



Vaccine Research Center (VRC)
**National Institute of Allergy and
Infectious Diseases**

Strategic Plan

**Research Toward Development of
Effective Vaccines**

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LIST OF ABBREVIATIONS

Ad	Adenovirus
AIDS	Acquired Immune Deficiency Syndrome
BALT	Bronchoalveolar-Associated Lymphoid Tissue
BARDA	Biomedical Advanced Research and Development Authority
cGMP	Current Good Manufacturing Practice
CDR	Complementarity Determining Regions
CTC	Clinical Trials Core
DC	Dendritic Cell
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr Virus
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-Linked Immunosorbent Spot Assay
FDA	Food and Drug Administration
GALT	Gut-Associated Lymphoid Tissue
GCLP	Good Clinical Laboratory Practices
gp	Glycoprotein
HAI	Hemagglutinin-Inhibition Assays
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus Type 1
HIV-2	Human Immunodeficiency Virus Type 2
HVTN	HIV Vaccines Trials Network
IAVI	International AIDS Vaccine Initiative
ICS	Intracellular Cytokine Assays
ID	Intradermal
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IM	Intramuscular
LTNP	Long Term Non-Progressor
MHC	Major Histocompatibility Complex
MPER	Membrane-Proximal External Region
MSM	Men Who Have Sex With Men
NHP	Non-Human Primate
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NP	Nucleoprotein
NVITAL	NIAID Vaccine Immune T-cell and Antibody Laboratory
PL	Proteoliposome
rAd	Recombinant Adenovirus
SARS	Severe Acute Respiratory Syndrome
SIV	Simian Immunodeficiency Virus
SP	Slow Progressor
TCR	T-Cell Receptor

TB	Tuberculosis
TLR	Toll-Like Receptor
TNF	Tumor Necrosis Factor
UNAIDS	Joint United Nations Programme on HIV/AIDS
USMHRP	United States Military HIV Research Program
VRC	Vaccine Research Center

EXECUTIVE SUMMARY

The Dale and Betty Bumpers Vaccine Research Center (VRC) at the National Institutes of Health (NIH), an agency of the U.S. Department of Health and Human Services (HHS), was established to facilitate research in vaccine development. The VRC is dedicated to improving global human health through the rigorous pursuit of effective vaccines for human diseases, especially HIV/AIDS. Established by former President Bill Clinton as part of an initiative to develop an HIV/AIDS vaccine, the VRC is a unique venture within the NIH intramural research program. Initially spearheaded by the National Institute of Allergy and Infectious Diseases (NIAID), the National Cancer Institute, and the NIH Office of AIDS Research, the VRC is now part of the NIAID organization.

The primary focus of VRC research has been and continues to be the development of vaccines for HIV/AIDS. The initial VRC strategic plan drafted in 2000 outlined the following goals: 1) Scientific design and rational development of effective HIV-1 vaccine candidates; 2) Evaluation and optimization of the immune responses generated by these candidates; 3) Advancement of the most promising candidates into human clinical trials. Since the initial VRC Strategic Plan was written in 2001, substantial progress has been achieved for each of these goals. Thus, the time is now opportune to build upon these past achievements and map out VRC goals and objectives for the future that include a broader mission in vaccine research beyond HIV/AIDS.

The updated VRC strategic plan is based on the premise that achievement of fundamental advances in immunology, virology and vaccine science will likely lead to the development of new and improved vaccines. The VRC's emphasis on vaccine product development has accelerated the identification of robust vaccine platforms and immune assays and has led to the development of numerous candidate vaccines that have been tested in non-human primate challenge models and in Phase I clinical trials. The VRC continues to focus on research toward the development of an effective HIV/AIDS vaccine while also expanding its effort to include several other emerging viral diseases, such as Ebola, Marburg and influenza.

The VRC has identified four main goals for 2009 and beyond:

- 1) **Determine whether a T-cell-based vaccine can protect against acquisition of HIV-1 infection or delay progression to AIDS in an effort to identify immune mechanisms and correlates of protection.** A series of Phase I and Phase II trials have been performed to evaluate both DNA and recombinant adenovirus serotype 5 vector (rAd5) delivery of genes encoding HIV-1 antigens. These studies have formed the basis for a proposed randomized, placebo-controlled Phase II vaccine efficacy trial projected for 2009. Alternative vaccine platforms are also in development, including alternative serotype adenovirus vectors.
- 2) **Develop an HIV-1 vaccine candidate that elicits neutralizing antibodies to circulating viral isolates and advance such a vaccine into clinical trials.** Recent studies suggest that it is possible to use our knowledge of atomic level structure to design better antibody-based immunogens.

- 3) **Identify improved T-cell vaccines that optimize HIV-1-specific immunity and are independent of anti-vector immunity.** Evidence is accruing from several studies to suggest that potent T-cell responses can be elicited by immunization and that the quality of the T-cell response is a critical factor in providing protection.
- 4) **Advance vaccine candidates for Ebola, Marburg and influenza into efficacy trials.** The VRC has developed DNA and recombinant adenovirus (rAd) vaccine approaches that can protect monkeys from lethal Ebola and Marburg virus infection. These candidates will be advanced into clinical trials. The VRC will also develop and test influenza candidate vaccines that may lead to improved efficacy and greater breadth of protection than conventional vaccines.

INTRODUCTION

The Role of the Vaccine Research Center (VRC)

The global epidemic of HIV/AIDS is one of the most significant infectious disease threats to human health in history. Although new AIDS diagnoses and deaths have fallen significantly in many developed countries, the HIV/AIDS epidemic continues to accelerate in the developing world. The Joint United Nations Programme on HIV/AIDS (UNAIDS) reported that globally, an estimated 33 million people were infected with human immunodeficiency virus type 1 (HIV-1) in 2007, with more than 2.5 million newly infected persons and more than half a million of those being children younger than 15 years of age. It was also estimated that AIDS caused 2.1 million deaths worldwide in 2007. Beyond the human tragedy of HIV/AIDS, the epidemic poses a significant impediment to the economic growth and political stability of many countries. In developing countries and in segments of the US population, anti-HIV therapies are frequently beyond financial reach. Accordingly, effective low-cost tools for HIV/AIDS prevention are urgently needed to bring the AIDS epidemic under control. A globally effective, accessible vaccine remains the best public health intervention for ending this pandemic.

The Vaccine Research Center (VRC) was conceived to address this growing epidemic. President Bill Clinton's 1997 speech at Morgan State University, Baltimore, announcing an HIV vaccine program led to the creation of the VRC, which opened in 2000. The initial VRC strategic plan goals were: 1) Scientific design and rational development of effective HIV-1 vaccine candidates; 2) Evaluation and optimization of the immune responses generated by these candidates; 3) Advancement of the most promising candidates into human clinical trials. Since the initial VRC Strategic Plan was written in 2001, substantial progress has been achieved for each of these goals. Thus, the time is now opportune to build upon these past achievements and map out VRC goals and objectives for the future.

The process that underlies vaccine development is inherently interdisciplinary, combining basic, translational, and applied research in immunology, virology, disease pathogenesis, molecular biology, structural biology, and clinical investigation, along with strong capabilities in regulatory science and clinical material manufacture to move novel technologies from the laboratory to the clinic in a safe and compliant manner. By housing all these activities within a single integrated research center, the VRC can effectively advance and accelerate the science of vaccine development. VRC scientists are leaders in their respective fields and are dedicated to the translation of the latest concepts in disease pathogenesis and immunology into new strategies for vaccine research and development, including immunogen design, adjuvant optimization, preclinical testing, clinical trials, evaluation and optimization of immune responses, and vaccine manufacturing, development and licensure. These areas complement each other and promote a spirit of collaboration and exchange, both within the center and with outside collaborators, providing an effective foundation for vaccine development.

The primary focus of activities at the VRC is the development of an effective HIV/AIDS vaccine. Using gene-based vaccine platforms, such as DNA plasmids and recombinant adenoviral vectors, VRC investigators developed and tested numerous vaccine

products and advanced the most promising candidates into clinical testing. In the process of addressing the need for an HIV/AIDS vaccine, innovative technologies (e.g., DNA vaccines, novel viral vectors, structure-designed proteins) have been developed. When appropriate, these technologies have been applied to the VRC's expanded role in the development of vaccines for biodefense and emerging infectious diseases, including Ebola, Marburg, SARS, West Nile Virus, improved smallpox vaccines, and influenza. Of these non-HIV/AIDS programs, the largest research effort is directed toward development of a vaccine to protect against Ebola and Marburg viruses. A new program on influenza vaccines aims to apply novel technologies and approaches to development of pandemic influenza vaccines for both human and veterinary use.

Previous VRC Goals (2000-2008)

The VRC began in 2000 with three main goals aimed towards creating an effective AIDS vaccine. The goals were as follows: 1) Scientific design and rational development of effective vaccine candidates; 2) Evaluation and optimization of the immune responses generated by these candidates; 3) Advancing the most promising candidates into human clinical trials. Substantial progress has been achieved for each of these goals.

