Antibody Durability in HIV Vaccine Development

Website summary

Researchers recently found that antibodies, though not neutralizing, were responsible for the low level (31%) of protection against HIV infection observed in the RV144 HIV vaccine trial—the first to show any vaccine efficacy. Over the past few years, researchers have also isolated scores of potent broadly neutralizing antibodies from HIV-infected individuals and identified common target epitopes. Progress has also been made in developing new antigens, adjuvants, and vaccination strategies that are capable of inducing higher titer, longer-lasting antibody responses. Yet the goal of developing a vaccine that can induce a long-lived antibody response capable of protecting against the extensive number of HIV variants in circulation has not been achieved yet, and remains a daunting task.

One of the many obstacles to the development of a feasible HIV vaccine is the issue of durability of immune responses, so on September 23, the Global HIV Vaccine Enterprise convened a group of immunologists and vaccine developers from industry, academia, and government research entities for a day-long think tank in New York City, part of the Enterprise's Timely Topics series, to address the challenges associated with developing a durable, protective HIV vaccine and to identify the areas of research that should be prioritized to extend the durability of vaccine-induced immune responses to HIV. The topic for the think tank was proposed originally by David Pauza at the Institute of Human Virology and the program was co-organized by Pauza, Chris Wilson of the Bill and Melinda Gates Foundation, Neal Nathanson of the University of Pennsylvania, Ripley Ballou of GlaxoSmithKline and Amapola Manrique of the Global HIV Vaccine Enterprise.

A select group of experts gathered for a wide-ranging discussion on what is known about the durability of antibody responses to existing vaccines; how durability of antibody responses to HIV vaccine candidates is affected by different vaccination schedules, antigens, and adjuvants; which immune responses could be used as a marker for antibody durability in future studies; and what studies could be conducted that would help improve our understanding about durability of antibody responses to HIV vaccines. The group started by discussing the performance of HIV vaccines in relation to established vaccine examples, described the scope of practical research efforts to extend vaccine durability, focusing on immunogen design, formulation, delivery and the roles for approved or experimental adjuvants and evaluated current research directions in vaccine research focusing on antigen stability/trafficking, distribution within lymph nodes, activating T follicular helper cell responses and stimulating naive B cells and T cell-B cell collaboration. At the end of the day there was a discussion to identify high priority areas likely to provide solutions for the HIV vaccine durability problem.

Much of the discussion centered on the basic premise of this meeting: Is durability of antibody responses to HIV envelope a discreet goal of HIV vaccine research or will this goal be achieved in the normal course of product improvement and optimization? Given that researchers have neither identified immunogen(s) capable of inducing broadly neutralizing antibodies nor obtained proof of principle that these antibodies can protect against HIV infection in humans, is the question of durability even justified at this time? Some participants concurred that the biggest issue by far is proof of principle that broadly neutralizing antibodies

can be induced and protect. Robert Seder of the Vaccine Research Center (VRC) at the US National Institute of Allergy and Infectious Diseases (NIAID) suggested that proof of principle that broadly neutralizing antibodies can protect in humans could come soon from passive administration studies the VRC and others are conducting with some of the recently isolated broadly neutralizing antibodies. Seder said durable memory was critical, but first you need proof of principle.

Furthermore, once the principle of virus neutralization as the mechanism of action for protective antibodies is established in humans, we still need to identify immunogens that can induce these antibodies through vaccination. Nathanson said any study of durability has to be built around research that provides at least some idea of how to induce broadly neutralizing antibodies, what he called the giant elephant in the room.

But when is the right time to be concerned with improving the durability of antibody responses to HIV, an issue that will continue to be important for the protective efficacy of any vaccine used on a broad scale? Dennis Burton of the Scripps Research Institute and Gunilla Karlsson Hedestam of the Karolinska Institute argued that the issue of durability is interconnected with work to develop antigens, delivery vehicles, adjuvants and formulations to elicit broadly neutralizing antibodies and should therefore happen in parallel. Others, including veteran vaccinologist Stanley Plotkin of the University of Pennsylvania, asserted that valuable information about the factors that lead to durable immune responses can be gleaned from studies of existing vaccines and then incorporated into HIV vaccine research, as well as vaccine development more broadly.

