Development of a mimotope-based synthetic peptide vaccine against HIV using plant viruses
by Uraiwan Intamaso

A dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Microbiology
Montana State University

Abstract:
Efforts to develop an HIV vaccine have been hindered by difficulties in identifying epitopes capable of inducing a broad neutralizing antibody response due to the high mutation rate and complex structure of HIV envelope glycoprotein. However, the CD4 binding site of gp120 exhibits a high degree of conservation, so epitopes at this site could potentially be used as a vaccine to induce a broad neutralizing antibody response. A random peptide phage display library was screened using monoclonal antibodies directed this site, to identify antigenic and immunogenic mimics of such epitopes of gp120. Peptides corresponding to the selected sequences plus a C-terminal cysteine were synthesized and coupled to carriers, such as the plant viruses CCMV and CPMV using heterobifunctional linkers. When these linkers were used with CCMV, low yields of conjugates and viral aggregation were obtained due to the many active lysine residues on the viral surface and the presence of excess linker. These problems were overcome by making a virus mutant with a single cysteine residue exposed on the surface of each capsid subunit (CCMV S130C). This cysteine was coupled to the peptides using a homobifunctional linkers. The peptides coupled to viral carriers and to KLH were used to immunize mice. Sera were tested for specific anti-peptide antibodies. All carrier-peptide conjugates induced anti-peptide antibodies. The amount of peptides attached to the carriers was not the only factor determining the anti-peptide antibody titers. All conjugates used were immunogenic when injected with adjuvant. However, when injected without adjuvant, CCMV S130C conjugate induced low anti-carrier antibody titers and no anti-peptide antibody titers, while KLH conjugates induced high anti-carrier antibody titers and low anti-peptide antibody titers. Anti-gp120 antibody titers were detected in sera from mice immunized with the virus-peptide conjugates but not in mice immunized with virus alone. However, sera from mice immunized with irrelevant peptide conjugates showed some anti-gp120 cross-reactivity. None of the sera showed binding to gp120 on HIV-infected cells or neutralizing activity. These results indicated that the peptides acted as antigenic mimics when screened but lost their mimicry when coupled to carriers.

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A dissertation submitted in partial fulfillment
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Doctor of Philosophy

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Microbiology

MONTANA STATE UNIVERSITY
Bozeman, Montana

May 2003

APPROVAL

of a dissertation submitted by

Uraiwan Intamaso

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies

Dr. Martin Teintze _____ 5/27/03
(Signature) Date

Approved for the Department of Microbiology

Dr.Tim Ford _____ 5/28/03
(Signature) Date

Approved for the College of Graduate Studies

Dr. Bruce McLeod Bruce R. McLeod 6-3-03
(Signature) Date

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ACKNOWLEDGMENTS

I would like to thank Dr. Martin Teintze who has been very invaluable to me. Not only with the research but also he has given me with the advice in the way of life. Thus only "thank you" doesn't express my gratitude to him. As a foreign student who has problems in English communication he has been very patient with me and helped me feel more comfortable speaking English. Moreover he also widened my attitude about understanding people who are different from me in terms of culture, religion and etc. I won't leave my good memories about him here but will bring them back with me forever. I would like to thank Dr. Seth Pincus who helped me understand that I can study English along with Science. Thus, I learned English any way I could and made me more comfortable with English. Moreover, thanks for his kindness and sincerity for helping me in research. Also, I would like thank Dr. Mark Young who gave me a chance to learn techniques in his lab which inspired me to learn more about viruses. I would like to thank Dr. Royce Wilkinson for his help with instrumentation. I also thank Wan for helping with some experiments and personal things. I would like to thank friends in Thailand who have never left me alone here, especially Dr. Siritida Porntosapon, Nong and Pom. Finally, I would like to thank my parents for their unconditional love and patience, most of all, for their confidence in their daughter and let me do what I really want to do.