The initial HIV-1 vaccine goals were based on hypotheses that: (i) a combination of broad and potent CD4- and CD8-mediated T-cell immune responses, and functional antibodies to the appropriate viral epitopes, will prevent HIV-1 infection or control HIV-1 disease; (ii) vaccine candidates can be constructed to elicit these responses in animal models and human subjects; and (iii) the definition of protective immune correlates will be paramount to the development of a highly effective AIDS vaccine. Based on these hypotheses, the VRC takes a rational approach to vaccine design aimed at devising vaccine strategies that induce protective immune responses.

VRC Achievements (2000-2008)

Goal 1. Scientific design and rational development of effective vaccine candidates

A. Develop and optimize gene-based vaccine platforms that elicit broad and potent cell mediated and humoral immunity

Based on scientific input from the international community, VRC investigators focused on development of an HIV-1 vaccine that expressed proteins from the predominant global HIV-1 subtypes: A, B, and C. Preclinical testing of numerous vaccine constructs led to the development of the current HIV-1 candidate vaccine that has been tested in Phase I and II trials and is advancing into a Phase II efficacy trial. It is a gene-based vaccine that combines both DNA and viral vector vaccination in a "prime-boost" approach that is more potent than either individual vaccine alone. In addition, development of HIV-1 vaccine candidates that elicit neutralizing antibodies continues to be a major area of research focus. Recent progress concerning antibody targets will be reviewed in the Antibody section of this Strategic Plan.

B. Utilize knowledge of HIV-1 envelope structure to design immunogens that elicit potent virus neutralizing antibodies through a program of rational structure-based design and screening of immunogens

VRC investigators made important progress in unraveling the atomic level structure of the viral Envelope protein to better understand how HIV-1 evades the body's antibody response and to understand how some antibodies are able to neutralize HIV-1. The goal of

developing a vaccine that generates neutralizing antibodies against HIV-1 remains a scientific challenge and is a major focus of VRC investigators. Viral structural information is being used to design novel immunogens that are currently being tested in pre-clinical studies.

Goal 2. Evaluation and optimization of immune response generated by candidate vaccines

A. Identify strategies that enhance potency, antigen presentation and immunogenicity

In order to assess the immunogenicity of vaccine candidates, the VRC developed, optimized and standardized several T-cell based assays such as the intracellular cytokine (ICS) and enzyme-linked immunosorbent spot (ELISpot) assays. The VRC also established and standardized the use of multicolor flow cytometry to better analyze and understand the quality of immune responses generated by T-cells. Similarly, standardized viral neutralization assays were developed based on well-characterized panels of reference viruses representing the major subtypes of circulating viruses.

Optimization of immune responses also involves studies examining routes of administration, and use of alternative Ad vectors, adjuvants and inserts, as well as assays to measure immune responses at mucosal surfaces. Progress in many of these areas will be discussed in detail as part of the main body of this Strategic Plan.

B. Develop the infrastructure to test the immunogenicity of candidate vaccines

Development and validation of immune assays was an essential prerequisite for evaluation of VRC vaccine candidates. The immunogenicity of VRC's candidate vaccines is assessed at the NIAID Vaccine Immune T-Cell and Antibody Laboratory (NVITAL), which provides validated immune testing of vaccine candidates from Phase I through Phase III efficacy trials. The Intracellular Cytokine (ICS) and ELISpot assays have been transferred to NVITAL for routine use. The NVITAL facility design complies with Good Clinical Laboratory Practices (GCLP) specifications.

C. Develop rational use of the primate model to assess vaccine strategies and define immune correlates

Studies in the monkey model of simian immunodeficiency virus (SIV) infection have focused on the pathogenesis of acute infection and correlates of vaccine induced protective immunity. VRC investigators examined the kinetics of acute infection and the effect on memory CD4+ T-cell populations, mucosal immunity and routes of immunization, and depletion of memory CD4+ T-cells in the gut. Human studies confirmed that HIV-1 infection results in massive infection and loss of memory CD4+ T-cells in multiple tissues. Using the SIV model of vaccination and challenge, VRC investigators showed that DNA/rAd5 immunization can preserve memory T-cells and prolong survival.

Goal 3. Advance the most promising vaccine candidates into human clinical trials

A. Conduct clinical evaluation of candidate vaccines

Preclinical studies showed that a multigene, multiclade vaccine was feasible and could elicit broad immune responses. The VRC was able to initiate its first clinical trial, VRC-001, a Phase I study evaluating a DNA vaccine encoding HIV-1 *gag-pol* administered by a needleless injection device, less than one year after officially opening its doors. VRC-001 was the initial trial in a clinical development that resulted in the development and testing of the multiclade DNA and rAd5 candidate vaccine incorporating *gag*, *pol*, *nef*, and *env* genes from HIV-

1 subtypes A, B, and C. The vaccine was subsequently optimized to consist of a six plasmid DNA prime (*envA*, *envB*, *envC*, and subtype B *gag*, *pol*, and *nef*), and four rAd component boost vaccine (subtype B Gag-Pol fusion protein, and Env from subtypes A, B, and C).

The current lead HIV-1 vaccine candidate (6-plasmid DNA prime, rAd5 boost) will advance into efficacy testing in 2009. The VRC has also developed vaccine candidates for avian influenza, Ebola, West Nile and SARS viruses. The VRC Clinical Trials Core (CTC) was established to facilitate Phase I testing of vaccine candidates on the NIH campus. This capability allows for expedited conduct of early-stage clinical trials. Since the VRC's inception, the CTC has successfully conducted over 35 clinical studies of promising vaccine candidates, including DNA and adenoviral vectors. The acceleration of timelines enabled by the VRC's integrated approach is illustrated by both SARS and influenza vaccine development. In the case of SARS, a potential vaccine was developed by the VRC and tested in people within 22 months of emerging infectious disease appearance. In the case of influenza, less than 18 months transpired from first appearance of an Indonesia strain outbreak to inoculation of human volunteers in the clinic with a VRC candidate vaccine.

B. Develop the infrastructure to produce and test vaccine products

The development of vaccine product manufacturing capability is an essential step in advancing vaccine candidates through clinical development. VRC vaccines are produced through collaborations and contracts with companies in the vaccine/biotechnology industry, as well as through manufacturing at the VRC Vaccine Pilot Plant. The VRC manufacturing plant complements external collaborations by providing a current Good Manufacturing Practices (cGMP)-compliant facility for the manufacture of candidate vaccines. This pilot plant focuses on production of vaccine products for Phase I and II clinical trials, and incorporates features that allow for larger scale operations capable of supporting Phase III clinical trials, if necessary.

Thus, in a relatively brief time span, the VRC has made progress in several major areas of vaccine research and development, including definition of markers and immune mechanisms that may protect against HIV-1 infection; influence of cellular and humoral immune responses; the conception, design, and preparation of vaccine candidates for HIV-1 and other infectious diseases; and the initiation of multiple human clinical trials to test the safety and immune response of these vaccine candidates.

Future VRC Goals for 2009 and Beyond

The VRC has four main goals for 2009 and beyond, including:

- 1) Determine whether a T-cell-based vaccine can protect against acquisition of HIV-1 infection or delay progression to AIDS in an effort to identify potential immune mechanisms and correlates of protection.
- 2) Develop an HIV-1 vaccine candidate that elicits neutralizing antibodies to circulating viral isolates and advance such a vaccine into clinical trials.
- 3) Identify improved T-cell vaccines that optimize HIV-1-specific immunity and are independent of anti-vector immunity.
- 4) Advance vaccine candidates for Ebola, Marburg and influenza viruses into efficacy trials.

These goals are complementary to other efforts in the AIDS vaccine research community and align with the scientific strategic plan of the Global HIV Vaccine Enterprise, which aims to coordinate the efforts of major research organizations in the field. The sections below provide detailed descriptions of each of these goals, followed by a series of selected scientific references.

GOAL 1. Determine whether a T-cell based vaccine can protect against acquisition of HIV-1 infection or delay disease progression in an effort to identify immune mechanisms and correlates of protection

Objective 1: Define the safety and efficacy of the multiclade DNA prime/rAd5 boost VRC candidate HIV-1 vaccine

A series of Phase I and Phase II trials were performed to evaluate both DNA and recombinant adenovirus serotype 5 vector (rAd5) delivery of genes encoding HIV-1 antigens. Advanced clinical study collaborations with DAIDS and networks sites in the HIV Vaccines Trials Network (HVTN), U.S. Military HIV Research Program (USMHRP), and the International AIDS Vaccine Initiative (IAVI) evaluated the safety, immunogenicity, and efficacy of the VRC candidate HIV-1 vaccine in Phase II trials in 11 countries. The vaccine candidate consists of a prime-boost vaccine regimen with a 6-plasmid DNA prime delivered with a needle-free injection device at monthly intervals (0, 1, 2 months), followed by a boost of 4 replication-defective recombinant adenoviral vectors (rAd5) at month six. The vaccine antigens expressed include Env, Gag, Pol, and Nef with the intent of inducing responses to multiple epitopes to diminish the chances of immune escape through genetic variation. Envelope sequences from HIV-1 subtypes A, B, and C are included with the intent of broadening the response and amplifying the response to conserved regions to make the vaccine relevant to diverse epidemic regions around the globe. The DNA/rAd5 combination elicits robust CD4+ and CD8+ T-cell responses that correlate with prolonged survival in the SIV macaque vaccine model. The clinical safety profile and consistent detection of HIV-1 specific CD8+ T-cell responses in the Phase I and II human clinical trials, and the animal model data suggesting clinical benefit, support the continued development and evaluation of this approach. Therefore, the VRC, together with NIAID-supported clinical trials investigators, has designed a Phase II efficacy trial (HVTN 505) with a projected start date in 2009.