Plotkin was also one of a few researchers who asserted that there is already cause to focus on improving durability of antibody responses: the fleeting protection observed in the RV144 trial. Efficacy of this viral vector/HIV protein prime-boost regimen was nearly 61% one year after initial vaccination but then declined to around 30% after three years, a problem that appears to be linked to waning antibody responses. The first thing to show is that we have a protective vaccine building on the RV144 vaccine, Plotkin said, adding that while antibody dependent cellular cytotoxicity (one mechanism through which the non-neutralizing antibodies induced by the vaccine regimen are thought to have afforded protection) is not the only response a vaccine should induce, the field should focus on how to extend these types of responses. Plans of future pox-protein trials are attempting to extend protection with adjuvants and an extra boost.

While there was plenty of disagreement throughout the day's discussions, nearly everyone agreed that a feasible HIV vaccine needs to elicit a durable antibody response and not require repeat immunizations to have the broadest public health impact, and therefore considered the issue of long-lived immune responses to HIV an important one. And in the end, the group coalesced on one concrete recommendation for the field. The participants agreed that the field should conduct studies in non-human primates comparing HIV Env to other viral glycoproteins to collect valuable information about the types and duration of antibody responses induced by HIV and whether they are inherently different from those induced by other viral proteins.

Lessons from licensed vaccines

The durability of protective immunity is a key factor in determining the success of vaccination programs. Some vaccines induce long-lived immune responses after a single vaccination, while others require periodic boosters.

But what is meant by durability of immune responses isn't entirely clear. Rip Ballou who summarized the durability of antibody responses to licensed vaccines proposed a much-needed definition for vaccine durability as: the time during which immune responses remain above the threshold required for protection. It doesn't necessarily matter how high the antibody titer is, as long as it remains above the protective level. Unfortunately, as Ballou, noted, this threshold is rarely defined until you have a successful vaccine and is far from being defined for HIV vaccines.

Ballou reviewed what is known about the durability of antibody responses using several vaccine exemplars. Based on a literature review of products for which there is at least two years of immunological follow up, he concluded that all of the vaccines had surprisingly similar antibody decay curves (a measure of the level of antibody over time). The decay rates were similar despite major differences in vaccine class (recombinant virus like particle vaccines, inactivated subunit vaccines, a polysaccharide conjugate vaccine, a purified inactivated virus vaccine, and a purified inactivated subunit vaccine were included), composition, and the peak antibody titers achieved by the vaccines. In general, these decay curves were biphasic with antibody responses peaking soon after vaccination and then declining rapidly to a level where they plateau and then decline much more slowly.

Ballou found that booster immunizations with most of the licensed vaccines recapitulated and sometimes exceeded the peak level of antibody responses following primary vaccination and often helped to slow the subsequent decay. Ballou's presentation showed the context for comparing HIV vaccine durability to existing examples, and showed the relationships between peak initial titer, titer after boosting and rates of decay in determining the duration of protection.

Despite these similarities, Stanley Plotkin pointed to many differences in the durability of antibody responses to licensed vaccines that aren't well understood. For example, the mumps vaccine has a problem with waning immunity while the rubella vaccine does not, which suggests the persistence of antibody responses is different for the two vaccines. Plotkin, who also cited others examples, argued that conducting deep immunological analyses of licensed vaccines might help identify the most important features of vaccine-induced immunity that are necessary for long-lived protection, and that perhaps this could be applied to vaccine development more broadly.

Mark Slifka of Oregon Health & Science University presented data on the smallpox vaccine, the gold standard in terms of inducing durable immunity, showing that vaccinated individuals maintain antibody response to Vaccinia virus up to 75 years after a single vaccination. However, it is not clear whether these antibody levels are still protective, as endemic smallpox has been eradicated.

