National Institutes of Health
National Institute of Allergy and Infectious Diseases
Division of Acquired Immune Deficiency Syndrome

AIDS VACCINE RESEARCH SUBCOMMITTEE

May 22, 2007
Fernwood Building, 10401 Fernwood Drive, Bethesda, MD

MEETING SUMMARY

The AIDS Vaccine Research Subcommittee (AVRS) met in public session on May 22-23, 2007, in Conference Room 2C-13 at 10401 Fernwood Drive, Bethesda, MD.

AVRS members present: Eric Hunter (chair), James Bradac (executive secretary), Jay Berzofsky (ex officio), Larry Corey, Susan Buchbinder, Kevin Fisher, Barton Haynes (ex officio), Paul Johnson, Jeffrey Lifson, Margaret Liu, Juliana McElrath, Timothy Mastro (ex officio), Bonnie Mathieson (ex officio), Nelson Michael (ex officio), Gary Nabel (ex officio), Louis Picker, Jerald Sadoff, Ian Wilson. Members not present: Nina Russell.

Other NIH personnel participating:
- Carl Dieffenbach, Acting Director, DAIDS, NIAID
- Peggy Johnston, Director, Vaccine Research Program, DAIDS, NIAID
- Alan Fix, Chief, Vaccine Clinical Research Branch, VRP, DAIDS, NIAID;
- Stuart Shapiro, Preclinical Research and Development Branch, VRP, DAIDS, NIAID.

Speakers:
- Sean Du, Maxygen, Inc.;
- Hildegund Ertl, Wistar Institute;
- Fred Frankel, University of Pennsylvania School of Medicine;
- Shiu-Lok Hu, University of Washington;
- David Knipe, Harvard Medical School;
- Bette Korber, Los Alamos National Laboratory;
- Norman Letvin, Beth Israel Deaconess Medical Center;
- Andrew McMichael, Weatherall Institute of Molecular Medicine;
- Chris Miller, School of Veterinary Medicine, University of California at Davis;
- George Shaw, University of Alabama at Birmingham Medical School;
- Joseph Sodroski, Dana-Farber Cancer Institute, Harvard Medical School;
- Indresh Srivastava, Novartis Vaccines and Diagnostics, Inc.

Opening remarks

Dr. Hunter welcomed participants and introduced three new member of the Subcommittee: Kevin Fisher, Jeffrey Lifson and Louis Picker. He asked other participants to introduce themselves (see attached list).

CHAVI Annual Report

Introduction
Stuart Shapiro, Program Officer, CHAVI, described CHAVI as the Vaccine Program's contribution the Global HIV Vaccine Enterprise. CHAVI attempts to tackle large outstanding questions using large cohorts, high-throughput assays, and large databases. CHAVI does not compete with R01 and P01 grants but rather complements them.

Overview and CHAVI Strategy
Bart Haynes, Director, CHAVI, reported that CHAVI is now at the end of year 2 with 94 investigators at 51 sites, including 13 clinical sites, and a centralized data center at the University of Washington. There are 12 protocols currently under way, focusing on the correlates of immunity in four populations:

- Acute HIV patients, prior to seroconversion (protocols 001 and 012);
- Exposed but uninfected patients (002, 003, 004, 007);
- Chronically infected patients (004, 006, 008); and
- Autoimmune disease patients (005).

CHAVI currently focuses on five critical questions:

1. *What type of consortium should CHAVI become?* Discovery-oriented big science to address bottlenecks and enabling technologies in an open and transparent partnership with DAIDS, VRC, IAVI, etc.
2. *What are the correlates of protection for HIV-1 infection?* CHAVI supports eight different programs to develop assays and study the genetics and transmission of HIV, in addition to CHAVI001, which will follow patients to several different endpoints.
3. *What virus to target with a vaccine?* It has become clear that the transmitted virus is the prime target for HIV vaccines, hence the need to push back the natural history of HIV. CHAVI's studies of the biology and signatures of transmitted virus will provide new strategies for aiming at this target.
4. *How to overcome HIV diversity with preventive (protective) T cell responses?* CHAVI pursues centralized "consensus" sequences that represent highly conserved, functional, antigenic, and neutralizing epitopes.
5. *Why are neutralizing antibodies (NAbs) so difficult to induce?* At present it appears that broadly NAbs are blocked by structural barriers or negative immunoregulatory controls. CHAVI seeks lessons in the ability of animals and humans with autoimmune diseases and defects in B-cell tolerance mechanism to produce NAbs to HIV. Two programs – structural biology and B cell immunoregulation – pursue this common goal.

