National Institutes of Health
National Institute of Allergy and Infectious Diseases
Acquired Immunodeficiency Syndrome Research Advisory Committee
AIDS Vaccine Research Subcommittee

Natcher Building, Rooms E1-E2
45 Center Drive, NIH
Bethesda, MD
February 6, 2013

MEETING SUMMARY

The AIDS Vaccine Research Subcommittee (AVRS) met in open session on February 6, 2013 in Bethesda, MD for presentations by the two newly funded Centers for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID). CHAVI-ID is the follow-up research consortium to CHAVI focused on immunogen design and funded by a seven-year award from NIAID. The research consortium is dedicated to immunological research directed at tackling the major scientific problems that hinder HIV vaccine design.

CHAVI-ID has been established at two centers: The Scripps Research Institute (TSRI) led by the principal investigator, Dennis Burton, Ph.D. and Duke University under the direction of Barton Haynes, M.D. CHAVI-ID encompasses investigators at several collaborating institutions.

WELCOME

The new AVRS Chairperson, Douglas Nixon called the meeting to order at 8:00 a.m. The Director of DAIDS, Carl Dieffenbach recognized the presence of two new members: Aftab A. Ansari, Ph.D., Professor of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA; and Donald Forthal, M.D., Associate Professor of Medicine, Division of Infectious Diseases, School of Medicine, University of California Irvine College of Medicine, Irvine, CA.

Dr. Dieffenbach acknowledged new ex officio members and the organization each represents at today's meeting: John Mascola, M.D., Acting Director, Vaccine Research Center (VRC); Bette Korber, Ph.D., Duke CHAVI-ID; Salvatore T. Butera, D.V.M., Ph.D., Scripps CHAVI-ID; Eric Hunter, Ph.D. and Rama R. Amara, Ph.D., Emory Consortium for AIDS Vaccine Research in Nonhuman Primates Dr. Dieffenbach also introduced to the audience the new Director of the DAIDS Vaccine Research Program (VRP), Dr. Mary Marovich.

DUKE CHAV-ID
Introduction to Overall Program
Barton Haynes, M.D., Director, Duke CHAVI-ID

Dr. Haynes presented an overview of the projects and teams, which compose the Duke CHAVI-ID, including the scientific leadership groups with two main foci: 1) the production of protective antibodies; and 2) the induction of protective T cell immunity. Dr. Haynes acknowledged Dr. Norm Letvin (1949-2012) for his founding work in HIV vaccine research, especially in the use

of nonhuman primates (NHPs) and for his enormous contributions to the CHAVI-ID initiative, both in the intellectual design and writing of the grant.

The scientific leadership group for the CHAVI-ID consists of Bart Haynes, Joseph Sodroski and Garnett (representing the Protective Antibodies group) and Andrew McMichael, Bette Korber and George Shaw (representing the Protective T Cell Immunity group). There are nine Scientific Research Support Components within the CHAVI-ID: Genomics (David Goldstein), Neutralizing Antibody (David Montefiori), Viral Biology (Beatrice Hahn), Structure-based B Lineage Immunogen Design (Steve Harrison), Mucosal Biology (Robin Shaddock), Antibody Sequencing (Andrew Fire), Computational Biology (Thomas Kepler, Alan Perelson, Bette Korber), Nonhuman Primates (Sampa Santra) and B Cell Biology (Garnett Kelsoe). The goal of the Duke CHAVI-ID is to design a preventive HIV-1 vaccine that will induce protective antibodies, especially broadly neutralizing antibodies (bNAbs) as well as T cell immunity.

Dr. Haynes stated a vaccine will not result from empirical studies as has been learned from the last 25 years of research. Scientists need to begin with basic science regarding, for example, the role of anti-HIV immune responses, and follow-up in the areas of immunogen design and formulation. The formulation of a vaccine will likely involve B and T cells as well as innate immunity. Dr. Letvin and others have demonstrated an innate cell population, apparently a subset of natural killer (NK) cells, which exhibits a type of immunological memory that may need to be harnessed as part of a HIV vaccine strategy (Gillard GO et al., *PLoS Pathog*. 2011 Aug). The Duke CHAVI-ID team interacts with many groups of similar interests, including the U.S. Military HIV Research Program (MHRP) and the Vaccine Research Center (VRC), NIAID/NIH.