Slifka said there is more than one way to maintain long-term protective immunity, which is a function of both the magnitude and duration of the adaptive immune response. He noted that in general, vaccines often produce lower immunity than the viruses themselves because of the limited persistence of the vaccine antigen as compared to the actual virus. This might, for example, account for the difference in the persistence of immunity induced by Vaccinia (the virus used to vaccinate against smallpox, a live virus attenuated in humans) as compared to modified Vaccinia Ankara (artificially attenuated), which is cleared quickly, Slifka said.

The persistence of antigen and its role in affording long-lived immunity came up several times during the day's discussions. Rolf Zinkernagel of the University Hospital Zurich argued repeatedly that the persistence of antigen is required to generate durable, high-titer antibody responses and therefore long-lasting

immunity. Others agreed this was an important factor, but did not share his view that persistence was the only factor. Ron Germain of the Center for Human Immunology at the US National Institutes of Health asked whether the persistent antigen was creating long-lived memory B cells or short-lived plasma B cells that continue to pump out antibodies in responses to stimulation from antigen. Zinkernagel said it was nearly impossible to decipher the difference, and that it was somewhat irrelevant because the biggest concern is functional antibody responses and not B cell memory.

Antigen and adjuvant selection and vaccination schedule

There are several ways researchers could approach improving the durability of antibody responses induced by HIV vaccine candidates. Principal among these are immunogen design, formulation and delivery. In humans, antibody responses to HIV Env appear to correlate best with short-lived memory B cells, both in HIV-infected individuals and in recipients of the viral vector prime-protein boost vaccine regimen evaluated in the RV144 trial (ALVAC-HIV vCP1521 prime, AIDSVAX B/E boost), according to Georgia Tomaras of Duke University School of Medicine.

As Tomaras pointed out in her presentation, clinical trials of different HIV vaccine candidates illustrate that the immunogen and adjuvant selection influence the persistence of antibodies, as well as the quality of vaccine-induced B cell responses, including the epitope specificity, isotype and subtype of antibody responses, and antibody functions (including neutralizing activity, antibody dependent cellular cytotoxicity, etc.).

The RV144 trial provides an important opportunity for researchers to study durability of immune responses to HIV because the efficacy dropped from 60.5% in the first year to 31.2% after 42 months of follow up, suggesting the immune responses waned dramatically in this time. And indeed analyses show that antibody responses directed to the V2 loop of HIV, which were seen in vaccinated individuals who were protected against HIV, declined by 10-fold over the first year.

But when Tomaras and her colleagues parsed the antibody responses, a clear difference emerged in the decay rates for individual subclasses of HIV-specific antibodies. The IgG3 responses to HIV Env induced by the RV144 vaccine declined earlier than Env IgG responses, while levels of IgG1, IgG2, and IgG4 were longer-lived. The IgG3 antibody subclass binds better to Fc receptors, indicating it may facilitate antibody dependent cellular cytotoxicity. Tomaras said this finding suggests that if researchers are trying to understand the correlation between responses and durability, they must evaluate the quality of antibody responses because discrete components of serum or mucosal antibodies may decay at different rates.

Adjuvants can also play an important role in improving the durability of immune responses. Susan Barnett of Novartis Vaccines and Steve Reed of the Infectious Disease Research Institute (IDRI) gave presentations on existing adjuvants and how they are being utilized in HIV vaccine research, as well as an update on research into new adjuvants. According to Barnett, the working hypothesis is that vaccine regimens can be optimized to elicit more potent and durable protective antibody responses to HIV, both through adjuvant selection and by altering the vaccination schedule.

To that end, Novartis is collaborating on a follow-up study to RV144 that will evaluate whether the MF59 adjuvant, a squalene-based oil in water emulsion adjuvant, is able to improve the degree of protection and the durability of protective immune responses when added to the prime-boost vaccine regimen. Barnett showed data from neonates indicating that an HIV gp120 protein administered with MF59 induced greater

than 20 times the levels of antibodies directed to the V1 and V2 loops at peak than peak responses for vaccinated individuals in RV144.

