

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Tackling influenza: diversity in viruses and hosts

What makes some influenza viruses able to efficiently transmit from person to person and others not? What determines the virulence of influenza viruses? Why does influenza cause severe disease in some people but not in others? How can we best respond to the emergence of a new influenza virus? These questions are the focus of active research, but influenza researchers face a number of specific challenges. The first is

the diversity of influenza viruses in nature, which limits how generalizable findings are from one strain to other strains or subtypes and necessitates experiments in different influenza virus backbones. Second, although several animal models can support the replication of influenza viruses, the extent to which they can be used to study disease or transmission varies, as does the availability of immunologic reagents to charac-

terize the host response to infection. In addition, although data from ferrets and monkeys are more relevant to humans than data from mice, the sample size in experiments with these species is often very small because they are expensive to purchase and house. Third, the unpredictability of the occurrence and severity of seasonal influenza makes it challenging to plan and execute studies in humans—a well-designed study

may not yield data in a mild influenza season. Therefore, it is prudent to plan and fund clinical studies to span more than one influenza season.

Influenza viruses are constantly evolving by reassortment and mutation. Infection of a new host species, as evidenced by equine influenza viruses isolated from dogs or avian influenza viruses isolated from pigs or humans, can be sporadic and can fail to spread, or the virus can spread efficiently in the newly infected species. When the latter occurs in humans, a pandemic can ensue. Unfortunately, we do not yet understand all of the viral and host factors that determine the transmissibility of influenza viruses in humans. With specific caveats in each case¹⁴, one can study transmission of human influenza viruses in humans¹⁵ and human and animal influenza viruses in animal models¹⁶.

Despite the controversy (primarily due to dual-use concerns) surrounding the publication of two recent studies describing mutations in H5N1 viruses that conferred transmissibility in a ferret model^{17,18}, transmissibility of influenza viruses is a crucial area for scientific research¹⁹ that we cannot afford to set aside entirely. One possible approach to studying the transmissibility of animal influenza viruses with pandemic potential is to begin hypothesis testing with low-virulence viruses, as such studies will not raise the same concerns as experiments with highly pathogenic H5N1 viruses. But limited experiments with a highly virulent virus (conducted safely under appropriate containment after careful risk assessment) will be needed to generate definitive proof. Such studies will require communication between influenza virologists and biosafety and biosecurity experts as well as the education of the scientific and general publics

on the importance of this research, with an emphasis on the safeguards that are in place.

Animal models have been used to answer many questions in influenza biology, ranging from pathogenesis and immune response to transmission and control measures. Each model has its pros and cons, and none faithfully replicates the clinical experience in humans. In part, the gap between data from animal models and the clinical experience is because laboratory research is carried out in influenza-naïve experimental animals, whereas humans beyond early childhood have prior experience with influenza. Studies in naïve animals led to an overestimation of the virulence of the 2009 pandemic H1N1 virus for humans^{20,21} and to the prediction that two doses of vaccine would be required to immunize the population. As it turned out, as a result of previous priming, a single dose of vaccine was sufficient to immunize all but very young children. Prior exposure should be modeled in animal models²², and models should be judiciously selected to address specific research questions rather than a one-size-fits-all approach in which data from ferrets or nonhuman primates are emphasized to the exclusion of other data.

The identification of viral determinants of virulence and an understanding of the events triggered in the host can lead to the development of new therapeutic agents. Viral determinants of virulence are typically identified by comparing pairs of influenza viruses with dichotomous virulence (and sequences) in an experimental animal model^{23,24}. Surveillance programs can monitor viruses for genetic changes that are associated with virulence. The challenge lies in synthesizing this information with a focus on the phenotype of virulence rather than the

genotype and in exploring how the mutations alter virulence to determine whether they are broadly applicable or unique to a particular isolate or clade.

Why does influenza cause severe disease in some people and not in others? A multidisciplinary approach that examines the influence of genetics, immunology and virus-host interactions in natural history studies²⁵ or human challenge studies²⁶ will probably shed light on this question. For human challenge studies, we need access to challenge virus preparations that meet regulatory requirements, to appropriate inpatient facilities and to a mechanism to maintain a pipeline of challenge virus pools to which subjects are susceptible. All of these require long-term commitment and funding.