HVTN 505 is planned as a randomized, placebo-controlled Phase II vaccine efficacy trial that will be performed in collaboration with the NIAID-sponsored HIV Vaccine Trials Network (HVTN). The study is designed to determine vaccine efficacy in a population of North American men who have sex with men (MSM) and who are circumcised and Ad5-seronegative. The two primary study objectives are safety and reduction in viral load using an endpoint-driven study design requiring 45 total infections among all subjects after week 26 (two weeks after the scheduled rAd5 boost). This cohort size should allow detection of ~1.0 log₁₀ reduction in viral load between vaccinees and placebo recipients who acquire infection.

Objective 2: Define immunological correlates of vaccine-induced protection from HIV-1 acquisition and/or disease progression to advance development of an HIV-1 candidate vaccine

If any level of efficacy is achieved in the HVTN 505 study, for either HIV-1 acquisition or reduction of viral load, VRC scientists will evaluate whether any measure of vaccine-induced immune response correlates with protection. In addition, among vaccinees who become infected despite immunization, the VRC will analyze immune responses with respect to viral load, CD4+ T-cell subset trajectories, time-to-antiretroviral treatment, and other clinical parameters. The primary hypothesis is that detection of HIV-1-specific CD8+ T-

cell responses will correlate with reduction in viral load, but measurement of multiple aspects of T-cell function and phenotype will be explored in an effort to identify an immune correlate. In addition, VRC researchers will evaluate the breadth and functional characteristics of the antibody response even though this particular vaccine regimen has not been shown to elicit neutralizing antibodies against most HIV-1 isolates.

HVTN 505 will also evaluate viral and host genetic parameters associated with protection. Full-length sequencing of breakthrough virus isolates in all subjects who acquire HIV-1 infection will be used to determine the breadth of protection and whether vaccine-induced protection may occur against select genetic variants in the diverse epidemic regions involved in the study. The study will compare the extent of genetic diversification over time in infected vaccinees to infected placebo recipients in the context of viral load levels during the acute and chronic phases of infection. In addition, the trial will evaluate host genetic factors that might impact immune responses to vaccination, adverse events to vaccination, or the major endpoints. The trial design will also be modified as new data becomes available from other non-human primates (NHP) or clinical studies that may impact its safety or efficacy.

Objective 3: Establish an HIV-1 vaccine development plan designed to address possible outcomes of the HVTN 505 efficacy trial

HVTN 505 is designed as a test-of-concept efficacy trial. Additional manufacturing process development, scale-up and validation will be needed prior to licensure of this candidate vaccine. If HVTN 505 shows a high level of efficacy in preventing HIV-1 acquisition, efforts will be made to rapidly satisfy additional requirements necessary for accelerated licensure. If less striking efficacy rates are demonstrated, the VRC will accelerate the evaluation of next generation products currently being explored in Phase I and II trials. These concepts are designed to improve the potency and breadth of vaccine-induced T-cell responses using gene-based vector delivery. They include new vector or combination approaches that may improve magnitude and functionality of the T-cell response. They also include new delivery approaches that may improve localization and kinetics of relevant vaccine-induced T-cell responses. Novel adjuvant approaches may improve the composition of vaccine-induced memory T-cell populations. Clinical studies of non-HIV vaccines will also be used to address general principles of delivery and schedule for the DNA, rAd and other vaccine platforms. The evaluation of new vector approaches will be facilitated by the use of a common gene insert (*envA*), that will be used in pilot studies of the VRC rAd35, and also in other vectors produced by the VRC and/or external investigators (see Goal 3).

If new generation T-cell vaccines are shown to have improved breadth, functionality, and durability in Phase I/II evaluation, they will be inserted into the overall vaccine development plan. If candidate vaccines with the capacity to induce broadly neutralizing antibodies are identified, the VRC will test these alone and in combination with the most promising T-cell vaccine candidate and advance them as quickly as possible. Existing and accruing clinical data will be used to increase our understanding of host determinants of immunogenicity for different vaccine delivery platforms. VRC scientists will analyze the distribution of single nucleotide polymorphisms in subjects with CD8+ T-cell responses elicited by DNA immunization compared to those subjects without CD8+ T-cell responses. Additional genetic studies are being considered to identify the molecular basis for the

frequency of selected immune responses and adverse events. These studies will inform future decisions for improving gene-based vaccine delivery systems.

Ultimately a vaccine for HIV-1 will need to be available to the most vulnerable at-risk populations. If there is evidence of safety and efficacy demonstrated in HVTN 505, the VRC may pursue additional studies in other cohorts, including at-risk populations.

GOAL 2. Develop an HIV-1 vaccine candidate that elicits neutralizing antibodies to circulating viral isolates and advance such a vaccine into clinical trials

Despite significant advances in eliciting T-cell immune responses, it is likely that an optimally protective HIV-1 vaccine will require the generation of neutralizing antibodies. Animal studies have shown that passive infusion of neutralizing antibodies provides resistance to infection. These studies suggest that if neutralizing antibodies were present before exposure to HIV-1, infection could be prevented or ameliorated. Unfortunately, antibodies capable of neutralizing most circulating HIV isolates have proven difficult to elicit. While many HIV-1 antibodies are elicited during natural infection, most of these are non-neutralizing. Such non-neutralizing antibodies either recognize internal viral proteins or surfaces of the envelope glycoprotein that are not exposed on the functional viral spike. Such surfaces or conformations appear to be immunodominant, whereas the viral envelope itself is labile and cloaked by N-linked glycans (sugars) that are generally not recognized by the immune system as foreign.

Objective 1: Determine the structure of a trimeric HIV-1 Envelope spike and delineate how oligomerization influences immunogenicity and evasion from antibody-mediated neutralization

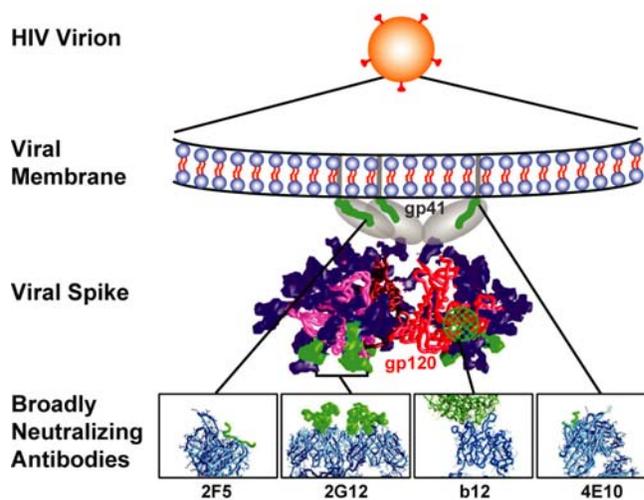


Figure 1: Schematic of the HIV-1 envelope glycoprotein with boxes showing the targets of broadly neutralizing antibodies

Figure 1 shows a diagrammatic view of the HIV-1 virion, its viral membrane, and viral envelope spike with structures and targets of broadly neutralizing antibodies. One long-held view is that particular properties of the HIV-1 spike form a set of decoy mechanisms that inhibit the elicitation of broadly reactive, neutralizing antibodies. These decoy mechanisms include liability of the envelope trimer, presence of non-functional spikes on the virus, immunodominant hypervariable loops, and conserved surfaces with relatively poor immune reactivity. It is possible that immunization with biochemically stable antigenic mimics of the functional viral spike would circumvent such mechanisms and elicit antibodies capable of neutralizing HIV-1. In this regard, trimeric molecules of the HIV-1 envelope ectodomain, especially those stabilized with COOH-terminal trimerization domains, exhibit increased antigenic mimicry of the functional spike relative to the monomer. They also elicit more effective antibodies than do monomeric immunogens. However, despite extensive effort, precise antigenic mimics of the HIV-1 viral spike have proven difficult to make. While the tools of modern protein design may overcome this impediment, such design is dependent on knowing the atomic-level structure of the viral spike, a trimeric heterodimer composed of three gp120 envelope glycoproteins non-covalently interacting with three gp41 transmembrane molecules.