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ABSTRACT

Efforts to develop an HIV vaccine have been hindered by difficulties in identifying epitopes capable of inducing a broad neutralizing antibody response due to the high mutation rate and complex structure of HIV envelope glycoprotein. However, the CD4 binding site of gp120 exhibits a high degree of conservation, so epitopes at this site could potentially be used as a vaccine to induce a broad neutralizing antibody response. A random peptide phage display library was screened using monoclonal antibodies directed this site, to identify antigenic and immunogenic mimics of such epitopes of gp120. Peptides corresponding to the selected sequences plus a C-terminal cysteine were synthesized and coupled to carriers, such as the plant viruses CCMV and CPMV using heterobifunctional linkers. When these linkers were used with CCMV, low yields of conjugates and viral aggregation were obtained due to the many active lysine residues on the viral surface and the presence of excess linker. These problems were overcome by making a virus mutant with a single cysteine residue exposed on the surface of each capsid subunit (CCMV S130C). This cysteine was coupled to the peptides using a homobifunctional linkers. The peptides coupled to viral carriers and to KLH were used to immunize mice. Sera were tested for specific anti-peptide antibodies. All carrier-peptide conjugates induced anti-peptide antibodies. The amount of peptides attached to the carriers was not the only factor determining the anti-peptide antibody titers. All conjugates used were immunogenic when injected with adjuvant. However, when injected without adjuvant, CCMV S130C conjugate induced low anti-carrier antibody titers and no anti-peptide antibody titers, while KLH conjugates induced high anti-carrier antibody titers and low anti-peptide antibody titers. Anti-gp120 antibody titers were detected in sera from mice immunized with the virus-peptide conjugates but not in mice immunized with virus alone. However, sera from mice immunized with irrelevant peptide conjugates showed some anti-gp120 cross-reactivity. None of the sera showed binding to gp120 on HIV-infected cells or neutralizing activity. These results indicated that the peptides acted as antigenic mimics when screened but lost their mimicry when coupled to carriers.

CHAPTER ONE

INTRODUCTION AND REVIEW OF HIV

Introduction

The human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS) in humans, which is spreading unabated in many parts of the world. The highest rate of HIV infection is found in sub-Saharan Africa where the lifetime risk of death from AIDS is 40-50% and life expectancy is reduced by up to 30 years (1).

Although the current generation of antiviral drugs has succeeded in fighting against AIDS, most HIV-infected individuals will never benefit from these therapeutic agents due to lack of access and/or the prohibited costs of these drugs. The AIDS epidemic is concentrated in regions of the world where insufficient financial resources prevent access to these drugs. Even in wealthy countries where these therapeutic agents are available, concerns have been raised regarding poor tolerance of the drugs and the long-term toxicity of therapeutic combinations. The ultimate solution for containment of the AIDS epidemic will require the development of a vaccine strategy. Almost all vaccines in use today are based on either a) killed or attenuated virus b) recombinant coat proteins. Neither of these approaches has been successful against HIV. Thus, it seems that the development of a vaccine against HIV will be more difficult than originally anticipated.

AIDS

Acquired immune deficiency syndrome (AIDS) is the syndrome resulting from the destruction of $CD4^+$ lymphocytes by HIV. The course of HIV-1 infection is characterized by a biphasic viremia. Following the dissemination and propagation of HIV-1 in the lymphoid tissues during primary infection, engagement of the host antiviral immune response coincides with a fall in the plasma virus load to a set-point level (2). Uncontrolled viral replication during primary HIV-1 infection initially leads to activation of a marked cell-mediated immune response, which is later accompanied by the development of a humoral immune response (3). Elevated levels of immune markers decline as the disease enters the chronic phase (4). Although the hallmark of HIV-1 infection and AIDS is immunodeficiency resulting from functional and numeric loss of $CD4^+$T lymphocytes, there is a heightened state of systemic immune activation throughout the course of chronic HIV-1 infection (5, 6, 7). The immune activation resulting from the presence of opportunistic infections, other inflammatory stimuli, or the antigenic stimulus of HIV-1 infection itself may provide an immunological environment that actually drives viral replication and disease progression in HIV infected persons (8). Immune activation has been a key theme in therapeutic approaches to the control of HIV replication and elimination of infection. However, the ability of HIV to replicate persistently in infected individuals and avoid immune surveillance has been one of major obstacles to achieving a therapeutic cure for HIV-infected persons (9).