The scientific, medical and public health communities must be able to respond rapidly to the emergence of a novel virus, and the only way to do this is to have a network in place that can be activated at short notice. A good example is the MOSAIC (Mechanisms of Severe Acute Influenza Consortium) network, which undertook clinical evaluation of the 2009 pandemic H1N1 virus in the UK²⁶. Despite the operational hurdles, we are at an exciting time in influenza research because increasingly sophisticated technology is becoming available to address complex questions in biology, medicine and public health. Rational discourse and application of specific models to elucidate the biological mechanisms underlying virulence and transmission will facilitate our understanding of human influenza. —KS

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Discovering and uncovering viruses

The methodical search for unknown viruses—or ‘virus discovery’—promises quick answers and shortcuts in the quest for viral disease etiologies. Understanding viral causation can be key to diagnostics, prevention and treatment of a disease. But the growing discrepancy between expectations and results in this field poses a crucial question that must be addressed: what can be learned from all of the new viruses—their sequences in particular—identified by new technologies such as next-generation sequencing? First, differences between viruses and viral sequences must be discussed and clarified. About 20 years ago, in the early days of PCR diagnostics, the phenomenon of reaction products ‘hopping’ between PCR tubes left virologists hesitant to accept the existence of

new viruses until isolates were obtained and characterized. The advanced technology used nowadays allows us to find sequences that often form new phylogenetic clades clearly different from laboratory strains, so they are unlikely to be contamination artifacts. Although next-generation sequencing will also detect integrated defective viruses and viral genome fossils²⁷, most of the encountered new sequences represent true viruses with the potential to replicate and cause disease in humans.

The threshold beyond which viruses can be declared ‘new’ still sparks debates^{27,28}, and these will continue as long as we lack general and prospective criteria to classify new viruses. Our improved knowledge of viral diversity obtained through virus discovery, however, should soon

enable a much more general approach to viral taxonomy based on full-genome phylogenies²⁹. Such more holistic taxonomy will have functional implications, for example when it comes to the investigation of cellular antiviral targets whose activities could be conserved within reasonably delineated taxonomic units of viruses³⁰.

In clinical virus discovery, there have been plenty of studies on respiratory illness and diarrhea—acute conditions where viruses can be caught red-handed in the body. For other diseases, such as multiple sclerosis and other chronic and degenerative diseases, we are far from solving their mystery etiologies, as if there is any virus involved, it may be long gone once symptoms develop³¹. The only traces of viruses might exist in immunity patterns such as anti-

bodies or primed immune cells. Although these patterns have already been investigated with the available methods, we have not seen the same technical revolution in immune diagnostics as in molecular detection, where next-generation sequencing has enhanced the depth of data by orders of magnitude. Therefore, our technical capability to find new virus sequences is no longer the bottleneck; instead, our biggest challenge is to appreciate the medical relevance of our results. Traditionally, it was believed that viruses are not part of the normal flora, causing a sentiment that newly discovered viruses should all be pathogens. But more recent studies have shown that the normal human microbial flora probably includes nonpathogenic and even symbiotic viruses³². In addition, innocuous contaminant viruses can also be detected in humans, such as plant, arthropod and fish viruses, most likely derived from diet³³.

Virus discovery studies must therefore entail appropriate clinical validation with statistical power to confirm the absence of new viruses in healthy control groups. We currently lack data on pathogenicity for a whole range of new viruses, including cardioviruses, klassevirus/salivirus, cosaviruses, several new anello-, circo- and cycloviruses (and other small single-stranded DNA viruses) as well as an expanding range of new polyomaviruses. The human bocavirus, discovered in 2005 and heavily investigated since, can be seen as a paradigm for this problematic field. The majority of studies on human bocavirus have focused on patients with disease, but those studies including a sufficient range of differential diagnoses found tre-

mendous rates of co-infection with a plethora of 'professional' respiratory and enteric pathogens, suggesting the virus to be a bystander³⁴.