VRC scientists previously found a probabilistic approach to be successful in the crystallization of the gp120 glycoprotein, especially when combined with small volume robotic screening of potential crystallization conditions. The VRC plans to apply these technologies to the crystallization of the ectodomain of the HIV-1 viral spike. Once suitable crystals have been obtained, structural determination by X-ray diffraction should be straightforward. While it will be challenging to translate atomic-level information into the design of oligomeric immunogens with increased antigenic mimicry, it is expected that correlation of immunogenicity with antigenic, biochemical, and structural features of the viral spike will reveal how oligomerization influences immunogenicity, and will provide additional clues as to how to elicit antibodies capable of recognizing the functional viral spike.

Objective 2: Develop immunogens and immunization strategies that elicit CD4-binding site-reactive antibodies that neutralize circulating viral isolates

The first step of infection by HIV-1 involves binding of the gp120 component of the HIV-1 spike to the CD4 receptor on the surface of host cells. Because the binding site for CD4 on gp120 must be both exposed and conserved in structure, it has been a target of vaccine design. Two recent developments have increased vaccine interest in the CD4-binding site. First, the structure of the broadly neutralizing, CD4-binding site-directed antibody, IgG1 b12, was determined in complex with gp120. This structure revealed that a distinct portion of the CD4-binding site—the initial site of CD4 contact—was exposed on primary viral isolates. It is this initial site of CD4 contact that forms a site of vulnerability on the functional spike. Second, analysis of sera from clinically stable HIV-1-infected individuals identified several with potent, broadly neutralizing activity. The broad neutralizing activity of these sera was attributed to antibodies that bound the CD4-binding site and the breadth of neutralization was greater than that elicited by the monoclonal antibody b12. These developments demonstrate that HIV-1 has a site susceptible to neutralization by antibody and that high titers of antibodies against this site can be generated in humans. The question then is how to create an immunogen capable of eliciting CD4-binding site antibodies that neutralizes circulating viral isolates.

During natural infection, CD4-binding site-reactive antibodies are often elicited. However, most of these antibodies are ineffective at neutralizing primary isolates. To elicit effective neutralizing antibodies, it may be necessary to understand the viral mechanisms of evasion that prevent most CD4-binding site-reactive antibodies from neutralizing circulating viral isolates. The structures of non-potent CD4-binding site antibodies in complex with gp120 will be instructive in this regard.

In addition to determining such structures, the VRC plans to proceed directly with immunogen design. Since the atomic-level structure of the site of vulnerability is known, one can use this information to create antigens that mimic and preferentially present this site. A number of immunogen-design strategies are available. These include:

- 1) conformational stabilization: restraining the flexible gp120 into a conformation that preferentially binds a particular broadly neutralizing ligand, e.g., CD4
- 2) epitope-scaffold transplantation: placement of the target epitope into a heterologous protein
- 3) antigenic cloaking: substituting the surface of gp120 not involved in antibody binding with amino acids from evolutionarily distant homologs
- 4) glycan silencing: adding N-linked glycosylation to antigenic surfaces involved in binding non-potent or non-neutralizing antibodies

Each of these design strategies can be iteratively optimized, either biochemically (for example, to improve solubility), antigenically (to improve affinity to a target antibody like b12 or to the CD4 ligand), or immunologically (based on immunogenicity data from small animal studies). These strategies can also be combined, for example, by using glycan silencing to divert the immune response from an immunodominant site in an epitope-scaffold transplant. Because the initial binding site for CD4 involves substantial backbone recognition and displays some sequence variability, it will be important to probe the relationship between sequence/clade diversity and CD4-binding site-directed neutralization.

Objective 3: Develop immunogens and immunization strategies that elicit neutralizing antibodies reactive with the conserved membrane-proximal external region (MPER) of gp41

The membrane-proximal domain of the HIV-1-envelope ectodomain is the target of two of the most broadly reactive anti-HIV-1 antibodies identified to date, 2F5 and 4E10. To understand the atomic-level details of 2F5 recognition, VRC scientists crystallized and solved the structure of 2F5 with gp41 peptides corresponding to its epitope. The structure revealed that 2F5 bound to only one part (face) of the extended peptide, and that this face was much more charged than the unbound hydrophobic face. The 2F5 CDR H3 loop, adjacent to the gp41 peptide, comprised a contiguous hydrophobic surface. Modeling of the gp41 peptide in the context of the viral membrane and surrounding gp41 sequences suggested an interaction proximal to the viral membrane. Biochemical analysis with solid phase Env-containing proteoliposomes either possessing or lacking a reconstituted membrane confirmed the involvement of lipids in 2F5-antibody-epitope recognition. VRC scientists also demonstrated that the binding of 2F5 as well as that of another membrane-proximal binding antibody, 4E10, could be enhanced almost two orders of magnitude by the inclusion of lipid membranes.

These structural and biochemical studies suggested that to elicit 2F5- and 4E10-like antibodies, considerations related to conformation, steric accessibility, and membrane context had to be taken into account. Based upon the model that the 2F5 epitope displays the extended loop structure visible in the 2F5-peptide structure, and that the membrane is associated with 2F5 and 4E10 antibody-antigen interaction, the VRC will pursue three distinct approaches in an attempt to translate this information into structure-based and rational immunogen design. First is the generation of MPER “miniproteins” designed to enhance interaction of the MPER with the plasma membrane, analyze their expression and antibody recognition, and then test selected constructs in the proteoliposome (PL) format. The second approach is to express miniproteins in a particulate and highly immunogenic lipid-containing platform, which utilizes the hepatitis B surface antigen S1 protein. In a third strategy based upon the 2F5-epitope crystal structure, VRC scientists will attempt to transplant the 2F5 epitope onto proteins possessing a similar surface-exposed, extended loop structural element.

Other scaffolding techniques such as protein grafting or *de novo* design can also be utilized. One advantage to the scaffolding approach on soluble proteins is that it targets the peptide backbone of the 2F5 epitope. Therefore, concerns about cross-reactivity with cardiolipin-self determinants as described recently may be alleviated. Other design options may be considered, such as introducing structure-based hydrophobic contacts to enhance the elicitation of antibodies possessing hydrophobic CDR loops similar to those possessed by 2F5. In principle, similar approaches to target the extremely hydrophobic 4E10 epitope could also be considered.

Objective 4: Define additional targets of broadly neutralizing HIV-1 antibodies

During HIV-1 infection, the first neutralizing antibodies to arise are against the patient’s initial infecting virus. In most patients, there is a broadening of the initial neutralizing antibody response to include heterologous strains of HIV-1, and in some patients, a potent and broadly reactive neutralizing antibody response develops. It is these broadly neutralizing sera that are of particular interest, since they represent cases in which natural immunity has successfully generated the type of antibodies that we seek to elicit via immunization.

To identify HIV-1⁺ sera that contain broadly neutralizing antibodies, the VRC will screen panels of sera from slow progressor (SP) and long-term non-progressor (LTNP) patients. Current data suggest that SP patients with some ongoing viremia for a minimum of several years tend to have the most broadly reactive sera. One possible explanation is that antibodies to numerous Env regions, including variable regions, accumulate over time leading to a broadly reactive antibody response. Alternatively, B cell affinity maturation may result in the evolution of antibodies to conserved Env determinants. The VRC will apply several methodologies to map the epitopes targeted by broadly neutralizing sera. Neutralization interference assays utilize linear peptides to block neutralization of known epitopes, such as the variable loop regions and the MPER of gp41. To map antibodies binding to discontinuous epitopes, conformationally intact recombinant Env proteins will be used for adsorption and elution of Env-specific antibodies. The specific epitopes targeted by serum antibodies can be identified based on binding to selected wild type and mutant proteins. Serum antibodies will be further defined using panels of chimeric Env pseudoviruses that either lack or include specific epitope regions. Examples include HIV-2 chimeras with the HIV-1 MPER or HIV-1 strains with mutated binding sites for monoclonal antibodies 2F5 and 4E10.

Existing data suggest that some broadly neutralizing sera contain neutralizing antibodies to the conserved CD4-binding site of gp120. There is currently only one known monoclonal antibody, IgG1 b12, which neutralizes HIV-1 via the CD4-binding-site region. Since these identified sera have CD4-binding site-directed antibodies that neutralize b12-resistant viruses, it is likely that unidentified neutralizing antibodies are present in the sera of these patients. These antibodies may target known epitopes in a novel way, or be directed to previously unidentified epitopes. The VRC will attempt to isolate and characterize monoclonal antibodies from patients whose sera contain broadly neutralizing antibodies. VRC researchers will use traditional methodologies such as Epstein-Barr virus (EBV) transformation and screening for antigen-specific cells as well as newer approaches that involve isolation of antigen-specific memory B cells by flow cytometry and cell sorting. VRC scientists plan to isolate antibodies from EBV-transformed B cell clones or by antibody gene cloning from specific B cells and reconstruct full IgG molecules in expression plasmids.

The epitope-mapping methods described above will also be applied to vaccine sera. Carefully mapping the epitope specificities of neutralizing antibodies elicited by novel immunogens will provide a way to understand the basis of the neutralization activity and will be important to provide information in designing improvements in immunogens. Current Env immunogens can elicit high levels of neutralizing antibodies directed against variable loop regions, but more information is needed to understand how other epitopes might be targeted. As novel Env immunogens are tested, e.g., those designed to elicit neutralizing antibodies against the MPER or CD4-binding-site region of gp120, epitope-mapping assays will be used to determine whether the specific antibody response elicited is targeting the expected epitopes. This will be an iterative process in which novel structure-based immunogen design is informed by epitope-mapping studies of vaccine sera.

