

**National Institutes of Health
National Institute of Allergy and Infectious Diseases
Division of Acquired Immune Deficiency Syndrome
AIDS VACCINE RESEARCH WORKING GROUP**

**Part I: Summary of Workshop on Strategies to Elicit and Analyze
Mucosal Immune Responses to HIV/SIV**

May 25, 2006

The AIDS Vaccine Research Working Group (AVRWG) met in an open session on May 25, 2006, in Conference Room 2C-13 of the Fernwood Building, 10401 Fernwood Road, Bethesda, MD.

AVRWG members present: Scott Hammer (chair), Deborah Birx (ex officio), James Bradac (executive secretary), Susan Buchbinder, Salvatore Butera (ex officio), Karen Goldenthal (ex officio), Eric Hunter, Paul Johnson, Margaret Liu, Bonnie Mathieson (ex officio), Nelson Michael (ex officio), Gary Nabel (ex officio), Nina Russell, Jerald Sadoff, Steven Wakefield, David Watkins, and Ian Wilson.

Speakers:

- Susan Barnett, Novartis Vaccines and Diagnostics
- Igor Belyakov, National Cancer Institute
- Satya Dandekar, University of California at Davis
- Ashley Haase, University of Minnesota
- Paul Johnson, Harvard University
- Rupert Kaul, University of Toronto
- Normal Letvin, Harvard University
- Jiri Mestecky, University of Alabama at Birmingham
- Linda Saif, Ohio State University
- Barbara Shacklett, University of California at Davis

On May 25, 06 the AIDS Vaccine Research Working Group (AVRWG) workshop was held on “The Strategies to Elicit and Analyze Mucosal Immune Responses to HIV-SIV”. This workshop was a follow up of the previous AVRWG workshop held in January 2006 on the “The preservation of mucosal CD4⁺ cells as a determinant of vaccine and therapeutic efficacy”. The consensus of that meeting was that the preservation of memory CD4⁺ T cells in blood or mucosa was a determinant of the degree of protection. However, it is a challenging task to sample mucosal sites for the monitoring and assessment of T and B cell immune responses, and more specifically, to monitor intestinal CD4⁺ subsets. Hence the present meeting focused on further analysis and elicitation of mucosal cellular immune responses in NHP and humans immunized with the vaccine candidates.

Vijay Mehra summarized the previous workshop and introduced the goals of the present workshop, that were: (1) to determine the relevance of mucosal immune responses in protection against HIV; (2) kinetics of viral replication; (3) induction, and measurement of CD4⁺ subsets and antibody responses from the mucosal sites; and (4) key challenges of sampling mucosal sites and how to address them.

This report presents the summary of the individual presentations, the broad lessons of the workshop, challenges faced for the analysis of vaccine-induced immune responses, and efforts to address them.

Lessons learnt from enteric viral vaccines and gaps for HIV

Dr. Linda Saif led off the presentations by reviewing the rationale for a vaccine that induces mucosal immunity. She presented an overview of an enteric viral vaccine, specifically the studies conducted with rotavirus vaccine in gnotobiotic pig model which demonstrated that protective immunity to rotavirus-induced diarrhea results from production of IgA by antibody-secreting cells (ASC) and memory B cells responses in the intestines. Sequence and route of prime and boost played an important role in inducing intestinal immune responses. The most effective vaccine regimen for clearing rotavirus infections involved oral priming with the live virus followed by intranasal boost with non-replicating virus-like particles expressing VP2 core and VP6 inner capsid rotavirus proteins. It was shown that separate subsets of IgA memory B cells reside in the ileum and spleen, but only the ileal memory cells correlate with protective immunity.

The notion that immunization at any mucosal inductive site would trigger immune responses at any mucosal effector site has been challenged by studies with porcine models of gastroenteric (TGEV) and respiratory (PRCV) coronaviruses. Though recurring PRCV exposure stimulated adequate immunity to the virus in the upper airways, this respiratory immunization did not filter down to the gut and failed to protect from infection with TGEV in the intestine. Due to compartmentalization, vaccination at one mucosal site may not protect at a distant mucosal site. Lymphocyte homing experiments in animals showed that lymphocytes primed in gut-associated lymphoid tissues tend to migrate back to many mucosal effector sites (intestine, urogenital tract, mammary glands, salivary glands and respiratory tract) while cells primed in the upper

aerodigestive lymphoid tissues preferentially populate the salivary and respiratory tract, but traffic poorly to the gut. Thus discrete homing and trafficking pathways exist within the common mucosal system, and chemokines determine this migratory pattern.