Genetic Diversity

The hallmark of HIV is its extensive genetic diversity. Heterogeneity in nucleic acid sequences is the result of the error-prone viral reverse-transcriptase (RT) enzyme as well as the high rate of virion production. Their heterogeneity allows for quick adaptation of viruses to environmental selection pressures.

HIV can be divided into two types: HIV-1 and HIV-2, which represent independent crossovers of simian retroviruses into humans (10). There are three branches in the phylogenetic tree of HIV-1 sequences, which constitute the M (main), N (new or non-M, non-O), and O (outlier) groups (11). Among these, group M viruses are the variants of HIV-1 responsible for more than 99% of infections worldwide. The M-group viruses have been divided into distinct genetic subtypes or clades, which are defined as groups of viruses that more closely resemble each other than other subtypes, across the whole genome (12). Using this definition, there are currently nine circulating genetic subtypes (A, B, C, D, F, G, H, J, and K) within group M. The viruses originally identified as subtypes E and I are now considered intersubtype recombinants. The prevalence of intersubtype recombination strains is increasing and creates even more HIV-1 antigenic diversity. Several recombinant viruses have now spread epidemically to establish distinct lineages. These are referred to as circulating recombinant forms (CRFs), nine of which have presently been identified (13). CRFs have a designation that includes the letters of the parent genetic subtypes (e.g., CRF01_AE) and in CRFs derived by recombination of more than three subtypes,

the letters are replaced by cpx (complex), e.g., subtypes E and I are now termed CRF01_AE and CRF04_cpx, respectively (12). Currently, strains belonging to the same subtype can differ by up to 20% in their envelope proteins, and differences between subtypes can be up to 35% (14). Moreover, this diversity is continually growing.

Globally, subtypes A and C account for most current infections followed by subtype B and the intersubtype recombinants CRF01_AE and CRF02_AG. Subtype C is currently infecting more people worldwide than any other and is common in Southern Africa and India. Subtype B is dominant in Europe, the Americas and Australia. Subtypes A and D infect large numbers of people in central and eastern Africa. In western Africa, an intersubtype recombinant, CRF02_AG, is the dominant virus type. CRF01_AE (which was previously referred to as subtype E) is the most prevalent virus in southern Asia. As HIV-1 continues to spread globally, the geographical restrictions are increasingly breaking down (11).

Superinfection.

Superinfection occurs when a cell becomes infected by at least two genetically distinct HIV virions, which may be of the same subtype or of different subtypes. HIV may increase its potential evolutionary success through recombination (15). During HIV recombination, the viral transcriptase (RT) enzyme responsible for reverse transcription of RNA into cDNA can switch from one viral RNA template to another, creating a new virion that is a chimera of the

parental virions (i.e. CRF). *In vitro* experiments have demonstrated that HIV-1 undergoes 2-3 recombination events per genome per replication cycle (16). The high rate of recombination allows HIV to evolve due to the introduction of a large number of genetic changes. Such changes can alter cell tropism, envelope serotypes, viral pathogenicity, antiretroviral drug susceptibility, and disease progression.

Viral Entry

HIV, like all retroviruses, is surrounded by an envelope consisting of a host cell lipid bilayer and virus-encoded envelope glycoproteins, organized into spikes on its surface, that are essential for viral entry into target cells. These envelope spikes consist of trimers of the surface glycoprotein (gp120), which contains the binding domains for cellular receptors, attached to trimers of the transmembrane glycoprotein (gp41) that anchor the spike into the viral membrane (17, 18).