New adjuvants that can elicit high titer and durable antibody responses in non-human primates are also in development, according to Barnett, who is evaluating the longevity of antibody and T-cell responses induced by a novel self-amplifying messenger (SAM)-based adjuvant for HIV in macaques.

Reed discussed studies of new adjuvants for HIV that his group is developing and testing in early stage clinical trials in collaboration with Barton Haynes at the Duke Human Vaccine Institute and with Robin Shattock at Imperial College London. He argued that focusing on adjuvant formulation was a better avenue to improving durability than studying existing vaccines.

Altering the vaccination schedule may be yet another way to improve the durability of immune responses. Barnett said they've seen better results when the prime and boost are co-administered, a strategy that is also being studied in one of the RV144 follow-up studies. The topic of optimal vaccination schedules prompted some discussion. Participants debated whether giving the prime in one arm and boost in the other arm or spacing the subsequent booster immunizations out over a longer period of time might help improve durability of immune responses. In the wrap up discussion at the end of the day, it was suggested that HIV vaccine researchers should further investigate vaccination intervals because this was something that could be done rather simply and provide information on whether it extends the durability of immune responses.

There was also discussion about whether an adjuvant could be used to mimic viral persistence by creating a longer-term depot for the HIV antigen that would therefore stimulate the immune response over a longer period of time. This approach would be similar to using a live viral vector, something that is currently being explored by AIDS vaccine researchers but as of yet has not induced a robust antibody response. Both Seder and Nathanson argued that this would be an attractive feature for an adjuvant.

In addition to developing new adjuvants, researchers including Seder asked that pharmaceutical companies like GlaxoSmithKline share their existing adjuvants, such as AS01, with researchers so that they could do head-to-head studies to benchmark how different HIV antigens perform with the same adjuvant.

Harnessing systems biology

Part of the day's discussion was also centered on the utility of systems biology in elucidating factors that influence durability of vaccine-induced immune responses. This discussion followed presentations by Damien Chaussabel of the Benaroya Research Institute and Germain. Both argued the value of measuring multiple parameters and generating as much data as possible. But some attendees were skeptical of precisely what the systems biology approach could contribute, suggesting more data does not correlate with more knowledge.

Chaussabel said one example of how systems biology could be employed in HIV vaccine development is to compare data from trials using different adjuvants to see what similarities there are in the immune response profiles. He argued for researchers to share as much of their data as possible and input into an analysis tool like the one developed at the Benaroya Institute so that such comparisons could be made.

Germain also defended using a more holistic approach to understanding durability of vaccine-induced immune responses and said that systems biology allows researchers to pay attention to the connectivity of

several issues, including antigen type, neutralizing titer, B cell repertoire, dose, and T follicular helper cell responses, among other parameters, and that measuring more things is better than measuring fewer things. However, many participants appeared not to be convinced.

Antigen trafficking

Another element of vaccine design is trying to understand antigen trafficking and how B cells are selected for in the germinal centers, issues that were touched on during the day's proceedings. Germain presented on antigen trafficking in the lymph node and discussed how antigen presentation affects the interactions between dendritic and follicular T helper cells and between B and T cells.

Michel Nussenzweig of The Rockefeller University presented on his work using photoactivation of a stable marker within single cells in mouse models to try to better understand the process of antibody development in the germinal centers, which is where B cell clonal selection and amplification takes place and somatic hypermutation occurs, mediated by activation induced deaminase (AID). The process of somatic hypermutation has proven to be an important one in the development of HIV vaccine candidates because the neutralizing antibodies to HIV that have been isolated in recent years are all extensively hypermutated. This hypermutation is what allows them to neutralize HIV so effectively; however, it may also be why these antibodies take so long to make.

Critical to the maturation of antibodies are a subset of CD4+ T cells known as T follicular helper (Tfh) cells. Rick Koup of the VRC presented on Tfh cells, which have been shown to be involved in the overall maturation of serum IgG antibody responses in chronic viral infections. However, Tfh cells have been difficult to study because they reside in lymph node follicles, leading researchers to look for T cells that occur in peripheral blood that are correlated with antibody responses. But as Koup pointed out in his presentation, the specific T cell populations in serum that correlate with antibody responses differ in every publication, leaving the current status of this search in flux. Koup said the marker researchers are really looking for is the presence of neutralizing antibody, and if you have neutralizing antibodies then you have Tfh cells. Remember, he said, our goal is to generate broadly neutralizing antibodies and we know how to measure them.