CHAVI Genetics Program – Genetics of Setpoint

David Goldstein explained that there are about 10 million common variants in the human genome, most of them single-nucleotide polymorphisms (SNPs), and some of these SNPs contribute to the considerable variability in viral control that has major consequences for the outcome of HIV infection (rapid progressors, normal patients, slow progressors, and "elite controllers" who seldom progress to full-blown AIDS). CHAVI is pursuing whole-genome association studies to identify the genetic variants associated with these phenotypes. Genotyping of over 550,000 SNPs has identified two SNPs with genome-wide impact on viral setpoint, namely HLA complex P5 (HCP5) and the HLA-C 5-prime region. The former is linked with HLA-B5701, which has a known association with HIV restriction, and the latter affects HLA-C mRNA expression levels; both provide better control of HIV replication. Progression analysis has confirmed that these SNPs have a significant effect on the time to a CD4 count below 350. Other analyses suggest that these two SNPs may explain up to 15 percent of variation in viral setpoint.

In response to questions, Goldstein added that it takes a large cohort – about 2,000 samples – to identify SNPs of interest. The cohort for the Merck vaccine trials is about 5,000, and it might be desirable to use samples from that trial for this analysis. CHAVI is developing a different chip to study variations in populations in developing countries. Within 18 months, investigators hope to identify additional SNPs that explain up to 50 percent of difference in setpoint.

Structure of Env

Joseph Sodroski described CHAVI's efforts to find surface protein epitopes that are exposed and conserved, and thus present targets for NAbs. HIV has evolved variable loops and other features that minimize the impact of Abs, most of which are weakly neutralizing. However, the b12 Ab is potently neutralizing and requires little entropy change to bind to the CD4 binding site on viral gp120. The b12 binding site is also well conserved in different strains of SHIV and HIV. Experiments have also demonstrated that b12 recognizes multiple conformations of HIV-1 gp120, fixing them into a more stable form that is more easily recognized and bound by other NAbs. Future efforts will look for similar structures of the gp41-interactive surface of gp120, as well as broadly NAb responses in the sera of selected HIV+ individuals.

In response to questions, Sodroski said that investigators had looked at variations in the b12 epitope, rather than the surrounding regions that alter access to the binding site. He agreed that it would be valuable to focus more tightly on the structure of the transmitted virus, which will likely be the target of a successful vaccine.

B-cell Immunology Studies

Bart Haynes described efforts to study Ab responses at the earliest stages of acute HIV infection (AHI). Recent research has shown that viral transmission occurs 12-21 days before viral ramp-up and up to 20 weeks before the appearance of autologous NAbs. Hence the need to study the Abs, both neutralizing and non-neutralizing, that appear soon after viral ramp-up. Unlike other viral diseases, however, Ab responses to HIV are delayed , and investigators suspect that virologic events may regulate Env-specific Ab responses. Class switching is one possibility –

IgG, IgM and IgA all rise at the same time (an implausible phenomenon), but IgM and IgA both fall off, and none of these early Ab responses is protective.

Investigators currently lack the cells to look into these events, but they will have the samples they need from CHAVI001. Their current hypothesis is that HIV induces a massive apoptosis before and during viral ramp-up, and that plasma microparticles (fragments of apoptotic CD3 and T cells) have a suppressive effect on Ab generation, thereby amplifying the apoptotic cascade. To counter this effect, what is needed is a B cell response that comes in hours or days, either from innate or marginal zone B cells, that will be able to prevent or limit the massive apoptosis that leads to immunosuppression. Current studies are designed to isolate immunological complexes in order to determine the immune repertoire, and to generate polyspecific B cells.

In response to questions, Haynes said that the timing of actual infection is unknown but appears to follow transmission by 11 to 28 days. Macaques develop infection more rapidly than humans, but they also receive a much larger innoculus. Evidence suggests that the need for NAbs is systemic, not in local tissues, but much remains to be learned about the natural history of HIV and the biology of mucosal response. For example, is there a difference between "priming apoptosis" and "pathogenic apoptosis"?

