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GUEST COMMENTARY

Why Do We Not Yet Have a Human Immunodeficiency Virus Vaccine?▽

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A 2-day meeting to explore why we do not yet have a human immunodeficiency virus (HIV) vaccine was held under the joint sponsorship of the Simons Center for Systems Biology at the Institute for Advanced Study and the International AIDS Vaccine Initiative, with additional support from Merck & Co., at the Institute for Advanced Study in Princeton, NJ, on 29 and 30 May 2008. The meeting brought together a diverse group of research scientists from both inside and outside the field of AIDS research to discuss the problems of making an HIV vaccine, with the goal of developing fresh approaches to vaccine development. The review that follows reflects only the author's view of the most important points made at the meeting with respect to identifying new questions, approaches, and directions in research that could lead to a better understanding of how to produce an HIV vaccine.

It was clear from the meeting that there are underserved areas of research that need to be engaged in this effort, and there are experimental questions that should be answered. It was also clear that a coordinated effort from basic research, translational research, and clinical research teams will be required to meet these challenges. The goal of our research effort should be to develop a rational basis to understand vaccine responses in humans. To do this, we need to bring new individuals with unique skills into the study of HIV/AIDS, utilize recent technical advances in molecular genetics employing human material, and develop a much better understanding of human and animal immune systems and a deeper appreciation of how retroviruses replicate in cells. This review of the meeting will provide more detailed examples of each of these research suggestions.

(i) Adaptive immunity. We reviewed the STEP (HVTN 502/Merck 023) trial at Merck, which attempted to engage the T-cell arm of adaptive immunity by measuring the end point of protection from future infection. Although this trial failed, we do not know why. One of the reasons for this is that there is not a clear consensus that we have adequate T-cell assays in vitro to measure the T-cell responses in vivo. Previous trials to elicit B-cell antibody using vaccines have also failed. A survey of the available HIV neutralizing antibodies demonstrates that there are only a few examples, and most individuals do not make those antibodies in detectable titers. Clearly, we need more examples of clones of neutralizing antibodies and some assurance that the neutralization tests in vitro, as well as those tests carried out in vivo in monkeys, are relevant to the neutralization of viruses in humans in vivo. Although the results to date could indicate that adaptive immunity will play only a minor role in future vaccine attempts, there is good evidence that this is not correct and that more needs to be learned. The existence of humans with the phenotype of elite controllers of HIV load during infection (and possibly prevention, which has not been tested) and its linkage to a specific set of HLA types demonstrates a role for class 1 and 2 molecules in adaptive immunity (and/or possibly linked genes) against this virus. A clinical trial of a vaccine using individuals with this HLA group could be instructive in learning from the few to apply to the many. At a minimum, we need more information about the epitopes involved and the nature of the responses in elite controllers.

Too few clinical trials have been done in such a way as to provide information about why the treatments studied failed to protect against the virus. To increase the number of clinical trials and get this information, new tests for T-cell and B-cell function in vitro must be developed and their meaning in vivo understood. We need to know more about neutralizing antibodies, why they are produced in few people at low titers and how they neutralize the virus (a study in virology). We need to know if the infected person has escape mechanisms from adaptive immunity. (For example, is the HIV agent found in exosomes? Is it passed from cell to cell by fusion? Are HLA molecules functional in infected cells, or are they modified?) We desperately need to know more about human immunology. Here, modern techniques of RNA microarrays and deep DNA sequencing of the T-cell and B-cell repertoire in normal, HIV-immunized, and HIV-infected individuals should be undertaken and interpreted by a strong systems biological approach. Single-nucleotide polymorphisms and genetic analyses of diverse groups that are infected and placed in a subset of sensible clinical phenotypes such as elite controllers should be undertaken and analyzed by systems biologists who can extract the signals from the noise. The modern tools of molecular biology now permit the human to be the best model organism to study biology.