T-cell Program Mosaic and Conserved Region Vaccine
Bette Korber, Ph.D., Laboratory Fellow, Los Alamos National Laboratory, Duke CHAVI-ID Computational Biology Scientific Research Support Component (SR SC), Scientific Leadership Group

Standing in for Dr. Andrew McMichael, Dr. Korber presented the two approaches to optimizing T cell vaccines: Conserved region vaccines (led by Dr. McMichael) and Mosaic full protein vaccines (led by Dr. Korber). Dr. Korber noted that the motivation for developing a conserved region vaccine derives from human biology and the relationship to HIV-1 specific T cell responses. Clinical studies carried out under the earlier CHAVI award have shown in acute HIV-1 infection, the escape rate of each epitope is determined by a pattern of immunodominance and epitope entropy (Liu MKP et al, *J Clin Invest*, 2013). Epitope entropy can be derived from an epitope database, showing the range of variability; the amount of diversity is used as a surrogate for the capacity of an epitope to tolerate mutational change at the population level. Low entropy values reflect greater conservation in the sites, and the CHAVI-led study provides insight into the effect of selective pressure by CD8$^+$ T cells on HIV-1, which turns over rapidly during early infection.

Thus, a desirable vaccine would focus immunodominant responses on low entropy epitopes and would ensure that these responses are activated early, thereby mimicking the protective responses associated with HLA B57, B58, B81 etc. The conserved region T cell vaccine is based on the design described in a paper by Letourneau et al (*PLoS One* 2:e984, 2007). The vaccine

design does not consist of an 'epitope string' rather each region is 26-130 amino acids (AA) long and can contain multiple epitopes. The conservation is less than 6 percent between clade consensus sequences. The gene coding for the HIV_{CONSV} protein was inserted into the three most studied vaccine vectors, plasmid DNA, human adenovirus serotype 5 and modified vaccine virus Ankara (MVA).

A small Phase I study was carried out testing the conserved immunogen approach with arms consisting of DNA/MVA/Chimp Ad, DNA/Chimp Ad/MVA, and Chimp Ad/MVA. And Dr. Korber presented the ELISpot responses for the simplest regimen, Chimp Ad/MVA. The details of the results will be given at Keystone 2013. The results potentially address the problems with the STEP trial rAd5 vaccine; unlike the rAd5 vaccine, the HIV_{CONSV} vaccine offers:

- Adenovirus with minimal sero-crossreactivity pre-vaccination
- T cell responses greater
- Focus on conserved not variable regions.

Also, both CD8+ and CD4+ T cell responses generated recognized HIV-infected cells and virus was inhibited by CD8+ T cells, particularly Pol-specific as well as Gag-specific T cells.

The mosaic vaccine is composed of polyvalent full-length proteins and is based on the design described in a paper by Fischer et al (*Nat Med* 13:100 (2007). Fischer et al reported how HIV evolution can be mimicked by *in silico* recombination derived from a genetic algorithm to optimize vaccine epitope coverage. Mosaics are *full-length* proteins that can elicit *both* B and T cell responses; while they are optimized for contiguous 9mer coverage, capturing local most-common combinations, the value extends to T cell epitopes of other lengths as well as B cell epitopes. Also, polyvalent mosaic vaccines yield more general reactive responses, and thus more "hits" per natural strain.

The breadth of Env-specific T lymphocyte responses was assessed in 12 monkeys from different experimental groups—number of peptide epitopes recognized per protein. The cellular immune responses elicited were determined by peripheral blood T lymphocytes recognition among 5 envelope sequences using a peptide/interferon-gamma (IFN-□) ELISpot assay. The peptides used for the ELISpot assays were designed to maintain their alignment so they could be directly compared between strains. In essence, HIV polyvalent mosaics elicit T cell responses with more breadth and depth.