Although the viruses mentioned above are quite different, Dr. Saif suggested that some of the lessons learnt from enteric viral vaccines can be applied to HIV. Since HIV is a mucosal infection, we should focus on the vaccines that induce mucosal immunity, and perhaps the goal should be to reduce the initial viral load. Hepatitis B and papilloma viruses may provide useful models, but experience has shown that even high levels of systemic immunity may provide little protection in the gut.

To target antigen delivery to mucosal inductive sites, the co-operative interaction between mucosal inductive (where immune response is initiated) and effector sites must be exploited by using prime/boost strategies and routes of administration that take advantage of the mucosal cell migratory pathways to produce the optimal immune response. Also, at present only a small number of adjuvants are available for inducing mucosal immunity, so this list needs to be expanded.

Assessment of Cellular Immune Responses at Mucosal Sites

Dr. Ashley Haase started this segment by discussing *in situ* tetramer staining technique that permits visualization of SIV-specific CD8⁺ T cells. Tetramer staining in combination with *in situ* hybridization, can be used to determine the location of SIV-infected cells. Combined together these methods acts as “microscopic GPS systems” to permit both the imaging of the spatial relationship between SIV-specific CD8⁺ T cells (CTLs) in various tissues to SIV-infected cells at various times of infection, and quantitation of effector (antigen-specific CTLs) and target (virus-infected cells) ratios within the tissues. The analysis of fresh vaginal tissue from over 50 monkeys has confirmed that unaided CTL response is too little, too late to prevent the loss of CD4⁺ T cells, and damage to mucosa. The peak viral replication occurs about 10 days post-infection, and by day 13 the population of activated CD4⁺ T cells is essentially depleted. HIV-specific CTL response does not appear until day 21, and by day 28 the local infection is under control, but the virus has already established itself in remote tissues.

These techniques have provided a better understanding of SIV/ HIV-1 pathogenesis, and are pivotal for developing and assessing efficacy of SIV/HIV-1 vaccine candidates. **However, a major limitation of these staining methods is that fresh unfixed tissue specimens are required to obtain optimal results.**

Dr. Paul Johnson discussed the status of potential homing markers for gut/genital homing lymphocytes and their utility as surrogate markers. Activated B and T cells express tissue-specific adhesion molecules and chemokine receptors that guide the homing of the lymphocytes to the distant mucosal effector sites through engagement of the corresponding ligands on the mucosal vasculature. The ability of HIV/SIV-specific T cells to home to the mucosal sites of viral replication plays a critical role in their ability to control viral replication. Gut homing CD8⁺ T cells gut-homing effector T cells selectively express $\alpha 4\beta 7$ integrin on their cell membrane. He also cautioned against the

use of only one marker as a determinant of homing specificity, rather advocating staining for a combination of molecules that act as “molecular zip codes” to dictate lymphocyte migration. For example, for T cells to target the respiratory tract both $\alpha_4\beta_1$ and CCL10 must be co-expressed which bind to their respective ligands VCAM1 and CCL28.

Pinch biopsies from inoculated macaques showed that $\alpha_4\beta_7$ CD8⁺ T cells do indeed migrate to gut-associated lymphoid tissues (GALT). However, this marker plays no role in genital mucosal homing, where the upregulation of $\alpha_E\beta_7$, CXCR3 or CCR5 may prove to be better homing markers. In each case, enrichment of the homing marker in the target site does not prove a causal relationship or a homing behavior. However they were able to observe specificity in the trafficking of activated cells within seven days after vaccination. Dr. Johnson commented that inducing and following specific cells *in vivo* would be a rather difficult task. However he suggested that to examine the behavior of infected cells *in situ* might be more feasible.

To track homing of lymphocytes, endoscope guided gut biopsies can be performed that do not require animal sacrifice, and hence, samples can be obtained multiple times, allowing longitudinal studies to be performed, and correlated with vaccine efficacy. In macaques vaccinated with an attenuated SIV, which replicates preferentially in the gut-associated lymphoid tissues, SIV-specific CD8⁺ T cells expressing $\alpha_4\beta_7$ integrin were detected both in peripheral blood as well as in gut biopsies but not in the peripheral blood of monkeys primed intramuscularly with DNA and boosted intradermally with MVA. Interestingly, at six weeks post MVA boost, the levels of SIV-specific cells in blood declined but decay rates in vagina and rectum were slow.

Thus analysis of $\alpha_4\beta_7$ expression provided some limited information about lymphocyte homing but it may not serve as a useful surrogate marker. Another unresolved question is the extent to which $\alpha_4\beta_7$ expression determines homing of long-term memory T cells to mucosal sites.