(ii) Innate immunity. Over the past 10 years, it has become clear that many species have developed an elaborate innate immune system for detecting and responding to DNA and RNA viruses. We are just now beginning to understand that cells have developed mechanisms to detect and respond to retrovirus DNA in the cytoplasm and RNAs that contain "foreign sequences" (AACpGAA) that are found in RNA viruses. These responses employ the JAK-STAT and NF-κB systems of regulation and produce cytokines that can limit virus replica-

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tion. Researchers in the HIV field (with the exception of some vaccine producers who have explored the addition of foreign nucleic acids to their vaccine preparations) have largely failed to explore this area of science and have not incorporated it into their designs for protection in vaccines. We do not even understand the cell types and their innate immunity receptors for detecting the first exposures of a human to a retrovirus, nor do we understand the full responses of these cells to retrovirus infection. This is a neglected area of vaccine research, retrovirology, and even immunology; it is often studied with the mouse as a model system, but it is only poorly studied with humans.

An organized effort in this area of research carried out in human beings is needed. The full complement of reagents (antibodies to the panel of cytokines, receptors, etc.) needs to be produced and made available to researchers in the field. We must understand how the organism and its cells detect the presence of a foreign retrovirus and how these cells respond.

(iii) Retrovirology. With the DNA sequence and the identification of the genes and proteins made by HIV during infection, the study of the virus replication cycle took a back seat to other questions asked by virologists. However, we do not know the details of how this virus replicates in cells. Indeed, we do not even understand how this virus causes the pathogenesis that leads to AIDS. This makes work on nonpathogenic primate models an important priority. We were reminded that many unknown cellular functions are employed by retroviruses during their replication cycle and that these functions need to be uncovered and, if possible, tested as drug targets to inhibit virus replication. It was clear that many questions remain to be answered using the primate models of virus infection. Here, there are the first clues to a possible model against virus reinfection and vaccine protection. Yet we do not know how this works. Some of the hybrid viruses produced for these studies were more complicated than we had thought. There was a surprising set of contradictory interpretations of simple questions about the consequences of HIV in chimpanzees. (Do they get an acute infection? Do they show any effects of chronic infections?) Given that the HIV comes from the chimpanzee virus and that in natural chimpanzee populations it does not appear (but are we sure of this?) to cause any disease, these questions should by now be settled in the field.

Although this report makes a strong case to study human biology, immunology, and virology, we need to continue the primate models, because they have much to teach us. We need to recruit new young virologists back into the HIV field to ask imaginative and central questions about the life cycle of the virus. How does it kill the T cell, and why does it do this so rapidly (within a day or so) in vivo? Which host proteins and functions does this virus utilize in the T cell, the macrophage, etc.? Do we know all of the functions of the nonstructural viral proteins? What are the chemical and structural properties of a protein that fails to elicit a strong B-cell or T-cell response? Do RNA and DNA retrovirus sequence variations alter the innate immune responses to this virus, as they do with influenza viruses? What are the genetic variations in a host that can alter an HIV infection? Do retroviruses activate stress responses (as do other RNA and DNA viruses) in the host cell, and if so, what is the nature of these responses and how does the virus protect itself against them? How does the feline leukemia virus vaccine work? Does this vaccine really work to protect kittens? Indeed, how do other vaccines that protect against persistent viruses, such as varicella-zoster virus, work? Ever since virologists left the HIV field to only a few laboratories, new and interesting biology has been learned in other fields but has not yet been applied to HIV research.

(iv) Systems biology. In response to large changes that have taken place in biology over the past 10 years, a new field is emerging to cope with the integration of a large number of observations and to develop the interpretation of these facts into a model that makes experimental predictions. The large data sets that are explored by systems biologists often combine molecular information (mRNA expression profiles, DNA sequences, epigenetic changes, single-nucleotide polymorphisms, etc.) with clinical information (CD4 counts, virus load, other infectious agents, drug treatments and changes in treatments over time, etc.) as well as geographic, temporal, and sampling variables. The young physicists, computer scientists, and mathematicians who have entered biology over the past few years are trained in examining biases in data sets, extracting signals from the noise, organizing data into patterns, and finding the rules that create these patterns. This often leads to predictions, models, and new experimental approaches. This work is complemented by the models produced by those who study the natural histories of infectious agents and the clinical responses to a virus infection. It was this type of study that permitted the conjecture that T-cell immunity played only a minor role in HIV responses, even before the STEP trial had failed. This approach is quantitative and is valuable in generating new ideas and directions in research and even opening the field to entirely new concepts. For example, it is now possible to use high-volume DNA sequencing to explore the human immune T-cell repertoire and to compare the naive and memory compartments and responses to cytokines and infections. However, without correcting the raw data for systematic errors, examining the frequency of repeated sequences, and exploring the statistical significance of rare events, interpreting these experimental data sets correctly would not be possible. The tools of the quantitative biologist and physicist are essential here. The HIV field has begun to generate some large data sets for the integration sites in the genome and a small interfering RNA screening of possible HIV regulatory sequences. These data may well be interpreted in useful ways by smart and knowledgeable systems biologists.