Mosaic constructs will be tested in two Phase I trials: 1. HVTN 099a) three Mosaic Env, Con S, and T/F 1059.B, b) DNA prime, NYVAC boost; the antigenicity and immunogenicity of HVTN 099 trial materials has been confirmed in rhesus macaques. 2. IPCAVD/MHRP (Dan Barouch & Nelson Michael) —a) two Mosaic Env/Gag/Pol, and b) Ad/Ad or Ad/MVA prime boost HVTN 099 trial materials are all immunogenic, eliciting both T cells and B cell responses; trial results will probably be available in two years. It has been shown that presenting only conserved regions in a vaccine did not enhance the magnitude nor the number of responses to those regions--shifts in immunodominance to conserved region vaccines may, however, confer a benefit.

Future work:

- Complete Phase I studies
- Directly compare the impact of isolating regions with beneficial epitopes, to using full length proteins carrying those same epitopes, in monkey SIV challenge studies.

Trimer Structure
Joseph Sodroski, M.D., Professor, Microbiol.& Immunol., Dana-Farber Cancer Institute, Duke CHAVI-ID, Scientific Leadership Group

Dr. Sodroski's presentation centered on single-particle cryoelectron microscopy (cryo-EM) studies of the HIV-1 envelope glycoprotein trimer. The trimeric HIV-1 Env glycoprotein spike mediates virus entry into host cells and is the primary target for virus-neutralizing Abs. The trimer is metastable and is induced by receptor binding to go down an energy pathway used to promote HIV-1 entry. Cleavage of a trimeric glycoprotein precursor, gp160 results in three gp120 and three gp41 subunits. The three gp120 and three gp41 subunits form a cage-like structure with a vacant interior surrounding the trimer axis. Interprotomer contacts are restricted to the gp41 transmembrane region, gp41 ectodomain, and a trimer-association domain of gp120, containing the V1, V2 and V3 variable regions.

Single-particle cryo-EM was used to gain more information about the structure of unliganded Env trimer. Cryo-EM employs many images of purified protein complexes to reconstruct a 3-dimensional structure. From their studies, the scientists learned: 1) CryoEM can be used to quality control Env trimer immunogens; and 2) The gp41 transmembrane region contributes to interprotomer interactions in the HIV-1 Env trimer and its deletion can influence the structure of the ectodomain.

The investigators determined that broad and potent neutralizing antibodies have certain characteristics, such as the angle of approach to an epitope, minimal steric clashes with adjacent protomers, and as a result, less conformational change in the Env trimer is required for optimal binding. Dr. Sodroski offered the following conclusions:

- The architecture of the unliganded HIV-1 Env trimer
    - explains its metastable nature and helps to understand receptor triggering of virus entry
    - reveals viral defenses against the binding of neutralizing antibodies (quaternary constraints)
    - provides a reference for quality control of Env trimer immunogens
    - may assist efforts to stabilize the metastable, unliganded conformation of the Env trimer (particularly T/F Env trimers) for immunogen design.

Dr. Sodroski stated that even with a perfect trimer in hand, it still might not elicit the desirable types of responses. A combination approach to vaccine design is required—lineage based design should be done as a starting point. Researchers have shown that the ectodomain *changes* with respect to antigenicity when trimer cleavage occurs. Dr. Sodroski noted in their studies, a cleavage-minus envelope trimer on a cell surface under glutaraldehyde cross-linking renders the same antigenicity as a cleaved trimer. This suggests that trimer cleavage allows denser packing of trimer subunits and less conformational flexibility. The denser packing and decreased flexibility blocks the binding of non-neutralizing antibodies and increases the binding of neutralizing antibodies.

Antibody-virus Evolution/Virus Evolution of CH505/ Patients CH505 CH103 Clonal Lineage
Barton Haynes, M.D., Director, Duke CHAVI-ID and Bette Korber, Ph.D., Laboratory Fellow, Los Alamos National Laboratory

Drs. Haynes and Korber discussed antibody-virus co-evolution in acutely infected patients followed through to bNAb induction.
Dr. Haynes noted that the bNAbs isolated thus far have one or more of the following characteristics:

- Highly somatically mutated
- Long third complementarity-determining region of the heavy chain (HCDR3s)
- Polyreactive/autoreactive
- Restrictive VH (immunoglobulin variable heavy-chain gene) usage
- 30-40% of bNAbs have large Indels (insertions or deletions or combination of) > 3 nts.