Dr. Mestecky summarized the studies to measure mucosal antibody responses. As HIV transmission occurs primarily across genital mucosal surfaces, inducing neutralizing antibodies directed against the virus at these vulnerable barriers may instantly halt the spread of the virus. He asserted that mucosal antibodies are the only molecules that can prevent HIV infection. While IgA isotype predominates in most mucosal secretions, the genital tract also contains a high proportion of IgG antibodies. Both intranasal and systemic immunization can produce genital immunity, and the genital tract also benefits from antibodies in circulation from other sites. Due to the absence of inductive mucosal sites in the human genital tracts, local humoral responses in the genital mucosa are weak and large fraction of antigen-specific IgG antibodies originate in the serum. In addition, the immune responsiveness of the female genital tract depends on the stage of the menstrual cycle. Hormonal changes regulate immunoglobulin levels and isotypes, and the response to vaginal vaccines. HIV-specific antibodies have been found in plasma, urine and vaginal wash. In chronic infection, the antibody secreting cells (ASCs) are present as much as 12 years after infection, yet they disappear within 10 days after immunization. The dominant HIV-neutralizing antibody is IgG, and IgA response is

consistently lower than IgG. It is unclear whether HIV damages the IgA response, or whether there is a selective IgA hyporesponsiveness in exposed individuals.

Dr. Jiri Mestecky urged the use of proper controls, and that the sample collection and processing should be performed stringently, otherwise the results of immunoglobulin levels will be skewed nonspecifically.

Dr. Satya Dandekar illustrated the promising rewards of applying high-throughput DNA microarray technology to assess gut-associated mucosal immune responses in HIV vaccine studies. Her studies showed that while DNA microarray technology allows us to obtain multiple measures from a small number of cells, however, sample selection, processing, and access to an expert in bioinformatics and biostatistics are crucial to the success of the technology. Challenges of applying this technology include limited access to mucosal samples, sample size, small numbers of cell populations, as well as the perception of the technology being a “fishing expedition”. Microarray technology is well-designed to extract multiple measures from small sample size, and can be extended to large cohorts. Using this technology Dr. Dandekar’s group showed that despite the containment of viral loads and preservation of immune functions in long-term non-progressors (LTNPs), downregulation in expression of genes associated with cell cycle regulation, lipid metabolism, epithelial repair and regeneration, nutrient digestion and absorption occurred in both LTNPs and chronically HIV-1 infected patients, and in macaques with high viral loads. Dr. Dandekar added that the one patient who became LTNP had a particularly low gene expression for inflammatory response. There have been no studies of nutritional deficiencies in infected patients, but they generally do poorly with gastrointestinal infections. It would be useful to compare gene expression patterns in GALT and PBMCs.

The functional genomic analysis provides evidence of impairment of the gut environment in LTNPs, and likely reflects either the effects of ongoing low-level viral replication or damage during primary HIV infection. To influence the outcome for HIV-infected patients, anti-viral therapy must be supplemented with growth factors, anti-inflammatory agents, and nutritional support for these individuals.

Challenges of Sampling Mucosal Sites

Dr. Barbara Shacklett discussed the challenges of sampling and monitoring mucosal sites for cellular immunity. She is concentrating on developing and optimizing techniques for assessing antigen-specific T cell responses in the GALT. Biopsies are obtained by Flexible Sigmoidoscopy, a minimally invasive procedure, but the sample size is small, yielding low number of cells, viability of cells is poor. Monitoring is further complicated by the presence of microflora at intestinal sites, the effects of hormones on cervical sites, and lack of experience with male reproductive sites. Most assays require multiples of 10^6 cells, the exception being tetramer staining (0.5×10^6 cells). The preferred assays, ELISA and cytokine flow cytometry (CFC), require on the order of 2×10^6 cells.

Dr. Shacklett characterized CD8⁺ T cells from genital mucosa of chronic HIV-infected cells by multi-color flow cytometry on freshly isolated cells alongside polyclonally expanded population that overcomes the barrier of low cell numbers from biopsies. Both rectal and blood compartments share considerable overlap in immunodominant epitopes, but rectal CD8⁺ T cell responses were greater in magnitude. Functional analysis of the Gag-specific CD8⁺ T cells revealed that rectal mucosa contained an elevated frequency of multifunctional CD8⁺ T cells capable of secreting cytokines IFN γ , TNF α , IL-2, MIP -1 β , and degranulation marker, CD107. **An apparent relationship was observed between CD8⁺ T cells functional complexity and the clinical status of the person. Individuals with low viral load seemed to have more functionally complex CD8⁺ T cells.** Five-function analysis, while not yet perfected, suggests that patients with low viral loads exhibit a more complex CD8⁺ response that can be strikingly different from that of patients with high viral load. The differences between the two will be informative as assays improve.