To initiate a new infection, nascent particles need to encounter a potential target cell that expresses the appropriate receptor structures. In the case of HIV, these are the CD4 molecule, found principally on T lymphocytes, macrophages and dendritic cells, and certain members of the chemokine receptor family, which are termed coreceptors. Initially, the specific CD4 binding domain of gp120 attaches with high affinity to the N-terminal membrane-distal domain of CD4. Binding to CD4 induces a conformational change that exposes the chemokine

receptor-binding domain of gp120, allowing it to bind the chemokine coreceptor. Following coreceptor binding, a more dramatic conformational change exposes the gp41 portion of the envelope previously buried in the interior of the complex. This transient species, termed prehairpin intermediate, is formed by folding of the N-terminal trimeric coiled coil, leading to the insertion of the N-terminal hydrophobic fusion peptide region into the target membrane. This prehairpin intermediate then collapses to form a six helix-bundle structure in which the C-terminal regions pack into the hydrophobic grooves of the N-terminal coiled-coil trimer in an antiparallel manner. The formation of this "trimer of hairpins" structure brings the viral and cellular membranes into close apposition, allowing them to fuse and causing release of the viral capsid into the cytoplasm (19, 20). Following virus entry, subsequent intracellular events (e.g., nucleocapsid uncoating and reverse transcription) take place, allowing virus replication in acutely infected cells.

Synthesis and Assembly of Envelope Glycoproteins

In the infected cell, the HIV-1 envelope protein is initially produced as an approximately 845- to 870-amino acid precursor in the rough endoplasmic reticulum. Asparagine-linked, high-mannose sugar chains are added to form the gp160 glycoprotein. Gp160 trimers are assembled and subsequently transported into the Golgi apparatus, where cleavage by a cellular protease generates the mature envelope glycoproteins: gp120 and gp41. The gp120 and gp41

glycoproteins are maintained in the assembled trimer by noncovalent, somewhat labile interactions between the gp41 ectodomain and a discontinuous structure composed of NH_2- and COOH-terminal gp120 sequences. When they reach the infected cell surface, gp120 and the ectodomain of gp41 are exposed on budding virus particles, whereas the membrane-spanning anchor and the long cytoplasmic tail of gp41 are inside the cells or virions (21).

Results from electronic microscopy show that mature HIV particles are icosahedral and possess about 72 spikes forming densely arranged knobs on the viral surface. The diameter of each knob is about 14 nm and its height is 9 to 10 nm; the distance from one to another is about 21 to 22 nm (22, 23)

The Structure of Gp120 Glycoprotein

A comparison of gp120 amino acid sequences from different viral origins revealed the existence of five variable regions (V1 to V5) interspersed with five conserved regions (24). Intramolecular disulfide bonds in gp120 result in the formation of the first four variable regions into large, looplike structures that are exposed on the surface of the protein, whereas the conserved regions fold into a central core. The structure of a gp120 core, lacking V1, V2, and V3 and the COOH and NH_2 termini, as well as most of the carbohydrates, and bound to the N-terminal domain of CD4 has been solved by X-ray crystallography (25). The gp120 core forms a globular structure, consisting of 25 β-strands, five α-helices and ten loops. The structure folds to form two domains, an inner and outer

domain, and a "bridging" β-sheet in between the domains (Fig. 1). The domain names reflect the likely orientation of gp120 in the glycoprotein trimer: the inner domain orients to gp41 whereas the outer domain is mostly exposed on the surface of the trimer. The CD4 receptor-binding regions, located in a depression formed at the interface of the outer and inner domains with the bridging sheet, contain residues that are well conserved among primate immunodeficiency viruses. These regions, and also the inner domain surface, are devoid of glycosylation. Although generally well conserved compared to the five variable regions, the gp120 core also shows variability disproportionately on the surface of the outer domain proximal to the V4 and V5 region. Such variability results from the δA, δC, δD and δE surface loops and also from the N-linked glycosylation sites.