What needs to be known about:bNAbs:

- We need to know bNAbs host control mechanisms
- We need to know if there are bNAb lineages that are more "normal"
- We need to find antigens that bind to clonal lineage members.

The strategy to learn about bNAbs involves a dataset of antibody lineages from chronically infected patients, allowing researchers to map the virus and antibody concurrently from the time of transmission. The CHAVI-17 virus/antibody evolution project has received specimens from subjects identified during acute HIV infection (AHI) located in eight African sites over the past seven years. The first 17 patients have been followed to bNAb induction and compared to 10 AHI subjects followed who have not developed bNAb activity. Single genome amplification (SGA) of viral sequences--about 400 to 500 over time—is performed. BnAbs or no breadth Nabs are isolated by memory B cell cultures using antigen specific probes and sorting. The lineages are then expanded by pyrosequencing.

Using bNAb isolates called CH103 and CH505 as examples, Drs. Haynes and Korber noted the evolutionary trajectory from the first autologous antibody responses against early/founder Env at 14 weeks post-infection (CH103) to heterologous neutralizing activity after several months. CH103 binds to the gp120 outer domain, and the diversity by six months in the CD4 binding region was greatest for CH505 among the 17 subjects followed from AHI. Viral evolution included immune evasion as shown with CH505 in which a CD8 T cell escape mutation occurred at week 14. The team found that unmutated common ancestors (UCA) frequently do not react with Env, and bNAbs take several years to develop.

The researchers concluded:

- The CH103 CD4 binding site antibody has fewer unusual traits compared to other bNAbs.
- Autologous neutralization activity appeared after 6.9% VH mutations; breadth at 13-16% VH mutations.
- The CH0505 transmitted/founder Env gp140 bound well to the UCA of the clonal lineage of antibodies and to all lineage members.

In the future, the plan is to:

- Map both virus and antibody evolution from the time of transmission to define the envelope changes that induce several types of bnAbs.
- Recreate this scenario with a vaccine + strong adjuvant.
- Key to study AHI through BnAb development

Dr. Haynes stated that in many chronically infected patients with high viral loads, by the time they developed bNAbs, the antibodies did not help much, particularly those who were usual or rapid progressors. Patients with hepatitis C (HCV) similarly have delay with autologous neutralizing antibodies; however, upon development of bNAbs, 70% of patients will control the virus for varying periods of time. The difference may lie in the fact that HIV is an integrating virus and the creation of the latent pool.

Transmitted/Founder Virus Biology
Beatric Hahn,M.D., Professor, Medicine and Microbiol., University of Pennsylvania, Leader of Virus Biology, Duke CHAVI-ID, SRSC

Dr. Hahn spoke about harnessing the phenotype of T/F HIV-1 for immunogen design. The overarching questions for Dr. Hahn's team are: 1) Are there any distinguishing biological features of the viruses that are transmitted through mucosal routes? 2) If so, could these properties be specifically targeted by a vaccine? The experimental approach is two-fold:

- Generate *env* genes (Envs) and full-length infectious molecular clones (IMCs) of transmitted founder (T/F) and chronic control (CC) viruses that are representative of major group M clades.
- Compare their biological properties in assays specifically designed to probe the earliest stages of HIV-1 infection.

The results from Env pseudotypes include the following:

- TF and CC Envs use CD4 and CCR5 equally efficiently (Keele, 2008; Wilen, 2011).
- TF Envs do not exhibit preferential tropism for particular CD4+ T cell subsets (Wilen, 2011).
- TF Envs do not exhibit enhanced sensitivity to neutralization (Wilen, 2011; Parrish, 2012).
- TF Envs do not interact with the integrin pair $\alpha 4\beta 7$ for cell entry (Parrish, 2012).
- Except for the preferential use of the CCR5 coreceptor, no other consistent phenotypic difference between TF and CC viruses has been identified.