Transmitted Virus Genetics

George Shaw noted that HIV transmission is an inefficient process that is difficult or impossible to study directly. The alternative is to study Env or the entire proteome in a large number of patients during the 14 to 28 days between transmission and viral takeoff. A study of 103 AHI patients from clade B, 50 of them phase 1 or 2, yielded 2,590 sample for sequencing by single genome analysis. Results showed a most sequences differed by zero, one or at most SNPs; most patients (77 of 93) showed a single strain, but a few (19) showed two strains and some (13) showed recombinant strains. Investigators inferred from this that, surprisingly, the viral Env is identical to the transmitted virus for 14 to 42 days after transmission, that the transmitted Env will still be an effective target at the time of peak viremia, and that (by extension) the consensus sequence at peak viremia will be identical to the transmitted virus. Further analysis showed that the transmitted virus shields V3 but displays R5, which makes R5 a logical vaccine target.

In response to questions, Shaw added that 80 percent of clinical infections, the source is a single viral particle. The observed recombinations occurred in the host, not in the donor – although the innoculus is phenotypically mixed, infection itself is a rare event (on the order of one in 10^4 or 10^5) and is accomplished by one or at most two virions, after which there is little or no genetic drift. No two hosts will have identical viral sequences, however, since they will have been infected by different virions. HIV replication takes 4 or 5 days. It would be useful to replicate this study with SHIV. It is difficult to draw broader conclusions because investigators did not have personal data (such as risk behaviors) and often did not know the sex of the patient from whom the samples were taken.

Transmitted Virus Signature Sequences

Bette Korber reported on a related attempt to identify the most recent common ancestor (MRCA) of virus phenotypes in 102 early AHI patients from clade B. Here again, 79 of 102 patients were homogenous, with little or no genetic diversity at 17 days from transmission, but the more heterogenous patients showed peaks at less than 10 and greater than 100 base pair differences – a diversity that is best explained by more than one transmitted virus. In fact, the great diversity of the hypermutated samples suggests that they received a "bolus of heterogeneity" and makes them worthy of further study (there is far more heterogeneity in acute HIV cases). Modeling solutions from the Los Alamos supercomputers produced signature-based phylogenetic trees, with full-genome analysis to reduce noise and false positives and negatives. The "loudest" signal peptides were at positions 18 and 29, with other signatures scattered. Further validation is needed for a second data set, and there are plans to replicate this study with clade C samples in order to study HIV evolution.

T-Cell and Innate Immunology Studies

Andrew McMichael reported on CHAVI studies of HIV-specific T cell responses using assays that provide improved sensitivity while maintaining specificity. Previous studies left uncertainties as to level of exposure; this study included behavioral questionnaires. Serological assays for cytokine responses revealed that IFN-alpha and IL15 levels rise early, while TNF-alpha, IL18 and IL10 rise later. T cell responses to HIV are quite different from those of other viral infections in humans. Dendritic cells are activated in the early stages of infection, while natural killer (NK) cells are activated about a month after peak viremia and 2 months after infection. Work will now concentrate on responses to transmitted virus, working backward from chronic infection to AHI. Results thus far have shown that the initial T cell response is entirely CD8, which broadens considerably between month 1 and month 6. HLA-C plays a key role in early response, but CD4+ T cell responses are absent. Investigators will now repeat the study with samples from CHAVI001, with the goal of identifying which kind of T cell response leads to protection.

In response to questions, McMichael added that specimen quality is more of a problem with flow cytometry, which produces better results with fresh samples. CHAVI clinical sites try to freeze samples within 3 or 4 hours, and QA has shown that 90 percent of them are viable at thawing. Generally the profile at month 1 is enriched at month 6, but none of the components disappears; investigators are scanning longitudinally to look for viral escape.

Correlates of Immunity

Norman Letvin reviewed the results of several animal studies conducted in the past few months, studying normal infection, superinfection and live-attenuated vaccine for SIV in nonhuman primates (NHPs). Preliminary studies of the role of NK, CD8+ T cells and B cells in controlling natural infection revealed that CD8+ are lost due to the loss of target cells in the gastrointestinal tract, and that CD8+ depletion increased both the peak load during viremia and the subsequent viral setpoint, with negative implications for survival. This points to the vital importance of CD8+ T cells in SIV control. NK cell depletion, on the other hand, has no effect on peak viral load or viral setpoint, and B cell depletion has no effect on viral load, although it does lower the Ab titer. Investigators suspect that some preexisting response may be protective in AHI.