Much of the sequence of the core is highly conserved, which is likely a reflection of functional constraints. In contrast, the sequence-variable loop regions appear to be more malleable. Even under the uniform immune pressure within the same infected individual, multiple sequences of the variable loops coexist (26, 27). This indicates that there are several different structural solutions in creating viruses that replicate and resist neutralization optimally. The multiplicity of variable loop structures facilitates the emergence of neutralization-resistant viruses, thus providing greater opportunities for rapid viral adaptation to selective pressure *in vivo*.

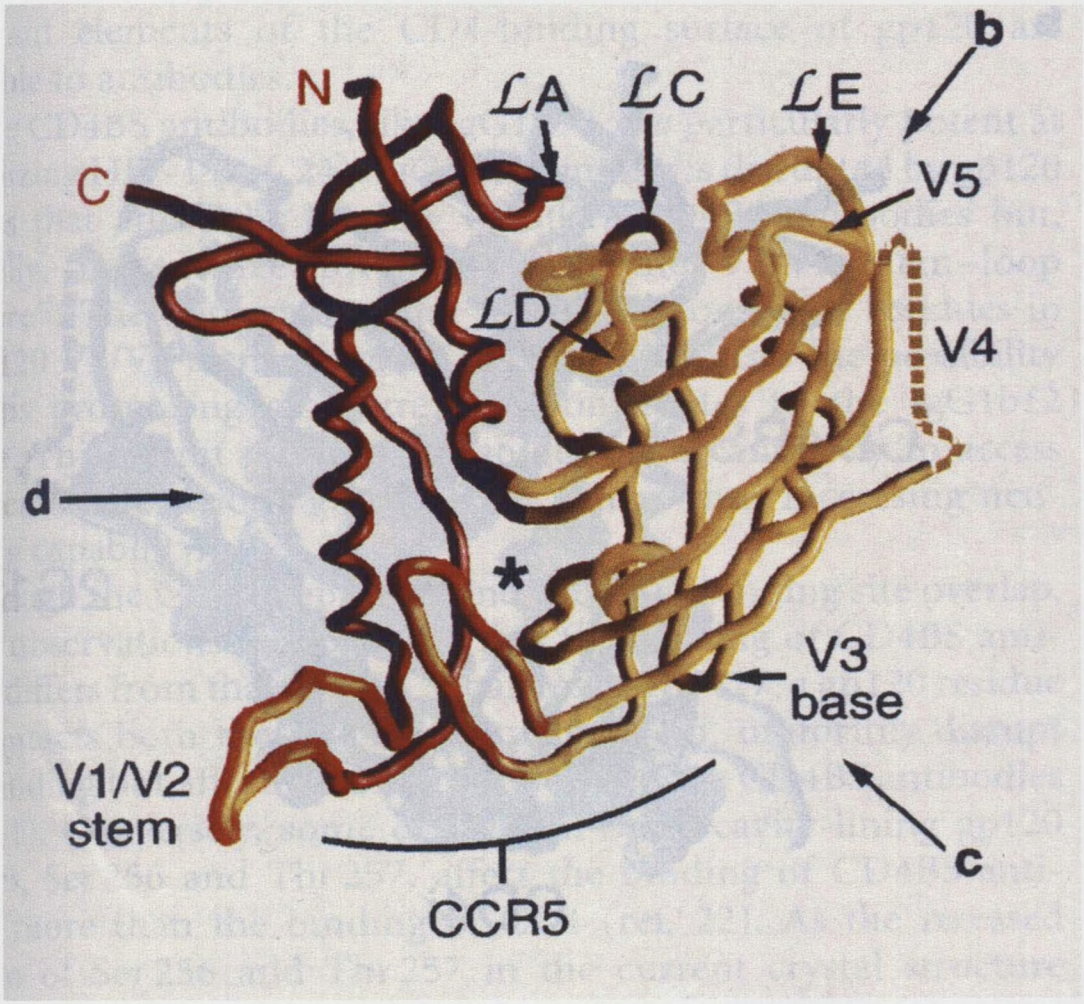

Fig 1. Structure of the HIV-1gp120 core. A Cα tracing of the gp120 core. The inner gp120 domain is shown in red and the outer domain in yellow; the bridging sheet is orange. The position of the Phe43 cavity involved in CD4 binding is indicated by an asterisk. The V4 loop was not resolved in the structure, and the V1/V2 and V3 were shortened in the "core" protein compared to the HIV sequence.

CD4-Binding Site.

CD4 binds in a recessed pocket on gp120, making extensive contact over ~800 $Å^3$ of the gp120 surface. There are two cavities at the gp120-CD4 interface. The larger cavity (about 280 $Å^3$) is shallow and filled with water molecules. It is lined with gp120 residues that do not form many direct contacts with CD4. This hydrophilic cavity is lined with variable gp120 residues. The second cavity (about 150 $Å^3$) is hydrophobic, and deeply buried within gp120. It is located at the interface between the three domains and is lined by highly conserved residues. Most important of these are Asp-368, Glu-370 and Trp-427, which surround the opening of the deep cavity and make contact mainly with Phe-43 and Arg-59 of CD4. These residues are all conserved among primate immunodeficiency viruses. Changes in these residues disrupt gp120 binding to CD4 and to certain monoclonal antibodies with epitopes at the CD4 binding site (25). When CD4 binds, its Phe-43 sidechain blocks the entrance of this cavity (28).

Coreceptor Binding Site.