Thus far, Dr. Hahn's team has concluded:

- TF viruses are slightly more infectious on a per particle basis, package slightly more Env, bind dendritic cells more efficiently and are relatively more resistant to IFN-α than CC viruses.
- Although these phenotypic differences do not completely differentiate TF and CC viruses, they likely act in concert to enhance cell free infection and virus replication in the face of an early innate immune response.
- Efforts are underway to harness these viral properties for immunogen design.

Humanized Mice--T follicular helper (TFH) CD4-T cells and bNAbs
Garnett Kelsoe, D.Sc., Professor, Immunology, Duke University, Duke CHAVI-ID Scientific Group, B-cell Biology SR SC

Dr. Kelsoe discussed immunization studies in humanized mouse strains—mice that carry human immunoglobulin gene loci. One of the problems in vaccine research has been finding suitable animal models for human studies. Mouse antibodies are substantially different from human serum proteins in their repertoire and forms. A solution is to place the human repertoire within the mouse, allowing the mouse to serve as a first-line investigative model. Three mouse lines are available for use:

1. 2F5, 4E10, and 48d knock-in mice
2. VelocImmune® (complete & V_H 1-69) mice
3. BAB5 YAC transgenic mice.

Dr. Kelsoe focused his presentation on the 2F5 and 4E10 knock-in (KI) mice as well as the VelocImmune® mice. The HIV-1 broadly neutralizing Ab 2F5 has been shown to be poly-/self-reactive *in vitro*, and it was previously demonstrated that targeted expression of its VDJ rearrangement alone led to B cell developmental blockade in 2F5 V_H KI mice resulting in central deletion of 2F5 H chain-expressing B cells.

The genes for the human Abs have been knocked-into the endogenous loci—the light chain allows for secondary rearrangements, known as receptor editing, and the location allows for somatic hypermutation. The 2F5 and 4E10 Abs represents the termini of B cell lineage, and the KIs represent the starting point. In addition to the B cell compartment, the mouse lines offer the capacity to study the interaction of these antigen-specific B cells with specific T cells.

H+L Double KI Mice characterization and analysis of the primary B-cell repertoire:
- 2F5 and 4E10 dKI mice carry human VDJ and VJ rearrangements at their proper genomic locations.
- 2F5 and 4E10 dKI mice exhibit a block in B-cell development.
- 2F5- and 4E10 GL KI mice have been generated.

One of the questions the team has attempted to answer is: If polyreactivity and autoreactivity are abundant in B cells, does that mean the corresponding epitopes cannot be incorporated into a vaccine design? The experimental results suggest the cells that escape into the periphery (10% of dKI 2F5 or 4E10 B cells) and do not spontaneously make antibody-like molecules, can be activated by immunization with liposome vaccine immunogens. The mouse models may also allow the identification of new receptors.

The conclusions thus far:

- Mice genetically modified to express humanized antibodies effectively mount serum antibody and cellular responses to HIV-1 vaccine antigens immunogens.
- Each of these models have some limitations, but offer substantial potential for identifying and developing vaccine antigens for additional testing in less tractable models.
- The cloning of Ag-specific, naïve B cells can aid the design of new HIV-1 immunogens.

Scripps CHAV-ID
Introduction to Overall Program/Broadly Neutralizing Antibodies
Dennis R. Burton, Ph.D., Principal Investigator, Scripps CHAVI-ID

The Scripps CHAVI-ID consists of two scientific Foci; Focus 1, Antibodies, headed by Dennis Burton and Michel Nussenzweig, and Focus 2, T cells, headed by Rafi Ahmed and Bruce Walker. The Scientific Leadership Group consists of Drs. Burton, Ahmed, Nussenzweig, Walker, and Dr. Ian Wilson. There are four Scientific Research Support Components; Data Management (Adam Godzk), Glycobiology (Chris Scanlan), Nonhuman primates (Dan Barouch and Guido Slivestri) and Vaccine Discovery (Julie McElrath). The central hypotheses of the Scripps CHAVI-ID are that a successful HIV vaccine should elicit protective antibodies, and that the combination of B cell and CD4+ T cell responses is critical for the induction and long-term maintenance of vaccine protection.