Using an NHP model of superinfection (infecting an HIV+ patient with a second strain of HIV), investigators tested the hypothesis that previous infection provides at least partial protection against a subsequent exposure. SIV+ monkeys were challenged with heterologous SIV in six weekly exposures; in 12 of 14 cases, the previously infected animal contained additional challenges, and 2 animals resisted them entirely. Viral load increased by 50 to 100 percent, compared to a sevenfold increase in the first infection, and there was no correlation with CD4+, cytokine or virologic factors. The same animals who resisted additional infections had also contained the primary infection. Much the same results were obtained with live attenuated vaccine – subsequent challenge was contained, producing a lower viral peak and setpoint, but there was no sterilization. This may be a useful model for the study of correlates of immunity.

Centralized Genes as Optimal HIV Inserts

Norm Letvin returned to discuss the diversity of wild-type Env genes, which has serious implications for the VRC strategy of developing multiclade vaccines. The centralized gene approach subsumes much of this diversity by using a consensus sequence that decreases the "distance" to any contemporary virus. Theoretically, his "Group M" consensus – which includes conserved functional, antigenic and neutralizing epitopes – should recognize more variants and induce more NAbs than any single-clade Ag. To test this concept, three groups of NHPs were injected with sham, specific (clade B) and consensus versions of the DNA prime + Ad5/Env boost vaccine and then challenged with ten different strains of HIV to test the breadth of reactivity. Epitope mapping showed that, while immunization with clade B Env elicits a clade B-specific response, immunization with consensus Env elicits a response with broader cross-clade specificity – a three- or four-fold increase in the number of epitopes recognized and a significantly higher Ab titer. Pending studies will test the effect clade B versus consensus boost and characterize the vaccine-elicited NAb response after boost.

In response to questions, Letvin could not comment on the quality of this immune response – investigators had only counted the number of epitopes recognized. Nor could he comment on "hot spots" in Env peptide recognition, such as Nef, since investigators haven't looked that carefully at the data.

PAVE 100 Update

Alan Fix reviewed the design and achievements of the PAVE 100 protocol, a Phase 2B clinical trial of a multiclade DNA-Ad5 HIV vaccine in 8,500 mixed-risk participants in East Africa, Southern Africa and the Americas. Enrollment began in 2003Q3 and results are expected in late 2011. Investigators continue to accumulate safety data on their vaccine regiment from HVTN-204, IAVI-V001 and USMHRP RV-172, and to date there have been no vaccine-related adverse events. Fix outlined the ways in which researchers have responded the three specific AVRS comments from its January 2007 meeting:

1. Reduce the duration of followup and revisit power calculation for correlates analysis;
2. Develop clear, prospectively defined analysis of immunogenicity data; and
3. Perform primary immunogenicity assay in a single laboratory.

Additional changes in implementation include extended followup for infected participants, a statistical plan that includes futility analysis for both operations and efficacy, and a 95-percent confidence interval of greater than 30 percent efficacy against relevant strains in each region.

In response to questions, Fix added that the protocol is powered to have validity in each region independently. He will provide an update to AVRS in August on emerging data relating to preexisting Ad5 immunity in Africa. Members suggested that the final design should include all of the features that will be needed to achieve licensure, if the vaccine is successful.

Programmatic Update

Alan Fix returned to report that several new products are currently in clinical trials, including new trials by VRC and SAAVI. Efficacy data from STEP are due in mid-2009, from HVTN in late 2010, and from PAVE 100 in late 2011.

Jim Bradac reported on contract and preclinical activities. Notably, the HIV Vaccine Design and Development Teams was not solicited in 2007 but will be resolicited in 2008. The HIV Database at Los Alamos has been renewed, and the joint DAIDS-DAIT program in B cell immunology is currently pending. The Simian Vaccine Evaluation Units (SVEUs) currently support 190 animals, with a capacity of 600-700, in support of a number of preliminary and comparative vaccine studies. The vaccine pipeline is relatively full, with five preclinical candidates due to enter clinical trials in 2007/2008 and three more after 2008.

The meeting recessed at 4:30 p.m. and reconvened the following morning at 8:30 a.m.