At present, there are several data sets of HIV sequences that demonstrate the diversity of sequence space in the HIV genome by geographical areas, and through different times, but rarely from the same individuals. In a few cases, these data sets are paired with clinical information, and in rare cases, there are serial samples from a patient that characterize both provirus and viruses in the serum. Unfortunately, these databases have significant ascertainment biases (the sampling bias is simply terrible), and even the accepted clade structure of worldwide HIV suffers from this biased sampling. An analysis of the DNA sequence databases for HIV and their comparison with the worldwide isolation and temporal exploration of influenza virus sequences demonstrates the serious limitations of the conclusions one can make about HIV vaccine development (which virus do I use?), the evolution of this virus in human populations, the random versus directed mutational pressure

on this virus, and many other questions that have been explored with influenza virus populations but cannot be explored properly with HIV using the existing data sets. It would now be very useful to plan a long-term (5- to 10-year), nonbiased collection of HIV strains from humans all over the world, along with excellent clinical data to produce a publicly available data set of HIV sequences. A subset of these sequences should come from long-term longitudinal studies of viruses from the same host over time. DNA sequences from the DNA in cells (proviruses) and viruses from the serum should be analyzed for the first time by high-volume sequencing to obtain the spectrum of viral sequences produced (defective viruses and viable viruses and their proportion in different hosts with time) and to look for reactivation of provirus sequences into the viruses obtained from the serum over time. The frequency of such events in different hosts (genetic backgrounds) should be measured. These studies can be associated with clinical changes, the use of new or different drugs, and the acquisition of infections by many different agents and with changes in immune function. As part of the clinical studies, it would be useful to measure the bacterial, fungal, and viral agents in the nasopharyngeal cavity (the flora) using the same DNA sequencing techniques (a swab taken at the same time as a blood sample). There is a need for a well-planned and organized study carried out in a collaborative effort among clinicians, translational and basic scientists, and systems biologists.

One might ask, what does this have to do with vaccine development, which after all has been successful even without such knowledge? The fact is that we have failed to make some vaccines (HIV, hepatitis C virus) because we do not understand the immune system, the virology, and the host in sufficient detail. We must carry out the hard work of doing good, even great, science; if we continue just taking "shots on goal" in the hope that we might get a small response to a vaccine, we will not be able to understand it or even improve it.

(v) Structure of the HIV field. It is clearly true that only a team with a large and diverse group of scientists and lots of funds can make and properly test a vaccine. It is also true that some types of projects, such as that described above, will require a large organization, excellent leadership, high levels of funding, and worldwide cooperation. However, the HIV field of basic and translational research has two structural properties that are not optimal for real novelty and progress. Because of the very large funding opportunities that come from several sources, laboratory sizes of some groups are very big. Having one leader and many researchers can narrow the direction and questions being asked in a field. A truly original and gifted scientist would not like to spend his or her career working on research problems formulated by others. The large groups compete well for funds, which may tend to drive talented new young researchers into other fields where they will have a greater chance to make an impact. Another consequence of large laboratories dominating a field is that research efforts become stale. Over the past few years, it has been rare that a new result reported in the HIV field has caught the attention of virologists or immunologists in related fields (perhaps an exception to this is knowledge gained in the area of innate immunity about the role of APOBEC-driven nucleotide changes or the interesting roles of Tsg101 and tetherin). A number of HIV vaccine researchers at the meeting held at the Institute for Advanced Study did not know all the researchers who were invited, nor had they heard about the research done by the immunologists and virologists who presented their work (which may be a problem in many fields). Related fields are moving on, and HIV research needs to keep up. This, of course, does not apply to everyone in the HIV field, but it is clear to many that the field has lost the vibrant nature it had in the late 1980s through the 1990s. A field that does not replenish itself with young, bright investigators is in trouble. There is a need to attract smart and interested young scientists to HIV virology, immunology, vaccine research, and systems biology. Not enough effort has been put into the planning of this component of the future of the HIV field. That is the best chance to gain the insights required for a larger group to develop a vaccine against HIV.

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