The overall mission of the CHAVI-ID is to define immunogens and immunization regimens that induce HIV cross-protective B cell and CD4+ T cell responses in preclinical models and thereby guide product development strategies for a preventative human AIDS vaccine.

The way forward is to examine the molecular interplay between the Env spikes and NAbs, taking cues from responsive infected individuals, and then using the understanding to design immunogens that when given to infected individuals would re-elicit bNAbs. The last three-four years have brought the isolation of many new bNAbs and thus potential targets so that the rules for immunogen design can be worked on.

Dr. Burton noted the examples of PGT121 and 126; two of the broadest and most potent Abs isolated to date. PGT121 is highly protective in the macaque model. Researchers have found inducibility is related to divergence from the germline along with breadth and potency. Deep sequencing has permitted researchers to note a positive correlation between the level of somatic mutation and somatic hypermutation (SHM) and neutralization (broader spectrum of HIV variants). SHM in either the heavy or light chain alone increases neutralization, which is then further increased when both chains are highly mutated. Neutralization with intermediate breadth and potency is achieved at levels of NAb SHM notably lower than the fully matured PGT121-4 mAbs. As the antibody matures, the neutralizing titer becomes even better, as if the antibody is being "honed" to converge on a highly conserved region or "sweet spot." Combination of Abs with moderate levels of SHM may give significant neutralization coverage of global isolates.

Mouse Models of Antibody Activity
Michel Nussenzweig, M.D., Ph.D., Rockefeller University

Dr. Nussenzweig discussed three CHAVI-ID projects: 1) Mouse models for HIV entry; 2) role of somatic mutation in antibody potency and breadth; and 3) human antibody "knock in" mouse models for vaccine development. The HIV *in vitro* entry system consists of an indicator, the luciferase gene, which has a "stop" in front of it so that it is turned off. A HIV pseudo-typed virus encoding Cre recombinase is used in the cell attack, and when Cre enters the cell, it finds the "stop", and the lights go on. The light emission can be quantitatively measured. The

receptors employed for HIV entry can be delivered by adenovirus or by transgene. In summary, the team has found:

- Adenoviral delivery of hCCR5/hCD4 allows assay of intravenous HIV challenge in mice and can be used to evaluate the *in vivo* potency of antibodies against entry.
- Transgenic mice that carry hCCR5/hCD4 and Floxed-Stop-Luciferase can be used to assay mucosal entry and to evaluate the potency of antibodies or drugs to block mucosal entry.
- Antibody concentrations required to block infection in mouse models is equivalent to macaque.

What has been learned from single cell antibody cloning?

- Memory B cell compartment contains up to 60 clones of anti-HIV antibodies many of which neutralize a fraction of viruses.
- Broad neutralization can be achieved by combinations of antibody clones or by individual clones.
- There are several different epitopes that can elicit broad and potent antibodies.
- High levels of somatic hypermutation are essential for broad and potent neutralizing activity for many of the antibodies.

The regions between the complementarity determining regions or hypervariable regions in the variable region are called the framework regions (FWRs). FWR mutation is essential for breadth and potency of the highly somatically mutated anti-HIV antibodies. FRW mutations alter essential contact residues and also enhance breadth by altering the shape or flexibility of the antibody.

Dr. Nussenzweig went on to discuss antibody therapy studies carried out in HIV-1-infected humanized mice (Klein et al, Nature, 2012) and combined ART and antibody monotherapy via AAV gene delivery.

Structure of Env and bNAbs
Ian Wilson, D. Phil., D.Sc., FRS, The Scripps Research Institute

Dr. Wilson noted 19 new bNAbs have been isolated and characterized from Protocol G blood donors (the International AIDS Vaccine Initiative [IAVI] Protocol G initiative). The broad and potent serum neutralization exhibited by these antibodies occurs in most donors through a limited number of specificities (1-2 per donor) and between donors (5-7). Across the donor panel, broad neutralization appears associated with six major sugar specificities. The antibodies recognize a particular Env sugar in different ways. A supersite of vulnerability appears to be present that is Asn322 dependent with variations of attack by the antibodies.