DAIDS HIV Vaccine Research and Design Program (HIVRAD)

Overview of HIVRAD
Jim Bradac reminded participants that HIVRAD was established in 1999 to answer questions important to HIV vaccine research and development in areas such as animal models, immunogen structure, mechanism of action, and vector development. The program makes P01 grants for 5 year at $1 to $2 million/yr, usually one or two awards each year. A total of 18 grants have been awarded; 4 have been completed, 12 are currently active, and 2 new grants were made in FY2007. Overall funding has reached $25 million in FY2007. He posed the following questions for AVRS members to consider during the day's presentations:

- Is the HIVRAD mechanism achieving the aims of the program?
- Does the program cover the most important areas of research?
- Do you have any additional questions or recommendations for HIVRAD?

Combined Approach to Broadly Protective AIDS Vaccines
Shiu-Lok Hu gave a review of his HIVRAD collaboration, which seeks to define the attributes of HIV that vaccines should target, the best way to elicit broadly NAbs to primary isolates, and the best way to optimize "prime-boost" immunization. It pursues these goals through four interrelated core projects:

1. Examine the phenotypes of clade A early infection and identify isolates suitable for vaccine development.
2. Test the concept of "quasispecies" Env immunogens that broaden Ab response by combining 15 sequences from different timepoints in HIV infection.
3. Explore Env modifications such as loop deletions and glycan changes to elicit more broadly NAbs.
4. Compare multiple "prime-boost" combinations for their ability to induce NAbs and suppress viral load in the SIV/macaque model.

In response to questions, Hu explained that NHPs are given intramuscular prime, dermal boost and intrarectal challenge. Preliminary data suggest that the characteristics of transmitted virus may be clade-specific, with little commonality across clades. The P01 grant mechanism offers logistical flexibility, but it also encourages scientific cross-talk. On the other hand, a large and complex project like this requires a lot of effort, and even cross-talk is demanding. External advisors have advised the team to shift their resources from NHPs to rabbits.

Targeting Neutralizing Epitopes in the HIV Envelope
Indresh Srivastava described his HIVRAD-funded project, which focuses on two core activities:

1. Induce conformational changes in Env that expose conserved neutralizing Env epitopes. Previous studies showed that structural modifications to gp120 (such as beta-sheet deletion or global deglycolization) and complexing with CD4 will neutralize both homologous and heterologous HIV-1 isolates. These immune-stimulating complexes (ISCOMs) elicit NAbs in rabbits. Current efforts aim to determine whether anti-complex Abs are responsible for neutralization in human sera.

2. Evaluate prime-boost vaccine regimens using immune-stimulating complexes (ISCOMs) in NHP challenge studies. The goal is to identify the regimen that induces the greatest titer and breadth of NAbs, complemented by Ag-specific CD4+ and CD8+ cells. Results indicate that rAd5 prime and protein boost induce the best immune response, and that pseudovirus boost produces the highest Ab titer.

In response to questions, Srivastava explained that the rationale for deglycolization was to determine empirically which sugars are blocking access to binding sites. The goal is to determine experimentally whether chemokine receptors will neutralize the primary isolate; thus far, bs12 doesn't seem as important as E51, and there may be better binding sites in other regions.

Development of Novel Immunogens for Vaccines to HIV-1

Sean Du reviewed his HIVRAD project, a collaboration among Scripps Research Institute and three biotechnology companies, Aldevron, Maxygen and Monogram Biosciences. It too has two research projects supported by two cores:

1. Create and evaluate combinatorial glyco-variants of JRCSF Env gp140, using directed molecular evolution (DME) select and improve the best immunogens. From a library of 10^5 to 10^8 clones, investigators have identified the 99 most promising variants for testing in rabbits. Results are forthcoming.
2. Compile libraries of native Env spikes and develop improved variants as immunogens in a virus-like particle format. Parental genes were JRFL, JTRCSF and SF162 – all clade B R5 primary isolates. Of the 50 clones selected for study, 27 produce infectious virions and 17 are fully replication-competent; the 10 clones with the highest Ab titers are now being tested for neutralization sensitivity. This effort will include determining the crystal structure of full-length gp120.

In response to questions, Du said that DME technology mimics natural viral evolution and has previously been used to study Venezuelan, Eastern and Western equine encephalitis, dengue and hepatitis B viruses. Investigators have avoided any bias toward a particular structural pattern. The goal is to establish a structural basis for improved immunogenicity, and the ultimate target is the transmitted virus. Monogram is currently working on a transmitted panel, but it can't yet be identified from shuffled sequences.