The elicitation of broadly neutralizing antibodies against a highly variable virus that survives in the face of a highly evolved immune response is a formidable task. While vigorously pursuing the goal of eliciting antibodies to conserved sites of the viral Env, VRC scientists will not ignore the fact that eliciting antibodies against the viral receptors, CD4 or CCR5, is a strategy that might also block HIV-1 infection of cells. Since these targets are host molecules, an entirely different set of challenges would arise regarding elicitation of inhibitory antibodies against these self proteins. At this juncture, this approach is a consideration, but one that is secondary to targeting conserved elements for neutralizing antibodies as described above.

Objective 5: Determine whether an antibody response against variable regions of HIV-1 Env can be generated that protects against diverse virus isolates

Whereas the previous four objectives focused on eliciting antibodies against conserved epitopes, or invariant surfaces, of either virus or host, the following objective highlights a different strategy: the targeting of sequence variable regions of HIV-1. Experiments will explore the relationship between sequence variability and antibody recognition and will evaluate if an appropriate antibody response against a variable surface would be able to contribute to neutralization of diverse strains of circulating virus.

Studies of the neutralizing antibody specificities of HIV⁺ sera show that not all neutralizing activity can be attributed to known conserved epitopes and it is possible that antibodies directed to variable regions contribute to the sum total of serum-neutralizing

activity. In addition, a number of recent immunization studies have suggested that neutralizing antibodies against specific domains of variable regions such as V3 and V1 can be readily elicited. Antibodies to the V3 epitope can recognize some heterologous viruses, but the V3 domain is almost always shielded within the context of the native viral spike and this limits antibody access. Though it has been difficult to achieve in practice, if viruses could be rendered sensitive to V3-mediated neutralization by a second antibody, anti-V3 antibodies could potentially contribute to an effective vaccine-induced antibody response. The VRC will continue to study the antibody specificities in HIV⁺ sera to better understand the polyclonal neutralizing antibody response, including antibodies to variable regions on the viral envelope. The VRC will also continue to study immunogens that have the potential to elicit neutralizing antibodies against highly immunogenic domains, including variable domains.

GOAL 3. Identify improved T-cell vaccines that optimize HIV-1-specific immunity and are independent of anti-vector immunity

The process of improving T-cell vaccines requires the characterization of effective antigen-specific responses, followed by iterative modifications of vaccine modalities to try to achieve those preferred responses. While some success has been achieved in the characterization of elicited T-cell responses to HIV-1-specific immune system stimulation, immunologic correlates of efficacy remain unknown for HIV-1. The characterization of protective immune responses can only be fully defined once a large-scale trial of at least a partially-effective vaccine has been performed in humans. Nonetheless, much information can be gleaned from the study of immune responses of SIV challenge studies in NHP and to a lesser extent, from studies of HIV-1-infected humans.

The VRC's scientific efforts have been influenced by the results of the STEP trial, where the rAd5 vaccine stimulated measurable T-cell responses but yielded no overall protection. Data from this study, as well as NHP studies, suggest that simply measuring the magnitude of the T-cell response is an insufficient immune correlate of protection. The VRC will therefore strengthen its efforts to identify other characteristics of T-cells that may correlate with protection, and seek alternative strategies to stimulate such responses. These efforts are also crucial in light of the STEP trial finding that some subset of vaccine recipients may have been at increased risk of acquiring HIV-1 infection. The VRC will make efforts to investigate the mechanism of this enhancement.

T-Cell Immunity

Objective 1: Characterize the magnitude, quality and breadth of T-cell responses required for protection

While it is known that T-cell responses are able to affect the course of SIV and HIV-1 infection, the requirements for optimal protection in terms of magnitude, phenotype, function, epitope targeting, T-cell receptor (TCR) diversity, and location of that response, or how best to engineer vaccines to elicit such responses, remains unclear. The VRC's major goal is to better define the full character of protective T-cell immune responses to HIV-1 and other pathogens, and to determine how best to stimulate such responses through vaccination. In pursuing these goals, VRC scientists will utilize a multi-faceted approach of studying human and NHP immune responses in acute and chronic infection with HIV-1, SIV, and other relevant pathogens, and investigate the immune response elicited by rationally designed vaccines in appropriate animal models, and ultimately, in human clinical trials.

T-cells are capable of a large repertoire of responses to antigens. Broadly, these responses include the abilities to proliferate or induce proliferation of other cells (through secretion of growth factors), to organize immune responses (by secreting chemoattractants or stimulatory cytokines), and to carry out direct effector functions (by directly killing infected cells, inducing programmed cell death, or secreting factors with direct antiviral properties). These T-cell functions can be expressed solely, or in complex combinations; together referred to as the "quality" of the T-cell response. This quality can be defined for an individual cell or for a population of cells. Evidence from the study of efficacious vaccines, HIV-1

pathogenesis, or novel vaccines in small animal models is rapidly accruing to suggest that the quality of the T-cell response is a critical factor in providing protection.

A priority in this regard is to define the complete palette of functional responses that antigen-specific T-cells can evince. Current studies typically characterize the secretion of three to five cytokines or chemokines, as well as quantify the cytotoxic and proliferative potential of the cells. Gene expression array technology as well as newly developed reagents will extend the range of responses that can be quantified. These functions need to be measured on a cell-by-cell basis in concert with phenotypic markers that identify the T-cell lineage, differentiation stage, and homing markers to best characterize the immune potential.

The other priority with regard to quality of T-cell response is to define the factors which influence the generation of different types of T-cell responses. Varying the adjuvant or antigen dose during the immunization phase can bias the resulting T-cell quality in different directions. The ability to reproducibly generate a given quality may be critical. For example, it is conceivable that while a certain quality of T-cell response is optimal for HIV-1, a different quality may be optimal for influenza virus due to differences in pathogen-host interactions.

The elucidation of correlates of immunological control of virus replication may serve not only to establish the rules that govern the dynamics of polyclonal antigen-specific T-cell populations *in vivo*, but also act as a basis for the rational design of vaccines guided by such principles. Escape from T-cell immunity through viral epitope mutation is a cardinal feature in the pathogenesis of HIV-1 infection, and a major obstacle to the development of vaccines that aim to elicit cellular immunity against retroviruses. The impact of antigen variation at the TCR contact surface is likely to be paramount. Thus, studies will examine the number and variability of epitopes targeted in natural infection, vaccine-induced T-cell responses, and the functional avidity of the responses to targeted epitopes and potential escape variants. Further studies will investigate whether the nature of antigen engagement by the TCR plays a formative role in the dynamic process of viral escape from T-cell recognition. Specifically, the question is whether diversity within the highly variable CDR3s that define TCR specificity could shape viral evolution and thereby influence the outcome of infection. TCR diversity and mode of epitope escape will be examined in early dominant responses to both SIV and HIV-1 after infection or vaccination. Further studies will determine the feasibility of vaccine strategies that direct the adaptive immune response to preempt viral evolution in SIV infection and will delineate the extent to which such principles might be applicable to human populations exposed to heterogeneous viral inocula.

Objective 2: Improve the immunogenicity of existing vaccines and develop additional formulations that will optimize protective T-cell responses

Currently, prime-boost immunization using heterologous vaccine formulations is an effective approach for improving the strength and quality of the cellular immune response. The specific type of vaccine formulation and the order in which they are given profoundly influence the type of T-cell responses generated. Thus, while the current VRC vaccination regimen with plasmid DNA encoding HIV-1 envelope or structural proteins, followed by rAd5 encoding similar proteins, elicits potent T-cell responses in humans, enhancement of such responses is desirable. As the primary immunization is critical for determining the magnitude and quality of T-cell responses after a boost, the VRC will direct its efforts toward improving the delivery and/or optimizing the vector inserts in DNA vaccines. In addition, the

VRC will undertake studies to address the mechanisms by which the primary immunization influences the functional capacity and durability of CD8+ T-cell responses following a rAd boost with emphasis on the role that CD4+ T-cells have in this process. Finally, as the ability of rAd vaccines to induce T-cell responses in vaccinated cohorts may be limited by pre-existing immunity in these populations acquired from prior adenoviral infection with the same serotype, the VRC will develop alternative rAd vectors using serotypes of low prevalence in the global population. In addition, to circumvent the issue of pre-existing immunity to rAd, the VRC will perform studies using other attenuated viral vectors and non-live vaccines (protein/adjuvant) that have been shown to elicit strong cellular immune responses.