One of things that need to be deciphered is the identity of the sugar at each of the contact positions. Sequencing is required at individual glycan locations to build position/site-specific information about the composition of the sugars. The geometry or configuration of the sugars provides additional information for epitope emulation in a vaccine design. So both crystal and 3M structures have been part of the CHAVI-ID project. The idea is to unite structure, molecular design, and immunogenicity to facilitate a 'rational' approach to vaccine design.

Epitope Focused HIV Vaccine Design
William Schief, Ph.D., Associate Professor of Immunology, The Scripps Research Institute

Dr. Schief's role in CHAVI-ID is centered on translation of research components into vaccine design by employing computational methods to reproduce the targeted epitopes bNAbs recognize. The starting material consists of the neutralizing antibodies and the associated structural information for use in immunogen design with the goal to re-elicit similar antibodies. Using the respiratory syncytial virus (RSV) for proof-of-concept, his team was able to computationally design immunogens that elicited protective antibodies. Specifically, they demonstrated:

- Artificial scaffold immunogens can induce potent neutralizing antibodies.
- Epitope structural mimicry and antibody affinity are important.
- Multimeric presentation is important.
- Germline-targeting not always necessary.
- Good model system to "learn the rules" for optimizing epitope-specific responses.

Dr. Schief discussed what might be required to induce potent bNAbs against CD4bs by vaccination such as, making the epitope immunoprominent; activating the VH1-02 germline precursor paired with the appropriate light chains; selecting the appropriate SHM by sequential heterologous boosts; and optimizing non-structural immunological factors affecting SHM, magnitude, and maintenance of plasma secreting cells. To develop a minimal CD4bs scaffold, the team engineered an outer domain (eOD), a platform to "shepherd" immune responses toward bNAbs. The eOD produced had several desirable properties: much smaller, very stable, and with similar binding affinities for all the VCR01 class bNAbs as the core gp120 that was used as the template. Thus, despite the many structural manipulations, the eOD has the correct binding profile, and the eOD is being tested with various immunogens in NHPs.

The team has also developed VH1-2 germline targeting eOD (eOD-GT) and find that the eOD-GT bind well to germline precursors of many VH1-2 *02 bNAbs (with higher affinity for the mature bNAbs in most cases). Additionally, they are assessing binding to a variety of non-neutralizing Abs, and the vast majoritydo not bind to the eOD. They are trying to avoid off-patch responses.

CD4 T cell Help and Cytotoxicity/ CD4 T cells Memory
Bruce Walker, M.D., Director, Ragon Institute; Rafi Ahmed, Ph.D., Emory Vaccine Center

Dr. Walker spoke about the other focus of Scripps CHAVI-ID: harnessing CD4^{+} T cell responses for long-term protective immunity against HIV. The task involves four aims:

Aim 1: To define the role of T follicular helper (TFH) cells in the development of bNAb responses in HIV- infected individuals.
Aim 2: To preferentially generate TFH cells by vaccination.
Aim 3: To modulate the multifaceted nature of CD4+ T cell help to B cells to preferentially evolve highly adapted BCRs giving rise to bNAbs.

Aim 4: To define novel functional attributes of HIV-specific CD4+ T cells that can control early focal HIV infection either directly through cytotoxicity or indirectly through CD8+ T cell help.

Currently, the team is in the tool development stage. In characterizing CD4 T cell responses, they had earlier identified a peptide within gp41 that is the major target of HIV-specific CD4 cells, in the context of four to five HLA Class II alleles. It is now known many different Class II alleles all present that same immunodominant epitope, recognized in nearly 50% of HIV-infected individuals. The next step is to examine the specificity at the single-cell level, and the development of Class II tetramers is a key component of the strategy to better define function.

In collaboration with scientists in Denmark, each tetramer is made on two different fluorophores so that the increased sensitivity allows detection of low frequency events. Additionally, Gag specific CD4 T cell responses have been identified by intracellular cytokine staining after peptide pool stimulation. Another goal is to dissect HIV-specific CD4 T cell-APC (antigen-presenting cell) interaction networks. They have also investigated the ability of CD4 T cells to help B cells in HIV-infected chronic progressors (off treatment/treatment naïve) versus HIV uninfected. The preliminary data suggest less survival and a reduced B cell differentiation and class-switching in HIV+ individuals compared to uninfected individuals.