Future studies

After a day of presentations and discussion centered on three areas—vaccine durability and deployment; vaccine design, formulation, and adjuvants; and antigen trafficking, B-T cell interaction, and induction of durable responses—the experts were tasked with identifying major issues involved in extending the durability of antibody responses to HIV and developing recommendations on how these issues could be addressed to advance the field. Many open questions were posed by Ballou, Plotkin, and Chris Wilson, who co-chaired the wrap up discussion.

Plotkin led off the discussion by quipping that it is often said immunology has contributed nothing to vaccinology. He said he thinks that observation is true, prompting laughter from some of the participants. Perhaps meetings like this one sponsored by the Global HIV Vaccine Enterprise, will use fundamental immunology to gain a rational understanding of what has been the empirical field of vaccine development and testing.

Plotkin then summarized his take on the day's proceedings. He drew a distinction between B cell memory and antibody responses that are functional and outlined several factors that might affect durability of

immune responses to vaccination, including the quantity of the initial immune response (the higher the peak antibody titer, the higher the persistent titer), the structure of the antigen, the selection of adjuvant, the persistence of antigen and where that occurs, the sub class of antibodies that are induced, and the role of T cells and Tfh cells in particular.

Plotkin implored researchers to collect more data from human clinical trials and from non-human primates when human studies aren't possible. He also argued for conducting studies of existing vaccines that generate both long- and short-lived responses in an attempt to elucidate how their immune response profiles differ, but it was unclear how many other participants shared this viewpoint.

Several other suggestions were also made. There was discussion about the need to standardize what antibody responses to HIV Env are measured (binding, neutralizing, ADCC, isotypes, etc.), in order to compare study outcomes, and to form a central group to promote standardization of monkey studies.

Hedestam suggested future studies be conducted to define the difference in immune responses induced by soluble versus particulate antigen and to look at how replicating versus non-replicating vectors affect durability of antibody responses but these suggestions always run into the obstacle of: should we learn more now or wait for the "best" immunogen that is "just around the corner."

There was also a debate about whether long-lived plasma cells are the best biomarker for antibody responses. Germain noted that in addition to generating long-lived plasma cells, you also need to have a niche where they can live. In the end, there was no consensus on whether persisting antigen driving the continual generation of new plasmablasts or long-lived plasma cells have a greater impact on antibody durability.

Toward the end of our wrap-up discussion, Wilson asked if the attendees thought HIV Env induces inherently less durable immune responses than other glycoproteins and whether this was something the field should explore. Plotkin's opinion was that gp120 is less immunogenic than other viral proteins. Germain said the heavy glycosylation alters how HIV Env is processed. Dennis Burton of the Scripps Research Institute said while it isn't known for certain if HIV gp120 is different from other viral antigens, evidence suggests it might be.

The attendees coalesced around the idea of conducting a head-to-head study in non-human primates comparing multiple trimeric HIV Env antigens, along with adjuvants, to other viral glycoproteins (possibly Influenza HA and another glycoprotein with exceptional durability such as Rabies) to see what can be learned about durability and whether HIV Env induces inherently less durable immune responses than other less heavily glycosylated viral glycoproteins.

It is undoubtable that durability of protective immunity is a critical barrier to successful vaccination against HIV; our panel was united in this view. However, unity around that theme belied clear differences between a camp expecting vaccine durability to improve during the normal course of product development and a camp believing that durability is a discreet aspect of vaccine science and should be approached as an unique research target that would enrich the success of many vaccines. By highlighting the differences in opinions of research leaders and showing the problems with simply defining vaccine durability or collecting relevant data, we emphasize the important of vaccine durability for the success of a future preventive vaccine against HIV.