In the acute phase of HIV-1/SIV infection, gut mucosal CD4⁺ T cell population becomes rapidly depleted, followed by a rapid influx of CD8⁺ T cells. The efficiency of these CD8⁺ T cells in combating the viral infection remains unknown. Surprisingly, in contrast to blood, the gut associated lymphoid tissues harbored HIV-specific CD8⁺ T cells expressing low perforin levels but normal granzyme A levels. However, the correlation between perforin secreting cells and viral load has yet to be determined. Either the CD8⁺ T cells trafficking to the gut mucosa belong to a different effector memory class or tight regulation of certain effector pathways occurs to maintain homeostatic balance in the gut. Already evidence of different trafficking pattern of memory cells has been accumulated. Cytomegalovirus (CMV)-specific memory cells occur in high numbers in HIV patients, but these CMV-specific CD8⁺ T cells do not traffic to the rectal mucosa. Thus all cells present in blood do not traffic to the gut mucosal compartment.

Dr. Rupert Kaul reviewed the challenges in measuring immune responses in the genital tract. He discussed studies conducted in Pumwani cohort to measure cervical HIV-specific CTL responses. In these studies HIV-specific CTL responses were detected in genital tract of both HIV-infected and exposed uninfected subjects, but the role of these CTLs in controlling viral shedding at mucosal surfaces or mediating protection against infection has not been elucidated. In HIV-infected men no association was observed between frequency of semen CD8⁺ IFN- γ response and HIV-1 RNA shedding in semen, but the total number of CD8⁺ T cells and levels of inflammatory cytokines in semen was associated with higher levels of HIV-1 RNA shedding in semen.

He addressed some of the difficulties in measuring weak T cell responses, especially in the context of clinical trials, and emphasized the need to develop more sensitive, reproducible T cell assays. His assessment was that the current assays (ELISPOT, ICS, and tetramer) lack sensitivity to measure low levels of CD8⁺ responses that are typical of current vaccines. No assay is currently available to monitor semen or penis samples.

Dr. Kaul suggested that coinfection with herpes simplex virus (HSV-2) imposes a further complication on the measurement of vaccine-induced CD8⁺ response. Also semen may

not be a perfect surrogate for the penile mucosa, but it's unclear where else to sample; perhaps new information will emerge from the circumcision trial in Kenya.

Dr. Kaul concluded that measuring genital immune responses in vaccine trials is currently not feasible. One possible solution is to determine in Phase I clinical studies whether vaccine-induced responses in blood reflect gut/genital responses, and if a positive association is observed, then perform the assays using blood. Failing this, new assays will be needed, based on either expansion/cloning of genital samples or new measurement techniques such as microarray technology.

Kinetics of viral replication and spread after mucosal exposure

Dr. Ashley Haase presented evidence that a narrow window of opportunity exists to contain the virus during the rapid acute phase of HIV infection. The earliest occasion to eliminate the virus is at the portal of entry where limiting both the size and expansion of the founder virus population will thwart seeding of distal sites, thereby foiling systemic infection. But these opportunities are lost within the first week of infection in the macaque model of Simian Immunodeficiency virus (SIV) infection. Data from monkey studies demonstrate that despite a large SIV inoculum, the mucosal barriers have shown extraordinary effectiveness in reducing exposure of target cells. Within the first 24 hrs after SIV delivery, virion RNA levels in cervicovaginal tissues are several orders of magnitude lower than the inoculum. At days 3-4 post-infection, virus is limited to rare foci in the genital tract. The immune system guards mucosal front lines from infection by richly supplying these areas with CD4⁺ T cells, which are ripe targets for the virus to establish infection. At days 4-6 post-infection greater than 90% of resting CD4⁺ T cells at the entry site become productively infected, which act as “amplifiers” broadcasting the infection to distal lymphoid tissues following the anatomic routes of dissemination. Once the virus successfully disperses to the distal sites, a reservoir of persistent viral production becomes established in the lymphatic tissue.