To date, the conclusions are:

- HIV-specific CD4 T cells can be readily detected in PBMC (peripheral blood mononuclear cell) with dual staining by class II tetramers.
- Complex cytokine networks regulate CD4-APC interactions.
- TFH cell epitope specificity remains to be defined.
- Despite the presence of TFH cells in PBMC, evidence of tetramer-positive responses is lacking in very preliminary experiments.

Dr. Ahmed continued the presentation with insights on CD4 TFH memory and the implications for designing rational prime/boost vaccination strategies. The function of CD4 TFH cells is to: 1) provide essential help for germinal center formation and maintenance; and 2) necessitate affinity maturation and differentiation of memory B cells and long-lived plasma cells. In terms of phenotype, the chemokine receptor CXCR5 is a functional marker for CD4+ TFH cells; Bcl-6 is a lineage regulator of TFH differentiation in CD4 T cells; other phenotypic markers include PD-1 and ICOS.

TFH function to provide help to B cells in response to T-cell dependent antigens; germinal centers are the primary location for B-cell somatic mutation, affinity maturation, and class switching. IL-21 is required for GC development (site where persistent memory B cells and long-lived plasma cells arise) and maintenance as well as the development of plasma cells to produce antibodies. Bcl-6 expressed by T cells is required to prime B cells for extrafollicular antibody responses.

Questions for future studies relevant to TFH cells and HIV vaccine design include:

1. What are the optimal prime/boost strategies for inducing effective and durable TFH responses?
2. How efficient are the different vectors (DNA, Ad, pox, etc.) in generating TFH responses? Acute viral infections give balanced TFH/Th1 responses. What do the different vectors and prime/boost strategies do?

3. What happens to TFH differentiation upon repeated boosting? Timing of protein boost?
4. What are the critical early signals in generating TFH cells? Role of DC subsets? Are distinct co-stimulatory signals involved? Role of adjuvants?
5. How do B cells influence the TFH response? Does the specificity (epitope) of the responding B cells determine the specificity of the TFH response and vice versa?

In answer to the question: Can you give a different adjuvant or signal to cause certain cells to convert to TFH?—Dr. Ahmed stated that cells may respond to signal and switch to the TFH phenotype, but the cells do not forget what they were because of prior changes at the epigenetic level. In terms of commitment to TH1, commitment is strong even though the cells are still elastic. Does a counterpart exist to IL-12 that induces BCL-6 and pushes assignment? IL-12 will push cells more toward TH1 (Th1). Dr. Shane Crotty is following up with experiments that test the effect of selective adjuvants on the process of differentiation and elasticity.

ADJOURNMENT

APPENDIX

AVRS Members present: Douglas F. Nixon, Aftab A. Ansari, Susan W. Barnett, Deborah L. Birx, Donald Forthal, Jeffrey D. Lifson, Nina D. Russell, Jerald C. Sadoff, William Snow, and Georgia D. Tomaras.

Ex Officio Members: Rama R. Amaro, Jay A. Berzofsky, Salvatore T. Burtera, Lawrence Corey, Eric Hunter, Bette Korber, John Mascola, Bonnie Mathieson, and Nelson L. Michael.

List of Speakers:

Duke CHAVI-ID

- Barton F. Haynes, Duke Human Vaccine Institute
- Beatrice-Hahn, University of Pennsylvania
- Garnett Kelsoe, Duke University
- Bette Korber, Los Alamos National Laboratory
- Joseph Sodroski, Harvard University

Scripps CHAVI-ID

- Dennis R. Burton, The Scripps Research Institute
- Rafi Ahmed, Emory Vaccine Center
- Michel Nussenzweig, The Rockefeller University
- William Schief, The Scripps Research Institute
- Bruce Walker, Ragon Institute
- Ian A. Wilson, The Scripps Research Institute