Objective 3: Determine the mechanisms by which vaccines elicit innate and adaptive immunity

A major step toward improving the immunogenicity of current vaccines and being able to rationally design newer formulations will be to understand the early cellular and molecular events by which the vaccines initially activate the immune response. In this regard, it is the innate immune response through activation of dendritic cells (DCs) and signaling through toll-like receptors (TLR) that has a critical role in shaping T-cell responses. The VRC intends to use mouse, NHP, and human *in vitro* and *in vivo* models to elucidate the role and mechanisms by which DCs and TLR signaling mediate T-cell activation. Studies will initially identify the mechanisms by which rAd5 vaccines elicit innate immune activation. As there appear to be differences in the potency and type of T-cell responses generated amongst different rAd serotypes, it will be important to study their ability to stimulate the innate immune responses compared to rAd5 vaccines. Furthermore, as pox viral vaccines have been shown to elicit a high frequency of multi-functional T-cell responses, VRC scientists expect to use a genomic approach to compare the innate mechanisms by which rAd and pox viral vaccines may differ amongst specific DC subsets. This approach may allow for re-engineering of current and future rAd vaccines to target specific intracellular signaling pathways that will optimize DC activation and improve the T-cell response. Finally, the VRC will emphasize developing non-live vaccines that can elicit potent T-cell responses. In this regard, protein-based vaccines that directly target DCs through cell-surface receptors or linking TLR ligands to proteins directly have been shown to enhance cellular immune responses.

Objective 4: Assess how vaccine delivery influences T-cell immunity

Additional variables that may influence the type of T-cell response generated include the route of delivery, dose and the interval of immunization. The VRC will use mouse, NHP, and human studies to assess how each of these parameters can be modified to elicit protective responses in a safe, deliverable and time-efficient manner. In addition, the VRC will study the factors that influence the durability of T-cell responses, including memory and effector T-cell responses. Defining the magnitude and quality of response required for protection will guide vaccine design for maintaining such responses.

Mucosal Immunity

Over the past ten years, our understanding of the pathogenesis of HIV-1 infection has been dramatically revised, particularly with respect to the kinetics and locality of CD4 T-cell depletion. It has become increasingly clear that the bulk of CD4 depletion from the body occurs within the first few weeks of infection and affects the majority of memory CD4 T-cells

in the body, which are located primarily in the mucosal tissues such as the gastrointestinal tract. The corollary of such observations is that an effective vaccine should elicit a mucosal immune response that will interfere with the initial infection events, such that viral replication within mucosal tissues is rapidly aborted, or at least greatly attenuated. The aim of the vaccine would be to reduce the number of infected memory CD4 T-cells.

Objective 1: Develop and optimize measurement of antigen-specific T- and B cell responses from mucosal sites

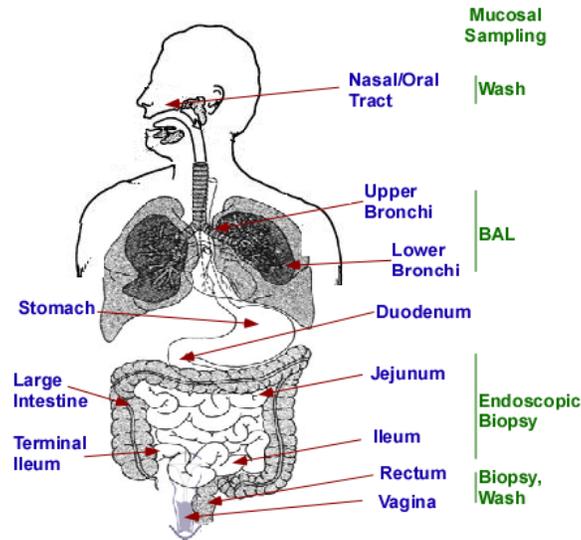


Figure 2: Tissue specimens that can potentially be sampled for analysis of mucosal immune responses

To this end, a major goal is to optimize analysis of resident mucosal immune cells and, more importantly, to optimize the measurement of antigen-specific T- and B cell responses from mucosal tissues. The determination of such responses will require direct sampling of these tissues using multiparametric immune-monitoring approaches. The VRC will carry out such studies in humans and NHPs infected with HIV-1/SIV at different stages of the disease course and, more importantly, in recipients of the various vaccine modalities developed by the VRC. The types of samples that can be analyzed are: 1) ileal Peyer's patches and lamina propria samples, acquired by endoscopy and biopsy of the terminal ileum; 2) bronchoalveolar lavage fluid; 3) rectal biopsies; 4) vaginal/cervical biopsies and cervical mononuclear cells isolated by cytobrush (Figure 2). There are a number of hurdles to overcome to achieve the level of sensitivity and specificity in detecting and assessing the function of cells from these samples as is currently possible with peripheral blood mononuclear cells. Thus, the VRC will first optimize approaches to measure mucosal HIV-1-specific T-cell responses by HIV-1 peptide stimulation, intracellular cytokine secretion and multiparametric flow cytometry. As MHC-multimers become more widely available, they will be used to assess HIV-1-specific CD8 T-cell responses. These techniques will be complemented by the more commonly

applied and simpler ELISpot technology, which can eventually be used by a wide range of researchers with whom the VRC will collaborate.

As the containment of HIV-1 replication is unlikely to be effected exclusively by T-cells, there is a need to broaden the scope of investigation. This would include assessment of the frequency, distribution, and phenotype of other resident mucosal immune cells in samples from infected individuals at different disease stages and with different rates of progression, and in samples from non-infected vaccinated individuals. The VRC will develop novel assays to assess humoral immunity, particularly at mucosal sites. VRC researchers are taking two complementary approaches in order to assess the quantitative and qualitative character of antibody production. Their first approach is to sample different mucosal sites (with brushes or washes) to quantify antibody secretion and characterize the relative contributions of the important subclasses (IgA, IgG). Secondly, the VRC is developing techniques to identify the antigen-specific B cells directly, in order to separate them viably and carry out molecular analyses. Importantly, VRC scientists wish to address whether the scope of the antigenic reactivity generated by the mucosal B cells is broader or more focused, or differently focused, than that of the systemic (blood-borne) immune response.

Objective 2: Define compartmentalization of central and mucosal immune sites for induction of antigen-specific responses

In concert with the measurement of antigen-specific responses from mucosal sites, the VRC will attempt to identify correlates of such responses in peripheral blood samples in order to extend these studies to larger vaccine trials where mucosal sampling would be logistically difficult. This effort will include identification of those rare cells in the blood that express mucosal homing markers, and restriction of functional analyses to those cells (compared directly to the resident mucosal cells in the same host). The identification of mucosal correlates of immunity could prove to be a powerful adjunct in the large-scale analysis of immune responses generated by our vaccines in Phase II/III trials, as it would provide information on associations between mucosal immunity and protection.

It will be important to understand the compartmentalization of the central and mucosal immune responses, particularly with regard to the induction of antigen-specific responses. Preliminary data suggest that the gut-associated lymphoid tissue (GALT) shows a good level of exchange with the systemic immune system, in that systemic immunization induces measurable immune responses in the gut. However, the rules of equilibration between these sites are not yet known. As an example, the question arises as to how one would selectively increase the gut-associated responses. In contrast, data suggests that the bronchoalveolar-associated lymphoid tissue (BALT) does not equilibrate substantially with the systemic or GALT immune systems—immune responses induced in the lung do not necessarily redistribute to other sites.

Since the protection from some diseases such as tuberculosis (TB) and influenza may require robust lung-associated responses, it will be crucial to understand how measurements of immune responses at these different sites interrelate. For example, it is possible that induction of immunity in the lung may protect against TB and influenza virus, but perhaps not against HIV-1.

Objective 3: Define strategies to elicit strong and persistent cellular and humoral responses at different mucosal sites

Combinations of priming and boosting at both systemic and mucosal sites may be necessary to induce optimal immunity at selected mucosal sites. This will require testing a wide range of combinations of systemic and mucosal priming with one vector, followed by boosting at the other site with another vector. Due to the differential anti-vector immunity expressed at different sites that adds yet another variable to this equation, the VRC expects to evaluate a substantial number of different immunization schemes.

With accruing evidence of the importance of memory immune cells at the mucosal site of viral entry, the VRC will focus its efforts on selecting immunogens and routes of vaccine delivery to optimize mucosal immunity through vaccination. While preliminary evidence suggests that systemic immunization with plasmid DNA and rAd5 induces cellular immune responses in mucosal compartments, systematic studies are needed to determine whether mucosal delivery of these immunogens increases effective mucosal immunity. Moreover, different strategies for mucosal delivery of immunogens need to be evaluated. Although studies in small laboratory animals have defined adjuvants that are particularly effective in inducing mucosal immune responses, additional studies are needed to identify adjuvants that will generate optimal mucosal immune responses in humans. Perhaps most importantly, the vectors currently being explored for inducing immune responses in humans have been selected for their ability to generate systemic immunity. It will be important to explore novel vectors for their ability to induce mucosal immune responses, including recombinant adenoviruses, mycobacteria, and resident microflora that specifically target mucosal sites. Finally, it will be productive to explore different routes of immunogen delivery—vaginal/cervical, rectal and respiratory—for inducing immunity in different mucosal compartments.