The conserved region of the coreceptor binding site on gp120 is located at an approximately 90° angle to the CD4 binding site (29) and is comprised principally of the bridging sheet, with an additional part from the base of V2 loop. Importantly, exposure of this site requires that the gp120 first binds to CD4. This induces a conformational change in gp120, which enables it to interact with one of several chemokine receptors. The CC-chemokine receptor CCR5 is the major

coreceptor for macrophage-tropic (R5) strains (30, 31, 32). During the early years of infection, CCR5 is an obligate receptor, and rare individuals that are genetically deficient in CCR5 expression are relatively resistant to HIV-1 (33, 34, 35). HIV-1 isolates arising later in the course of infection often use another chemokine receptor, CXCR4. Using CXCR4, T-cell-line-tropic (X4) viruses play a role in disease progression or propagation in tissue culture (36, 37, 38, 39). Studies of chimeric envelope glycoproteins demonstrated that the V3 loop sequence of gp120 is a major determinant of which the chemokine receptor is used (40).

The Immune Response to HIV in Infected Individuals

One key factor in the rational development of a vaccine against HIV is the characterization of the immune correlates, and delineation of the specific portions of the virus that elicit this response. It has been found that a few individuals remain seronegative despite frequent HIV exposure, and a small population (~5%) of HIV-infected individuals, termed long-term non-progressors, remains asymptomatic for more than 15 years after infection. The study of these people has indicated that they show high levels of non-major histocompatibility complex (MHC)-restricted virus suppressor activity, together with strong polyepitopic cytotoxic T lymphocyte (CTL) responses and broadly reactive neutralizing antibody responses. These immune responses are able to maintain low viral loads, stable CD4+ T cells, and the structure and functional organization of lymph

nodes (41). Both neutralizing antibodies that prevent infectivity, and cytotoxic T-cells (CTL), which eliminate virus-infected cells, are considered important elements for protection against HIV infection.

Cell-Mediated Immune Response.