Virus discovery has also extended to animal viruses and the investigation of viral reservoirs, which has raised big expectations as to the translational value of these data. Although some of these studies are aimed primarily at understanding the ecology and evolution of viruses³⁵, there is hope (and there are big promises) that virus discovery may give us a head start against the next pandemic to emerge³⁶. It is reasonable to endeavor to make a census of all viruses lurking in animal reservoirs, as this will help identify viruses during future outbreaks. For example, our expanded knowledge about bat-borne coronaviruses has made the identification of a new human coronavirus that recently emerged in the Arabian Peninsula much easier than the identification of the SARS coronavirus 10 years ago^{37,38}. But the sequencing of reservoir-borne viruses can only be a very first step in viral risk assessment, as complex processes such as viral entry and virus-host cooperation at the cellular level, as well as the many components of epithelial and systemic host defense versus viral immune evasion, also determine whether a virus can infect and spread in humans. The unfiltered presentation of new viruses can create an unjustified air of alarm, and we should therefore investigate biological functions to provide surrogates of transmission risk along with initial descriptions of reservoir-borne viruses. Initial studies working along these lines have now been published, such as the description of a new henipavirus that seems to lack an essential anti-interferon pro-

tein, or a new influenza virus from bats whose replicative proteins seem to function in the context of human cells^{39,40}.

An improved viral sequence inventory may address one of the largest technical challenges currently encountered in viral discovery: the lack of template sequences against which we can compare our data. We routinely see next-generation sequencing reads that cannot be aligned to any known member of public gene databases because their genetic distance to any banked virus is just too large. There is still a genetic terra incognita where we cannot recognize next-generation sequencing reads as viral sequences. The shortness of these reads also precludes more sensitive database comparisons based on prediction of encoded protein structure. Thus, even though the collection of reservoir animal 'metaviromes' seems like a large effort with little direct utility, it may in fact constitute the most direct way to better understand the human virome.

Our collective current approach to viral discovery is dominated by the fascination around new technology, which is evolving at a rapid pace. Nevertheless, the results we obtain are still closer to raw data than to conclusive analyses of etiological problems. More clinical and functional investigations must be included routinely in discovery studies to prevent misinterpretation of data and to fulfill our most elementary translational claim—the identification of meaningful viral etiologies. —CD

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Filling the holes in RSV prevention and treatment

Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis and pneumonia in children. The WHO estimated that in 2005, RSV infection resulted in 34 million cases of acute lower respiratory disease in children less than 5 years old, with about 10% requiring hospital admission and 66,000–199,000 deaths⁴¹. Despite years of intense study, there are no vaccines to prevent RSV disease and few options for treatment. Although passive administration of virus-neutralizing antibodies can reduce RSV-related hospitalizations in infants at high risk for RSV disease^{42–44}, translation of this protective correlate into a safe and effective pediatric vaccine has not yet occurred.

Several problems account for this lack of success. The highest rate of RSV hospitalization is among infants less than 3 months of age⁴⁵, which is when the ability to elicit a strong anti-

body response to vaccine may be compromised by the presence of maternal antibodies and the immaturity of the infant immune system. In addition, vaccine formats that might be capable of eliciting strong RSV-neutralizing serum antibody responses in children, comparable to those achieved with antibody immunoprophylaxis (such as parenteral administration of properly folded viral protein subunits formulated with adjuvant), would be difficult to clinically develop, at least in part, because of concerns that these vaccines may potentiate RSV disease. In the 1960s, a formalin-inactivated, alum-adsorbed whole-virus vaccine was tested in infants and children; however, it not only failed to protect against RSV disease but also resulted in immune-mediated disease enhancement⁴⁶. The subsequent development of animal models of RSV vaccine disease

enhancement suggested that parenteral vaccination of RSV-naïve animals with subunit vaccines could result in enhanced pulmonary pathology after RSV challenge⁴⁷. In contrast, live, attenuated RSV vaccines and chimeric viruses expressing RSV antigens^{48,49} did not seem to enhance the risk for RSV disease in vaccinated animals. It is these live, intranasally administered vaccine candidates that have advanced into clinical studies in seronegative infants (ClinicalTrials.gov NCT00767416, NCT00686075 and NCT01459198).