They observed that the peak of viral infection occurs at 10 days after intravaginal infection with SIV, and by day 14 the CD4⁺ T cell population in lamina propria is nearly wiped out. Virus-induced apoptosis of CD4⁺ T cells and epithelial cells lining the small and large intestine irreversibly damages the gut mucosa and gut-associated lymphoid tissues. Driven by the peak viral replication, CD8⁺ T cells population expands but by then it is far too late, even then insufficient in magnitude to control viral replication. At 4 weeks post-infection robust CD8⁺ T cell responses to both Gag and Tat are noted, that correlate with a progressive decrease in number of infected cells in tissues. This vigorous response turns out to be of sufficient magnitude to partially control virus by over two orders of magnitude at the portal of entry. At 15 weeks post-infection, Gag response persists but mutation in Tat results in markedly diminished Tat responses, eventually leading to disappearance of Tat-specific response. **If virus-specific CD8⁺ T cell response can be evoked in the first week of viral rather than day 14, then the host may achieve a favorable outcome.** To counteract the intense immune activation occurring during days 7 and 28 post-infection, a period of peak viral replication, T regulatory response amplifies to rapidly dampen host defenses. **Thus for the host a partial virus control is the best achievable outcome.**

Inducing Mucosal Immunity to SIV/HIV-1

Successful mucosal immunization can be achieved by

- (i) Effective delivery of antigen to the mucosal immune induction site
- (ii) Enhancing mucosal immune responses through the use of safe mucosal adjuvants
- (iii) Adopting a strategy and routes of immunization capable of inducing protective responses not only at the desired mucosal site but also systemically
- (iv) Choosing an adequate formulation for the vaccine during optimization of the mucosal immunization regimen

Dr. Norm Letvin discussed number of potential vectors for induction of mucosal immune responses including recombinant vesicular stomatitis virus, adenovirus, mycobacteria, enteric bacteria, and plasmid DNA. Studies have already shown that it may not be necessary to have a mucosally delivered vaccine to generate mucosal T cell and antibody responses. For instance, DNA prime /MVA boost or DNA prime/Ad5 boost in rhesus monkeys, through the systemic route, elicited robust mucosal T cell responses.

Early studies at Wyeth with *vesicular stomatitis virus* (VSV), a mucosally transmitted rhabdovirus, showed significant neurovirulence with intranasal administration, but attenuated VSV showed a good safety profile in animal models. VSV-based HIV vaccine candidate is scheduled to be in Phase 1 human trials in Q1 FY2008.

Several serotypes of recombinant adenovirus (rAd) are being studied by Merck, VRC, and others. Replication-incompetent rAd5 has produced a good response in nonhuman primates following mucosal administration, and rAd41 has produced good response in mice in a prime-boost regimen with rAd5. Since pre-existing vector immunity limits the usefulness of Ad5 vectors, Gary Nabel and his colleagues are developing a novel vector based on serotype Ad41.

Marjorie Robert-Guroff has demonstrated in macaques that the replication-competent rAd is a promising oral vaccine and is expected to induce mucosal immunity. It is still in the early stages of development but is expected to move forward to Phase I trials.

Bart Haynes and colleagues at Duke have used *Mycobacterium smegmatis* and BCG constructs expressing HIV env and observed good T-cell responses in the reproductive tract of female mice.

Enteric bacteria such as salmonella and shigella have not been consistently immunogenic as antigen delivery vehicles in animals due to the unavailability of appropriate animal models, but they remain a viable choice to be explored further for inducing mucosal immune responses. In response to questions, Dr. Letvin added that, while it is rational to expect these vectors to work, there is no experimental data to show that they will induce mucosal immunity.

Adjuvanted *DNA* vectors delivered intravaginally have been shown to elicit T cell responses in mice.

Dr. Letvin also discussed the sites of mucosal sampling and their limitations. Mucosal sampling for detecting antigen-specific mucosal T-cells is done from the distal colon, duodenum, vaginal vault/cervix, and broncho-alveolar lavage. He emphasized several challenges faced in obtaining sufficient samples, including the small biopsy size, small number of T cells, their fragility, and heterogeneity of cell populations in different mucosal sites. In addition, the background signal is high, making it difficult to measure antigen induced responses. At present the most feasible assays are tetramer staining and ICS.