CTLs are known as killer T cells because they recognize, bind, and kill cells that display foreign antigens. A CTL response is highly desirable in an AIDS vaccine because CTLs can eliminate or reduce virus production by killing viral producing cells. Evidence for this protective role comes from a variety of observations. Early studies demonstrated that CD8+ T lymphocytes from HIV-1 infected individuals could suppress HIV-1 replication in autologous CD4+T lymphocytes *in vitro* (42). A temporal association was identified in HIV-1 infected patients between the appearance of virus-specific CD8+CTL in the peripheral blood and decline of primary viremia in acutely infected patients (43, 44, 45). Moreover, in those chronically infected with HIV-1, a high virus-specific CTL activity is correlated with the maintenance of low virus load and a stable clinical status (46). Since MHC class-I molecules present viral peptide fragments to CTLs, some investigators have reasoned that certain class I molecules may bind and display HIV-1 peptides more efficiently than others (47). In fact, correlations have been demonstrated between certain HLA haplotypes and the rate of clinical disease progression in HIV-1 infected individuals. HLA-B27 and HLA-B57 expression were associated with slow disease progression (48), whereas HLA-

B*3501 and HLA-Cw*04 expression were associated with rapid disease progression (49).

Whereas the CTL responses observed in chronically HIV-1-infected individuals are potent and persistent, they ultimately fail to control virus replication. The virus can escape from CTL recognition through mutation of viral epitopes (50). Furthermore, HIV has evolved mechanisms to downregulate expression of CD4 and class I MHC, cell surface proteins that are essential to CTL recognition of viral antigens on viral producer cells (51). Thus, vaccines that depend strictly on a CTL response may be countered by viral adaptations that allow the virus to evade detection by T cells.

Humoral Immune Response.

Because CTL responses alone are unlikely to provide complete protection, it will be important for an HIV vaccine to also elicit neutralizing antibodies (Abs) to the virus. Such Abs are dependent on memory B cells, a long-lived cell population that can divide and differentiate into Ab-producing plasma cells upon re-exposure to antigens, thus conferring long term protection. Another advantage of Abs is that they have the potential to inactivate the virus before it has a chance to infect the cells of the host. Antibodies may also neutralize virus indirectly by binding complement or promote antibody-directed cell-mediated cytotoxicity (ADCC), thus leading to killing of infected cells. Much of the evidence about the importance of the humoral immune response was obtained from studies in nonhuman primates. Neutralizing monoclonal antibodies (mAbs)

have been shown to confer passive protection to rhesus monkeys against challenge with a highly pathogenic SHIV (a chimeric simian-human immunodeficiency virus that expresses the envelope glycoproteins of a primary HIV-1 isolate) (52). Although it has been possible to generate antibodies against the envelope protein of HIV, such antibodies have had limited efficacy. They neutralize laboratory-adapted strains, but are not effective against primary isolates. Moreover they are often strain-specific. In natural infection, generally, primary peak viremia declines before neutralizing Abs directed against HIV envelope glycoproteins are detectable (53). HIV-infected individuals may generate potent neutralizing Ab responses to their autologous isolates, but such responses take a long time to mature. Thus, by the time such neutralizing Abs are generated, they are no longer able to suppress neutralization-escape mutants that have arisen. To develop immunogens that elicit broadly neutralizing antibodies and CTL's, the extensive genetic diversity among different strains and clades of HIV must be taken into account.

Gp120 Glycoprotein as an Antigen and Immunogen

The success of HIV in achieving persistent infections implies that the viral envelope glycoproteins have evolved to be less than ideal immunogens. The analysis of the structure of core gp120 revealed multiple potential mechanisms of immune evasion. Structures on the viral glycoprotein that are conserved among diverse viral strains are poorly exposed to the humoral immune system. Studies

of monoclonal antibody binding suggested the existence of two faces on gp120: a neutralization face and a non-neutralization face (54, 55). The non-neutralization face is buried within the gp120 trimer, but well exposed at the surface of soluble monomeric gp120. Indeed, this face induces a strong Ab response in infected individuals, but these Abs do not exhibit neutralizing activity. Spontaneous dissociation of gp120 from gp41, or release of gp120 from dying, infected cells therefore results in presentation to B cells of highly antigenic segments of molecule. However, Abs elicited to these regions are irrelevant to neutralization (56) because such Abs cannot bind the assembled, functional envelope glycoprotein complex (57).