Mechanisms of Protective Immunity Induced by Live Attenuated SIV Vaccines

Paul Johnson explained that live attenuated SIV (LASIV) has proven to be the most effective vaccine in the SIV/macaque model, providing sterilizing protection against intravenous, rectal and vaginal challenge. However, this protection is less effective against heterologous challenge, and protection appears to be time-dependent, decreasing with attenuation of the original vaccine strain. Previous studies of LASIV have been limited in design, assays and power, leaving questions about the role of NAb responses, ongoing Ag stimulation, site of immunization, and innate immune responses in mediating protection, as well as how to protect against heterologous challenge. Consequently, he and his HIVRAD collaborators are returning to LASIV with new tools and techniques arrayed in three central research projects:

1. Examine the mechanisms of mucosal protection induced by LASIV, including both adaptive and immune responses and the effect of prolonged B-cell depletion, with a particular emphasis on vaginal challenge and responses.
2. Elucidate the contribution of anti-Env responses to protection by LASIV, using complementary genetics approaches to study responses to closely matched and mismatched sequences.
3. Investigate mucosal immunity and heterologous protection induced by single-cycle SIV. For example, does the site of immunization determine the mucosal homing properties of virus-specific T cell response and the ability to protect against challenge? Would immunization with a mixture of antigenically diverse strains of SIV broaden the response and protection against heterologous challenge?

In response to questions, Johnson added that his group is coordinating their work with other HIVRAD groups as well as CHAVI, GHVE etc. There is a possibility that protection may decline due to clonal exhaustion, but this would take 5 to 7 years and can only be elucidated in a long-term study. The NHP and mouse models may be of little use in defining the correlates of protection in humans, but the emphasis should be on earlier and earlier stages of infection. There is no evidence of CD4 depletion, although there may be some CD20 depletion; they have not done a CD8 depletion experiment.

Mechanisms of Protection in Live Attenuated AIDS Vaccines

Chris Miller described his group's efforts to build on SIV-macaque results using live attenuated SHIV (HIV envelope, SIV core) to immunize against SIV challenge. This HIVRAD project has three projects to achieve three specific aims:

1. Define SIV-specific T cell and B cell responses in tissues of SHIV-immunized animals before and after challenge.
2. Define innate antiviral responses and T regulatory cell responses in tissues of SHIV-immunized animals before and after SIV challenge. Innate responses include Type 1 IFN and other effectors, proinflammatory cytokines and NK cells.
3. Define the identity, number and distribution of SHIV- and SIV-infected cells, SIV-specific T cells and T regulatory cells in tissues of SHIV-immunized animals before and after SIV challenge.

Results to date have shown that, in NHPs, the time of peak viremia varies depending on isolate and challenge. There is a clear benefit to vaccination, with 90 percent of tissues showing T cell activity. Immunized animals have a lower peak viremia and a lower setpoint, but some animals remain relatively unprotected. There is little Ab response in vaginal tissues, but vaccination does induce polyfunctional Gag-specific CD8+ T cells in the genital tract, along with TNF-alpha, IL-2 and IFN-gamma. Response in PBMCS is different from that in tissue and will be pursued in future.

In response to questions, Miller said that further study will be needed to clarify questions of infectivity. However, the virus that infects is not the virus that's later found in the blood. Progesterone appears to increase protection in females but reduce protection in males. Precise

interactions among site of immunization, route of challenge and level of protection remain to be determined. However, intravenous challenge seems to produce more or less the same response as intranasal and intrarectal – an initial viremic "blip" followed by long-term progression. Mesenteric challenge is technically difficult, and Miller hopes that other groups will attempt it.

HIV-1 Vaccine Based on Chimp Serotypes of Adenovirus

Hildegund Ertl explained that, after the discovery preexisting Ad5 immunity can block the effect of HIV vaccines based on the human Ad5 vector, researchers found that vaccines based on chimpanzee adenovirus (AdC) was not blocked. As a result, Ertl and her colleagues received a HIVRAD grant to develop AdC vectors as carriers for HIV immunogens. Work has been carried out several vectors – AdC68, AdC6, AdC7, and AdC1/C5 – showing that they are not affected by preexisting Ad5 immunity, they induce good T cell response in humans (but weaker B cell response than Ad5), they can be boosted to target the genital tract rather than the gut, and they induce dendritic cell maturation more efficiently than Ad5 vectors. Because the AdC vector persists in humans, there is a well-sustained CD8+ T cell response in the liver, spleen and muscles. Comparative studies have shown that effectiveness depends on prime-boost regimen, but Ad5 prime + AdC boost provides a better immune response than Ad5 + Ad5. Future plans include a final NHP trial to compare AdC7 + AdC6 against AdC6 + AdC7, a Phase 1 dose-escalation trial with AdC6 gag and AdC7gag, and a Phase 2 prime-boost trial with AdC6 and AdC7 both given twice. Investigators will continue to study the biology of AdC vectors, including replication-competent vs. replication-defective.