Dr. Susan Barnett reported vaccine/challenge studies in macaques conducted at Novartis Vaccines and Diagnostics (NOVAD, formerly Chiron) to develop a HIV vaccine based on a chimeric alphavirus vector VEE/SIN (V/S) derived from Venezuelan Equine encephalitis (VEE) and Sindbis (SIN) viruses, expressing HIV envelope plus SIV Gag for priming and envelope protein for boosting. Using combinations of mucosal and intramuscular routes of immunization for prime and boost they aimed at inducing both cell-mediated antiviral responses and neutralizing antibodies that would prevent dissemination of the virus to secondary sites as well as control infection. Macaques vaccinated via intranasal, intrarectal or intramuscular priming routes followed by intramuscular boost with Env protein, were subsequently challenged 4 weeks later intrarectally with SHIV. Although all routes induced neutralizing antibodies, the highest magnitude was observed with the intramuscular route. **The systemic route also resulted in the most dramatic reduction in viral load.** The amounts of pre-challenge neutralizing antibody titer inversely correlated with the acute phase viral load. In addition, adjuvant LTK63 (detoxified *E. coli* labile enterotoxin), was evaluated in mice using intranasal prime/ intramuscular boost. HIV Env adjuvanted with LTK63 induced high-titer vaginal IgA and serum IgG antibodies in mice. This immunization strategy will be tested in a phase I clinical trial sponsored by EU.

Dr. Igor Belyakov summarized his several published studies to suggest that vaccine-induced, high-avidity CD8⁺ CTLs can delay viral dissemination from the mucosa. In murine models, rectal but not subcutaneous immunization induced HIV-specific CD8⁺ lymphocytes at mucosal sites that protected against mucosal viral challenge with recombinant vaccinia virus expressing HIVgp120. Furthermore, the antigen-specific IFN γ secreting T cells elicited in the gut of macaques by the rectal vaccination with HIV Env, SIV Gag and Pol peptides significantly reduced viremia after rectal challenge with pathogenic SHIV.

In monkeys, a viral peptides prime followed by poxviral (NYVAC) boost slowed the spread of SHIV from the gastrointestinal tract to the peripheral tissues. Significantly, a strong inverse correlation was noted between vaccine-induced CD8⁺ T cells in the colon before challenge and the viral load in the blood after challenge; there was no such correlation between serum CD8⁺ T cells and viral load. Incorporating cytokines such as IL-12 or GM-CSF, CpG adjuvant further augmented the immune responses. These

findings suggest that vaccine-induced, high-avidity CD8⁺ T cells in the mucosa, are the key to delaying viral dissemination and may make it possible to eradicate the infection before it becomes systemic. These observations suggest that the mucosal CD8⁺ T cell responses may be important in controlling mucosally acquired HIV-1 infection.

Conclusions of the workshop

Dr. Johnson identified a number of important points made during the workshop:

1. Mucosal immune system is compartmentalized - oral, nasal, gut, genital. The diversity and complexity of immune responses exists not only between compartments but also within compartments and there is need to appreciate the differences between and within them.
2. Mucosal T cell responses display similar specificities to those observed in PBMC, but may differ in magnitude, decay rates (rapid in peripheral blood, slow in rectal mucosa), and function (virus-specific T cells express low levels of perforin), reinforcing the need for mucosal sampling.
3. Virus-specific B cell responses in mucosal tissues are short-lived.
4. The long-term memory responses for both B and T cells need to be examined as AIDS vaccine ought to induce long-term humoral and cellular immunity.
5. The site of priming matters for prime/boost regimens designed to induce mucosal immune responses. Mucosal (intranasal) priming followed by systemic (intramuscular) boost appeared to achieve the most effective mucosal immunity.
6. The tolerogenic environment of the gut may suppress the development of effective antiviral responses.

Recommendations for Research Priorities:

1. Better characterization of factors affecting induction, trafficking, and maintenance of T and B cell responses at mucosal sites, especially long-term memory responses.
2. Development of better and novel vectors able to induce mucosal immune responses.
3. Development of improved techniques for analysis of mucosal immune responses.
4. Establishment of consensus protocols SOPs for collection and processing samples.
5. Need to develop better models of male transmission and analysis of immune responses in male reproductive tract.
6. Rigorously establish whether induction of virus-specific memory T cells at mucosal sites improves protection against or control of SIV infection.
7. Continued exploration of novel mechanisms of protection at mucosal sites - use of microarrays, analysis of innate immune responses, etc.
8. **Maybe Program Staff can help improve cross-talk between basic immunologists, particularly mucosal immunology and AIDS immunologists/vaccinologists, to especially facilitate translation of lessons learnt from one system to another.**

In conclusion, though progress has been made in understanding the role of mucosal immunity in HIV infection, developing immunological technologies and assays that can be applied to monitor mucosal tissues has been a challenging task due to difficulties of

obtaining tissue samples, small sample sizes, and heterogeneity of cell populations in different mucosal compartments. Thus there are several challenges to sampling and assessment of mucosal immune responses that need to be addressed, including development of more sensitive T cell assays that can be performed using small numbers of cells, search for surrogate markers, and design multiparameter analysis.