The neutralization face induces Abs exhibiting neutralizing activity. However, to escape the Ab response, this face is relatively occluded. To replicate, HIV-1 must interact with the CD4 receptor on target cells; thus, the virus needs to keep the CD4 binding site conserved and exposed enough for receptor binding. However, at the same time, HIV must hide this region from antibodies. The first difficulty for Abs is to access the CD4 binding site recessed within the gp120 core. The Fab of an Ab molecule is wider than CD4: two Ig domains compared to one. In addition, the binding site is flanked by variable and heavily glycosylated regions (28). Since most carbohydrate moieties appear as "self" to the immune system, this may reduce the potential of these regions to serve as an immunogenic target. The large hydrophilic cavity at the CD4 binding site tolerates gp120 mutations, and this may facilitate viral escape from

anti-CD4 binding site Abs. Some of the residues of gp120 contact CD4 through their main-chain atoms. This may allow the variation of these residues to facilitate escape from neutralizing Abs without detrimental effects on CD4 binding. However, some human monoclonal Abs characterized by mutation analysis can recognize discontinuous epitopes at the CD4 binding site, called CD4 binding epitopes (58, 59). This indicates that some broadly neutralizing Abs could access the more recessed elements of the CD4-binding pocket.

Subunit Vaccine Strategies to Block Viral Entry

The exposure of HIV envelope glycoproteins on the surface of the virions makes them prime targets for antibodies that potentially bind to the HIV envelope, thereby blocking interactions between the virus and cellular receptors, and preventing viral entry into the cells. Because protein subunits or peptides are not very immunogenic by themselves, they have to be coupled with a variety of carriers and formulated with adjuvants to increase their immunogenicity.

Recombinant Proteins.

In view of the successful use of a recombinant protein as an immunogen for eliciting protective immunity against hepatitis B virus (60,61), considerable effort has been devoted to developing a recombinant protein vaccine for HIV-1. Such recombinant proteins can be produced by expression of the viral gene encoding envelope glycoproteins in tumor cell lines or insect cells. At least 13 different monomeric gp120 and gp160 candidate vaccines have been evaluated

in prophylactic trials. Initial research focused on gp120, because it was simpler to produce and did not pose any obvious disadvantages over gp160. The first generation vaccines have been safe and immunogenic in diverse populations and have induced neutralizing Abs in almost all recipients. However, the antibodies recognized linear epitopes exposed on denatured gp120 and did not neutralize primary viral isolates (62, 63). In contrast, neutralizing Abs isolated from the serum samples of HIV-infected patients recognize primarily conformation-specific discontinuous epitopes (64). The reason for this is that the native envelope exists as a trimer rather than a monomer and the most immunogenic regions of envelope are hidden on the trimeric, virion-associated form. This observation has led to the hypothesis that viral proteins maintaining a tertiary conformation similar to the native virus may be attractive as vaccine immunogens. Thus, the use of oligomeric forms of gp120 as immunogens is one strategy to improve the quality of the neutralizing Ab response. In order to mimic the quaternary structure of envelope glycoprotein, gp120 and gp41 can be stabilized by an intermolecular disulfide bond (65). However, whether this protein will be superior to existing imunogens still awaits clinical testing.

Because gp120 is heavily glycosylated (>50% carbohydrate by weight), it has been proposed that the removal of carbohydrate moieties on gp120 may expose hidden epitopes and lead to greater immunogenicity and to the production of a better neutralizing Ab response (66). Another way to expose