In response to questions, Ertl said that researchers are unable to explain the persistence of the AdC vectors. It doesn't seem to be from replication or recombination, and in any event it is below the level that FDA worries about.

Engineered Listeria Monocytogenes as an AIDS Vaccine

Fred Frankel reviewed the efforts of his HIVRAD partnership to evaluate vaccination strategies for attenuated *Listeria monocytogenes* (LM)-vector vaccines in mice and NHP. Animals are immunized three times (weeks 1, 6 and 19) either orally or oral prime + intramuscular (IM) boost, and followed to week 36. Results showed that the vaccine is safe in rhesus monkeys, with no viable organisms shed in stool and no diarrhea. Oral immunization produced T cell responses, while oral + IM induced Abs but not T cells. Overall T cell response was weak, even when the experiment was repeated with replication-competent and expression-boosted strains of LM. DNA prime + LM boost produces a much larger immune response, and there is no sign of oral tolerance.

In response to questions, Frankel said that investigators have not yet experimented with multigenic vectors, although they are currently working on tat, nef and gp120 immunogens. In mice, LM prime + Ad5 boost produced the greatest vaginal effect, particularly when the boost rather than the prime is vaginal. This regime produces gag-specific CD8+ T effector cells that persist as memory cells. These experiments have been repeated in NHP with the same results, and with protection against SIV challenge.

Herpes Viruses as Vaccine Vectors for AIDS

David Knipe described the efforts of his HIVRAD team to develop recombinant herpes simplex virus (rHSV) as a vector for HIV vaccine and to evaluate immune responses and protection in NHP immunized with rHSV. Infection with herpes simplex virus 2 (HSV-2) is a significant risk factor for HIV acquisition, but replication-defective HSV could provide a safe and persistent vector for an HIV vaccine due to its high level of foreign gene expression and wide tropism. In an early study, rhesus macaques immunized with HSV-1 vectors expressing SIV env and nef showed no detectable SIV RNA after rectal challenge with SIV five months after immunization. In a second study, rHSV-2 containing the d106 mutation (six immediate and early genes) expresses even fewer HSV proteins yet maintains high expression of SIV transgenes, inducing a immune response that reduces viral load in challenged NHP. The next study, using DNA prime + rHSV boost, produced robust anti-Gag and anti-Env cellular responses and significantly lower viral loads in immunized NHP. Immune predictors of viremic control were magnitude of anti-SIV NAbs during vaccine phase, presence of anti-Rev T cells on day of challenge, and rapidity of anti-Tat T cell response after challenge. The current study compares four prime + boost regimens of DNA and d106-rHSV-2 in six NHP each, using two primes (weeks 0 and 4) two boosts (weeks 12 and 24), and SIV challenge at week 36. Preliminary results parallel those of the smaller studies – immune responses from all four regimes, but the DNA + HSV group showed the highest magnitude of cellular immune response, plus weak neutralization activity and induction of lymph node responses, central memory CD8+ T cells. It remains to be seen how these differences in immune response predicate the outcome after SIV challenge.

Discussion

Participants expressed satisfaction that HIVRAD is achieving its goals, pursuing promising research in important scientific areas, and stimulating valuable information exchanges among HIV researchers. They suggested that it would be useful for a group of HIVRAD researchers to present to AVRS every six months, that the presenters be invited to attend both days of the meeting, and that the HIVRAD portion have a scientific focus (e.g., live attenuated vaccines). Alternatively, AVRS might sponsor theme-centered meetings among HIVRAD researchers. Crosstalk between the CHAVI and HIVRAD investigators is also desirable. It's difficult to build and maintain interactive teams; HIVRAD and other longitudinal funding mechanisms are a great help. Sponsors also need to be patient and reasonable – not all of these projects will lead to clinical trials, but all of them are valuable.

The meeting adjourned at 1:00 p.m.