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**May 26, 2006
10401 Fernwood Road, Bethesda, MD**

Part II: MEETING SUMMARY

The AIDS Vaccine Research Working Group (AVRWG) met in public session on May 26, 2006, in Conference Room 2C-13 of the Fernwood Building, 10401 Fernwood Road, Bethesda, MD.

AVRWG members present: Scott Hammer (chair), Deborah Birx (ex officio), James Bradac (executive secretary), Susan Buchbinder, Salvatore Butera (ex officio), Karen Goldenthal (ex officio), Eric Hunter, Paul Johnson, Margaret Liu, Bonnie Mathieson (ex officio), Nelson Michael (ex officio), Gary Nabel (ex officio), Nina Russell, Jerald Sadoff, Steven Wakefield, David Watkins, Ian Wilson.

Presenters:

- AVRWG members Susan Buchbinder, R. Paul Johnson, and Nelson Michael.
- Mary Allen, Vaccine and Prevention Research Program (VPRP), Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID);
- Jorge Flores, VPRP, DAIDS, NIAID;
- Barney Graham, Vaccine Research Center, NIH.

Dr. Hammer called the meeting to order at 8:30 a.m. and reviewed the agenda.

VPRP Preclinical/Clinical Timelines

Jorge Flores reviewed the timelines for ongoing preclinical and clinical vaccine trials. Merck's four rAd5 vaccines have completed Phase I, the first Phase II trial (HVTN 502) has begun in the Americas, and a second Phase II trial (HVTN 503) will begin 2007 Q3 in South Africa. VRC's DNA/rAd5 candidates have completed a series of Phase 1 trials since 2001 with an eighth currently underway; advanced development will culminate with the PAVE 100 commencing 2007Q3. The RV144 Phase 3 trial in Thailand completed enrolment in November 2005; the independent Data Safety Monitoring Board (DSMB), which recommended continuation in October 2005, will meet again in June 2006. Efficacy data should be available from RV144 in mid-2009, from HVTN 502 in mid-2010, from HVTN 503 at end-2010, and from PAVE 100 at end-2011.

Six other products are in Phase I or II trials, some of them prototypes being tested to see if they are worthy of further development. A number of additional products are in the preclinical pipeline with IND applications expected between 2006 Q4 and 2007 Q4.

Clinical Trial Updates

Susan Buchbinder described the status of HVTN 502, which is testing the Merck Ad5 trivalent vaccine against clade B HIV. Screening and enrollment are underway in the United States, Canada, Puerto Rico, Dominican Republic, Haiti, and Peru, and will begin soon in Brazil, Australia and Chile. At an eventual rate of 200 per month, enrollments should reach 3,000 by end-2006. Subjects are diverse, although males are largely homosexual and females largely heterosexual. Ad5 titers have been 57 percent high (>500) and 43 percent low (<500), with a goal of 50 percent in each arm. Interim analysis for fertility and efficacy will be conducted after 30 endpoints in each arm, probably mid-2007 to early 2008.

Mary Allen described the status of HVTN 503, which is testing the Merck trivalent DNA vaccine against clade C HIV at three high-incidence sites in South Africa. This trial will enroll more women and adolescents than HVTN 502, with an eventual total of 3,000 subjects between 18 and 35, more than 50 percent women, and more than 40 percent with baseline Ad5 NAb less than 200. Endpoints include likelihood of infection and viral setpoint after seroconversion. Initial analysis for immunogenicity will take place after 600 volunteers are enrolled and 300 vaccinated, including 180 with high Ad5 NAb titer and 60 with low titer. Interim analysis for primary efficacy (infection) will occur when 60 cases of HIV infection have occurred, and interim analysis for secondary efficacy (setpoint) will occur after 35 cases of HIV infection have occurred in subjects with Ad5 NAb titer <200 and viral loads are available.

In response to questions, Ms. Allen said that the go-no go decision would be based on the percentage of responders (ELISpot) both to the clade B and C peptide pools. Merck has done variability studies on ELISpot in South Africa and will publish the results. Memory T cells were not included as a secondary marker of immunogenicity because of the lack of a validated data. Working Group members suggested that investigators reevaluate the criteria for 503 based on the interim efficacy results for 502, which are due in early 2007.

Summary and Recommendations from HVTN NHP Workshop

R. Paul Johnson presented the results of this workshop, held in April, 2006, the purpose of which was to discuss the use of the nonhuman primate model (NHP) for making decisions on moving vaccine products into Phase I clinical trial.