Objective 4: Determine if mucosal immune responses have independent predictive power for HIV-1 vaccine efficacy

The VRC will evaluate whether mucosal immune responses have independent predictive power for vaccine efficacy. It is likely that evidence for this will come mainly from animal models, as the wide range of sampling required makes human clinical trials more difficult. In the case of influenza virus or TB, lung-associated immune responses are likely to be important. For HIV-1, it is likely that gut-associated responses are most important. Based on animal data, it will be possible to conduct selected tissue sampling in human subjects to validate the concept that local immune responses will predict overall vaccine efficacy.

GOAL 4. Advance vaccine candidates for Ebola, Marburg, and influenza viruses into efficacy trials

Ebola and Marburg Viruses

Objective 1: Develop filovirus vaccines and immunization strategies in NHPs that will support ongoing efforts to define an optimal human vaccine

In 2002, the U.S. Food and Drug Administration (FDA) instituted a new rule (21 CFR 601, Subpart H; the “Animal Rule”) that allows approval of products for dangerous pathogens based on “evidence of effectiveness derived from appropriate studies in animals” when human efficacy trials cannot be ethically or feasibly conducted. In place of human efficacy trials, marketing approval for a new product can be based upon safety demonstrated in human clinical trials, along with efficacy demonstrated in an animal species that has been shown to predict relevant responses in humans. The VRC will use this process of animal efficacy studies to pursue approval of filovirus vaccines for Ebola and Marburg viruses. Ebola virus infection in cynomolgus macaques represents an animal model that satisfies the FDA requirements for vaccine licensure because the disease time course, pathophysiology, and clinical symptoms closely mimic those observed in human Ebola infections. The NHP infectious challenge model for Ebola is the most appropriate predictive tool to assess efficacy under the Animal Rule, and, thereby, to advance potential vaccine candidates from animals into humans.

Of five possible Ebola virus subtypes that might be targets for immunization, the subtypes Zaire and Sudan/Gulu have the highest priority for vaccine development based on incidence and high pathogenicity. Two subtypes, Ivory Coast and Reston, have low virulence or have not been shown to cause disease in humans, respectively. Tests of a prototype vaccine in cynomolgus macaques showed that addition of the GP (major surface glycoprotein) gene from the Ebola virus Sudan/Gulu strain to an Ebola Zaire GP vaccine did not reduce efficacy against subsequent challenge with Zaire. Further experiments have demonstrated that addition of the NP (nucleoprotein) gene to the vaccine did not improve protection. Therefore, candidate vaccines based on GP from Ebola Zaire, Ebola Sudan/Gulu, and Marburg have been advanced into clinical trials.

The NHP model has been used to evaluate regimen and dose range for vaccine efficacy and tolerability, and this information informs the design of human trials. The NHP data, together with considerations of manufacturing scalability, and feasibility of distribution, will determine priorities for advanced development.

Clinical trials of candidate vaccines based on the rAd5 vector platform have suggested that natural immunity to Ad5 may limit the immunogenicity of rAd5-based vaccines. The influence of prior immunity was tested for the Ebola rAd vaccine and it was observed that immune protection using rAd5 alone was diminished in Ad5 pre-immune NHPs. The VRC is evaluating several strategies in parallel to overcome this issue of pre-existing immunity. Testing vectors based on alternative Ad serotypes with low seroprevalence in humans, such as Ad35 and Ad26, has shown promise for eliciting robust immunity in Ad5-positive NHPs. Experiments show that DNA priming prior to rAd immunization enhances the magnitude of adaptive immune responses. Therefore, a second approach will test whether DNA and/or

alternative gene-delivery vector prime-boost strategies will improve immune potency in Ad5-immune subjects. Finally, the VRC will investigate alternative routes of rAd immunization to evaluate whether specific tissue compartments provide a more favorable environment for rAd delivery of vaccine inserts. VRC scientists will advance strategies yielding potent protective immunity in Ad5-immune NHPs in parallel human safety and immunogenicity clinical trials that are staged to immediately follow successful animal studies. All studies will assess the frequency and magnitude of the Ebola and Marburg vaccine-induced humoral immune response by standardized ELISA assays. ELISpot and multi-parameter flow cytometry, including intracellular cytokine staining and surface markers of memory phenotype, will be used to measure the frequency, magnitude, and phenotypic characterization of Ebola and Marburg vaccine-induced T-cell responses.

As a first step in human studies, a Phase I randomized human clinical trial was conducted using doses ranging from 2 to 8 mg of Ebola DNA GP constructs. The range of antibody titers was comparable to those observed in NHPs vaccinated with a similar vaccine, indicating that the NHP model may be a valid predictor of human immune responses against Ebola virus antigens. The VRC is evaluating additional DNA and replication-defective adenovirus vectors as components of candidate Ebola and Marburg vaccines. VRC scientists will perform dose-ranging experiments and define the optimal schedule for eliciting an antibody response to Ebola and Marburg GP. The VRC will also evaluate a combined Ebola/Marburg vaccine strategy following assessment of individual candidate vaccines for Ebola and Marburg.

Objective 2: Define cellular and humoral immune responses that correlate with protection against filovirus infection in NHPs and survival in humans

Epidemiological data from human outbreaks suggest that the majority of infected individuals are unable to mount effective immune responses against Ebola virus infection. However, the timing and magnitude of Ebola infection-induced inflammatory cytokine and humoral responses distinguished survivors from fatalities in an outbreak of Ebola Zaire and suggested that protective immunity could be generated in some hosts.

The use of multiple rAd serotype vectors generates an array of immune responses that can be evaluated to elucidate correlates of immune protection. The hypothesis is that immunity could correlate with the quantity, quality, or breadth of T-cell responses as assessed by intracellular cytokine analysis, or with the humoral immune response as evaluated by measuring the magnitude and subtype of Ebola-specific serum antibody. In the Ebola vaccine-challenge model, VRC scientists showed that vaccine-induced cellular and humoral immune responses are higher in animals that survive challenge. Using methods that capture multiple qualitative and quantitative delineators of immune cell function, VRC researchers will identify components of a vaccine-induced immune response that predict survival after challenge with a lethal dose of Ebola virus.

The first demonstration of vaccine-induced protection against Ebola infection showed a correlation between survival in mice and antigen-specific humoral responses as measured by ELISA IgG. Later studies in an NHP model confirmed that pre-challenge Ebola-specific antibody levels are a potential surrogate marker for immune protection. In order to define the immune correlates, the VRC plans to identify vaccine conditions that achieve “breakthrough”

infection and partial protection, with a range of immune responses yielding a corresponding range in disease outcome.

One approach to achieving breakthrough is to refine the vaccine dose to yield partial protection. In this setting, the VRC has evaluated both humoral and cellular immunity. Preliminary analyses identified antibodies against the envelope GP as a primary immune correlate. The quality of cellular immune responses measured by multi-parameter flow cytometry provided additional parameters associated with immune protection. The aim is to dissect qualitative and quantitative differences in Ebola antigen-specific cellular responses that will predict survival in vaccinated subjects. Since VRC scientists are especially interested in pre-challenge immune responses elicited by a single immunization, they have focused their initial investigations on memory responses. The quantity and quality of vaccine-induced cellular responses will be evaluated further in subsequent studies by identifying the range of cytokine responses that are elicited following immunization. Therefore, VRC researchers have amplified the number of phenotypic markers and cytokines measured to better characterize the lineage, memory phenotype, state of activation, and cytokine-producing attributes for each antigen-responsive cell. The resulting data are used to perform an analysis capable of capturing multiple functional aspects of vaccine-induced T-cell responses.

Similar studies undertaken to evaluate immune responses in survivors of natural Ebola infection will help to elucidate immune mechanisms of protection, especially memory responses that are critical in a prophylactic vaccine. The VRC has obtained blood samples from individuals who survived the 1995 Ebola outbreak in Kikwit, Democratic Republic of Congo, and has begun measuring a variety of human humoral and cellular immune responses to compare to those obtained in NHPs. VRC scientists are obtaining serum samples from African subjects at high risk for Ebola exposures (e.g., bush meat hunters or carcass handlers) to explore characteristics of humoral immunity that may effectively prevent infection. Using additional samples obtained from close contacts of Ebola patients, the VRC will seek to identify components of both cellular and humoral immunity associated with asymptomatic infection.

Objective 3: Elucidate the mechanisms of protective immunity induced by filovirus vaccines in the NHP model of infection

Ebola virus poses unusual difficulties because outbreaks occur unpredictably in remote geographic locations and are often self-contained, terminating before research teams can be mobilized for the collection of rare clinical specimens. A large epidemic spanning a six-month period in 1995 allowed one of the first opportunities for international teams to intervene and treat patients, while also providing support for careful sample collection, documentation of disease outcomes, and retrospective analysis of host responses in surviving and fatal cases. In an analysis of innate immune responses, serum samples from fatalities exhibited elevated proinflammatory cytokine levels, comprised of IFN- γ , IL-2, IL-10, TNF- α , and IFN- α compared to survivors or uninfected controls, suggesting that exaggerated and uncontrolled immune activation might be deleterious if unaccompanied by the rapid development of antigen-specific immune effector functions needed to clear infected cells and prevent virus propagation. A study of convalescent patients from the same outbreak showed a correlation between elevations in IgM or IgG and the disappearance of viral antigen, providing an indication that adaptive immune responses may be involved in controlling viral

loads during Ebola infection. Samples from a 1996 Gabon outbreak showed that early activation of T-cells was fatal if unaccompanied by the effector activity of cytotoxic T-cells or in the absence of IgG specific for viral proteins. IgG was detected against NP in all survivors after the onset of symptoms and against Ebola VP35 and VP40 proteins in most survivors during the convalescent period. Since immunoglobulin directed against internal virion proteins will not be neutralizing, it is likely that the humoral response measured in these patients reflects the induction of broad antigen-specific cellular immunity leading to both B- and T-cell effector functions.