However, identifying immune correlates of activity and protection will probably be difficult for live intranasal vaccines that remain localized in the airways. Only 44% of infants intranasally vaccinated with a live, attenuated RSV vaccine candidate had detectable serum antibody to RSV, in spite of evidence that most of the vac-

cine recipients were protected against subsequent challenge with the vaccine virus⁵⁰. Past experience with live, attenuated influenza vaccines illustrates the difficulty of identifying correlates of protection for vaccines administered locally. Numerous assays have been evaluated over the past 20 years, including various measures of serum antibody responses, nasal IgA and interferon- γ enzyme-linked immunosorbent assays; however, to date no absolute correlate of protection has been identified for these influenza vaccines. The problem may be that current assay systems are insufficiently sensitive to detect local immune responses. Although it is possible to develop a vaccine without a correlate of activity, it is challenging to do so. The inability to show that a vaccine induces a robust immune response in the majority of recipients makes the decision to proceed into large, expensive efficacy studies very difficult. If a vaccine is licensed and no correlate of immune protection is established, the demonstration that subsequent manufacturing and/or formulation modifications do not have a negative impact on vaccine potency requires the conduct of additional efficacy studies.

It would be highly desirable to have an RSV therapeutic capable of reducing disease severity, halting disease progression and/or shortening the time to disease resolution for RSV-infected children after they are diagnosed. This is especially important with the continuing absence of a RSV vaccine. A central concern in RSV antiviral development, however, is whether any antiviral compound can affect disease progression or outcome when administered after a child is symptomatic. A recent study of RSV infection in the US indicated that children brought to emergency departments and pediatric offices often already have moderately severe disease⁵¹. It has been proposed that RSV pathogenesis, which involves airway obstruction by exudate containing damaged epithelial cells, is driven both by viral replication and the host immune response to infection, so that targeting one and not the other would fail to provide therapeutic benefit. Much of what we understand about RSV pathogenesis, and the role of host responses in RSV pathology, comes from mouse and cotton rat infection models. But these animal models are imperfect at best and are unlikely to accurately reflect disease progression in children. The continuing debate about whether RSV replication drives disease after respiratory symptoms are apparent may be discouraging the development of new antiviral compounds for RSV. Antivirals capable of shutting down virus replication are more likely to be effective later in the disease course than agents designed to block virus entry and spread, and it is through clinical testing of replication inhibitors that the drivers of RSV disease may be elucidated. Furthermore, agents

that act on the virus and not the host may provide the safest strategy for treating young children with RSV disease.

Although RSV is largely known as a pediatric infection, it also causes substantial disease in the elderly, adults with chronic heart and lung disease and those who are immunosuppressed, specifically with T cell deficiencies⁵². As the humoral response to RSV seems intact in adult patients⁵³, it is likely that effective vaccine strategies targeting adult populations will differ from those for infants. The presence of RSV-neutralizing antibody in adults would prevent infection of the upper airways with the live, attenuated RSV vaccines that are being developed for seronegative children. Development of therapeutic interventions for adults will require rapid diagnostics appropriate for this population, as adults shed lower quantities of virus and for shorter periods of time compared to children⁵⁴. Many RSV diagnostics developed for pediatrics (there are about 20 different commercial RSV diagnostic kits) are insufficiently sensitive to detect RSV in adults. PCR methodologies that have allowed detection of RSV in adults are extremely sensitive and have led to observations by some investigators of persistent RSV infection, particularly in adults with chronic obstructive pulmonary disease⁵⁵. Persistent infection with RSV remains a controversial topic that, if substantiated and found to be clinically meaningful, could have profound implications for the development of RSV vaccines and therapies.

RSV is a complex pathogen that poses multiple translational challenges. These include the lack of a small-animal model that recapitulates human infection and disease, the different human target populations requiring different diagnostics and intervention strategies, and the fact that its pathogenesis and immune responses must be studied in a rather inaccessible compartment such as the human airway. As is the case with other complex pathogens, it will be the consolidation of data from multiple sources each of which independently provides an incomplete picture, along with the application of new technology platforms, that will lead to vaccines and therapies for RSV. In addition, because RSV largely affects the very young and the old, success against RSV will require a commitment to better understanding immune responses to infection early and late in life. —JS

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