During the meeting, two breakout groups were formed to discuss the model for either immunogenicity testing or protection from virus challenge. In the immunogenicity group, a fairly good consensus favored NHP testing for immunogenicity prior to preclinical toxicology and GMP work. The vaccine should be the HIV product, not a prototype with SIV insert, and should be administered at the high-end dose by the same route as humans. Assays should be conducted at peak and plateau of infection, using standard assays that will allow for comparisons among products. The tests should set a low bar for immunogenicity – is the product immunogenic? – rather than a definitive test or ranking against other products.

There was less consensus in the challenge group. There was general agreement that the current R5 SHIV strains are not the best challenge model for T cell vaccines, but less agreement on which challenge strain should replace them. Perhaps 80 percent of participants favored SIVmac 251, which is difficult to neutralize, over SIVmac 239 or E660. There was disagreement over route of challenge, as well, with 60 percent favoring intravenous (simpler and more reproducible) and 40 percent favoring rectal (more relevant).

There was consensus on the desired endpoint, namely a one-log reduction in set point viremia. Participants did not address timing, although implicitly this should be fairly early, perhaps 8 to 12 weeks. For mucosally administered vaccines, the group favored the same SIVmac 251 strain, preferably with intrarectal or intravaginal challenge, and the same endpoint. In the end, participants recognized that NHP testing should serve as an initial screen before prototype vaccines go to Phase 1 human trials. Macaques are not people, so NHP trials cannot rank human vaccines. Instead, there should be an iterative process between NHP and human trials to establish correlations.

In the discussion that followed, Dr. Hammer said that AVRWG hopes to develop this workshop report into a peer-reviewed document. It will have an appropriate comment period because of its potential impact on vaccine researchers and developers. Dr. Watkins agreed with the need for a better challenge strain and asked if it might not be useful to have a wider range of challenge. What's really needed is an SIV that behaves like HIV. Dr. Sadoff suggested that NHP challenge be a screen for Phase 2, rather than Phase 1, so as to avoid delaying the development process.

U.S. Military HIV Research Program

Nelson Michael reported on USMHRP, which partners with, NIAID, CDC, and the Department of State, as well as academic, governmental and NGO entities worldwide. The vast majority of its activities are Phase I, II and III trials in Thailand and East Africa, with the focus currently shifting to vaccines based on DNA + rAd5. In Thailand, a Phase III trial of canarypox vaccine with gp120 protein boost has completed enrollment and will be giving injections in June and July, with evaluation scheduled to be completed by July 2009. In Africa, it will be possible to test vaccines against a broader range of HIV genotypes in populations where incidence ranges from 1.3 to 1.8 percent. Cohort development is underway in Uganda, Kenya and Tanzania. DNA + rAd5 trials currently underway for RV 156 (Phase I, 31 volunteers), RV 172 (Phase I/II, n=324), and PAVE 100 (Phase 2b, n=9,000 to 12,000), the latter in collaboration with HVTN and IAVI. In addition, USMHRP is preparing to initiate a series of clinical trials to test prime/boost regimens of various DNA constructs and the modified vaccinia Ankara (MVA) vaccine developed by Walter Reed and NIH. Data from the Phase I trial, RV 158, will be presented in Amsterdam in August/September 2006. Additional DNA+MVA trials are being conducted in collaboration with the Karolinska Institute.

In response to questions, Col. Michael reported that CTL responses are strong in several of these studies. He explained that volunteers are civilians rather than military personnel, although some of them are drawn from military families. Subjects are re-consented for

rollover from DNA to MVA boost. The current assay used to measure immune responses is chromium release cytotoxicity, which while old-fashioned is well-established; USMHRP will eventually migrate to newer validated assays. Endpoint analysis will employ newer, standardized assays.

VRC Clinical Trials Update

Barney Graham reported on the status of two VRC pilot studies, VRC 004/009 (four-plasmid DNA prime, rAd5 boost at month 24, N=10) and VRC 007/009 (six-plasmid DNA prime, rAd5 boost at month 9, N=4). Preliminary results show a stronger response to DNA+rAd5 than to DNA or rAd5 alone, with a four-log boost in specific antibodies following rAd5 boost. Neutralizing titers remain low, possibly below the effective threshold. There is a threefold decrease in the response to rAd5 boost in subjects who are Ad5 seropositive. In general, subjects that respond more strongly to prime will also respond better to boost. Several additional studies are planned to study different doses and routes. Data are not yet available to compare four- and six-plasmid primes; that data will be available in July.

Plans for AVRWG session in Amsterdam

AVRWG will meet for three hours on August 29 before the opening session of the vaccine conference. DAIDS staff will work with Dr. Hammer to arrange the agenda for that meeting.

The meeting adjourned at 12:15 p.m.