The VRC is using the studies of vaccine-induced protective immunity in NHPs to help elucidate the precise mechanisms of immune protection against Ebola infection. Specific cellular and humoral responses can be manipulated to help assess protective immunity against Ebola infection mediated by antigen-specific T and B cells revealing important mechanisms of immunity. Current studies being conducted in NHPs utilize methods for selective inhibition of specific immunity such as T- and B cell depletion, inhibition of T-cell help, and passive transfer of immunoglobulin. In order to ascertain the potential role of B and T lymphocytes in protection against Ebola infection, it may be important to completely abrogate any potential contribution of each component by depleting the cell subset from circulation. In challenge experiments, animals will be immunized with rAd5 Ebola vaccine to elicit cellular and humoral immunity. CD3+, CD4+, CD8+ T-cells and CD20+ B cells will subsequently be depleted, either alone or in combination, and the animals will be challenged with a lethal dose of Ebola virus. The experiments will be designed to evaluate the quantity and quality of cellular and humoral immune responses before and during infection.

A potential drawback of the depletion approach lies in the fact that there may be protected “pockets” of antigen-specific T-cells that are not depleted and are not accurately measured by enumeration of T-cells in the peripheral blood. This possibility would be most problematic if depletion had no effect on vaccine-induced protection. However, the observation of even a partial effect implicates an influence of T-cells in immune protection. Further studies of the tissue distribution of antigen-specific T-cells, either via biopsy, post mortem analysis, or whole animal imaging, may provide a more complete understanding of partial protection.

Objective 4: To develop a NHP model for mucosal and cutaneous infection with Ebola virus, and to identify vaccine protection for these routes of exposure

Oral and conjunctival infectious challenge has been reported in NHPs, but these models have not been developed for reproducibility or for the study of immune responses. Intramuscular immunization against Ebola virus generates peripheral blood antibodies that are primarily IgG. However, antibodies at the mucosal surface, including IgG, secretory immunoglobulin A (SIgA) and IgM, may be critical for protection against mucosal pathogens. These antibodies can prevent pathogen entry, or can work at later stages to reduce virus spread. Since protective immune responses may differ depending on the route of exposure, the VRC will develop models of mucosal and cutaneous Ebola virus infection in cynomolgus macaques in order to assess antigen-specific immune responses.

Studies have shown that oral immunization can generate IgA in tears, saliva and nasal secretions even in the absence of detectable serum IgA. Therefore, measurement of only peripheral blood immune responses may underestimate immunity at mucosal surfaces. The

VRC will evaluate antibody and T-cell responses in nasal secretions and bronchoalveolar lavage to assess the relative efficiency of intramuscular and mucosal vaccines to elicit antigen-specific immune responses against Ebola virus at mucosal surfaces. In addition, the VRC will perform imaging studies in the new NIAID BSL-4 facility, currently under construction, to evaluate virus distribution at mucosal surfaces and the interaction of virus particles with immune cells and antibodies. Lastly, these studies will be complemented by field studies in which VRC collaborators in Africa (Republic of Congo, Democratic Republic of Congo, and Cameroon) are collecting blood samples from subjects reporting high-risk activities for mucosal and cutaneous exposure.

Influenza Virus (Avian and Seasonal)

Objective 1: Determine the safety profile of influenza DNA and adenovirus vector vaccines in healthy adults

VRC DNA candidate vaccines encoding proteins from HIV-1, SARS, Ebola and West Nile virus have been shown to be safe and well tolerated and to induce both antibody and T-cell responses in healthy adults. The VRC has also recently developed avian influenza and seasonal influenza virus candidate DNA vaccines using these previously evaluated DNA expression plasmids. The first Phase I trial of a candidate avian influenza vaccine, using DNA encoding hemagglutinin for an H5 Indonesian strain, was initiated in 2006. Other influenza vaccine trials are planned in the near future. For example, in cooperation with the HHS Biomedical Advanced Research and Development Authority (BARDA), the VRC has obtained clinical materials for studies to determine whether a prime-boost strategy using H5 DNA and inactivated H5N1 vaccine induces a broader or more durable immune response than either candidate alone. A Phase I study of this prime-boost platform commenced in November 2008. For seasonal influenza, the VRC plans to assess a prime-boost strategy (trivalent seasonal DNA, boosted by inactivated seasonal vaccine). Such a vaccine might induce a more vigorous immune response in older persons than inactivated influenza vaccine alone.

The VRC will assess safety and tolerability of influenza vaccine candidates at a range of doses and injection schedules and by various routes of administration in Phase I clinical trials. The dose, route, and injection schedules for a series of influenza vaccine Phase I trials will be determined based on data from pre-clinical and human trials for similar VRC candidate vaccines. The VRC is evaluating doses from 500 µg to 4 mg and intramuscular (IM) or intradermal (ID) delivery with needle and syringe or alternative devices (e.g., the needle-free injection system, Biojector®).

Objective 2: Determine the characteristics of the immune response following vaccination with influenza DNA vaccines in healthy adults

ELISA, neutralizing antibody assays, and hemagglutinin-inhibition assays (HAI) will be used to measure the magnitude and frequency of the influenza vaccine-induced humoral immune response. Additionally, autologous and heterologous influenza strains will be important to understand the potential for antibody-mediated cross-protection between strains of influenza virus.

Based on studies of licensed influenza vaccines, ID delivery is well tolerated and has improved immunogenicity with dose-sparing compared to IM delivery. The VRC will test in

Phase I clinical trials whether ID delivery with a variety of devices will improve the magnitude, frequency, and phenotypic characterization of vaccine-induced antibody or T-cell responses. VRC researchers plan to use ELISpot and ICS using multi-parameter flow cytometry to assess T-cell function and to measure the cross-reactive potential of vaccine-induced T-cell responses by stimulating peripheral blood mononuclear cells with peptide pools representing both autologous and heterologous influenza strains.

The quality of the mucosal immune response to a candidate influenza vaccine may be important in determining the vaccine's efficacy. Although this type of assessment can be difficult both clinically and in the laboratory, the VRC will address this important exploratory question as the Phase I trials of VRC candidate influenza vaccines progress. VRC scientists will utilize bronchoalveolar lavage and nasal mucosal biopsies to assess the quality and frequency of vaccine-induced respiratory mucosal immune responses in collaboration with the Laboratory of Clinical Infectious Diseases, NIAID, NIH. Samples will be measured for humoral and T-cell immune responses by ELISA, HAI, and intracellular cytokine staining assays using influenza, proteins, and peptide pools from both autologous and heterologous virus strains.

Objective 3: Determine the potential for influenza DNA vaccines to prime for inactivated influenza virus vaccines in a heterologous prime-boost regimen

Avian influenza antigens tend to be less immunogenic than seasonal influenza antigens. In trials of inactivated avian influenza candidate vaccines, high doses have been required to induce marginal immune responses using traditional adjuvants. In Phase I clinical trials, the VRC has begun to assess the potential use of H5 DNA vaccines as a priming vaccine to improve overall immunogenicity or to achieve dose-sparing for an inactivated virus-vaccine boost.

The frequency and magnitude of immune responses to seasonal influenza vaccination is reduced in older adults. The VRC will evaluate DNA as an influenza vaccine prime (trivalent seasonal DNA + H5 DNA), boosted by inactivated seasonal + inactivated H5 vaccine, as a means of improving the frequency and magnitude of the immune responses to influenza vaccination in older adults.

Objective 4: Determine the feasibility and develop prototypes for a universal influenza vaccine

There is increasing evidence that neutralizing antibodies that show greater breadth than those typically observed in antisera after natural infection can be detected in recombinant antibody libraries and in selected infected individuals. This finding raises the possibility that it may be possible to elicit such antibodies by vaccination. Based on an understanding of influenza hemagglutinin structure and genetics, and building on the VRC's HIV-1 structural biology efforts, the VRC will explore the molecular basis for such increased reactivity and will utilize structure-based design approaches to develop vaccine candidates. In addition, the VRC will analyze next-generation vaccine candidates for the breadth and magnitude of their antibody response as these candidates progress in human trials. VRC scientists will also evaluate different influenza A and B strains for their susceptibility to neutralization to such antibodies using lentiviral vector reporter and microneutralization assays, and compare them

to the traditional hemagglutinin inhibition immune correlate currently in use for licensed influenza vaccines.

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