Chapter-2

Review Of Literature
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2.1 Introduction to HIV-1 Env

Among genes within the HIV-1 genome, the env gene evolves at a particularly high rate (1–2% per year) at the population and the individual level (Korber, Muldoon et al. 2000). After HIV infection, genetic diversity in env begins low with an average of 2.0% (Ritola, Pilcher et al. 2004), undergoes a drop as much as 0.40 to 3.02% (average, 1.08%) (Learn, Muthui et al. 2002) and then increases to a peak as much as 3 to 5% several years into the infection (Shankarappa, Margolick et al. 1999). Consequently, env is highly genetically diverse, posing a significant challenge to vaccine development.

The HIV-1, the env forms the glycoprotein precursor, gp160, results from the addition of N-linked, high-mannose sugar chains of approximately 845- to 870-amino acids. The gp160 is initially synthesized as a polyprotein precursor and is a primary translation occurs in the rough endoplasmic reticulum. Then undergoes posttranslational modifications including glycosylation, oligomerization. Following oligomerization, the gp160 glycoprotein is transported to the Golgi apparatus, where cleavage by a cellular protease generates the surface unit, gp120 and the transmembrane unit, gp41 glycoproteins, which remain associated through noncovalent interactions (Earl, Moss et al. 1991). The gp41 contains ecto-, transmembrane, and cytoplasmic domains, and gp120, is noncovalently linked to the ectodomain of gp41 (Kowalski, Potz et al. 1987). The mature envelope (Env) glycoproteins are transported to the cell surface, where they are incorporated into the virus as an oligomeric complex. The evidence indicates that the mature oligomer consists of and functions as a trimer of gp120-gp41 heterodimers (Farzan, Choe et al. 1998).
The Env glycoprotein complex promotes viral entry into host cells by binding cellular receptors and mediating the fusion of the viral and cellular membranes (Dalgleish, Beverley et al. 1984; Deng, Liu et al. 1996; Dragic, Litwin et al. 1996). The gp120 binds the CD4 molecule, which serves as a receptor for the virus and facilitates the interaction of gp120 with a second receptor (typically, the chemokine receptor CCR5 or CXCR4, which are seven-transmembrane G-protein-coupled receptors). The interactions between gp120 and the cellular receptor molecules are believed to trigger conformational changes in the Env glycoprotein complex important for the membrane fusion process. These changes result in the interaction of the gp41 glycoprotein with the target cell membrane, resulting in fusion of this membrane with the viral membrane.

2.2 Structure of HIV-1 Env

The entry of HIV into cells requires the sequential interaction of the viral exterior Env glycoprotein, gp120, with the CD4 glycoprotein and a chemokine receptor on the cell surface. These interactions initiate a fusion of the viral and cellular membranes. The gp120 molecule derived from a gp160 glycoprotein is noncovalently associated with the transmembrane glycoprotein, gp41, to form trimeric complexes on the virus or cell surface. The gp120 exterior Env glycoprotein contains five successive constant (C1 to C5) and variable (V1 to V5) domains (Leonard, Spellman et al. 1990). The transmembrane subunit consists of fusion domain, two heptad repeats (the N-heptad repeat HR1 and the C-heptad repeat HR2), transmembrane domain (TM) and the long
cytoplasmic tail. The trivalent Env complex consists of 3 extracellular glycoproteins (gp120) that are noncovalently associated with 3 transmembrane glycoproteins (gp41) by hydrogen bonding.

The x-ray crystal structure of the gp120 core derived from the HXBc2 strain of HIV-1, in a tertiary complex with two-domain CD4 and a neutralizing antibody, has been solved (Kwong, Wyatt et al. 1998). In spite of high genetic variability in HIV env, the need to specifically recognize highly conserved cellular receptor structures restricts the degree of variation that the virus can tolerate at selected gp120 sites. Such conserved regions include the CD4-binding site, a discontinuous region at the interface between the inner and outer domains of gp120 (Kwong, Wyatt et al. 1998), and the so-called "bridging sheet," which connects the two gp120 domains and contributes the largest surface of the coreceptor-binding site (Kwong, Wyatt et al. 1998; Rizzuto, Wyatt et al. 1998). Figure 1 shows a series of ribbon diagrams of the gp120 core, from the perspective of the CD4 glycoprotein. The inner and outer domains are shown in red and yellow, respectively, and the bridging sheet is colored blue.

Figure 1: gp120 core; inner domain (red), outer domain (yellow), bridging sheet (blue).
CD4 binding by HIV Env is a high-affinity interaction that results in a conformational change in the shape of both gp120 and gp41, exposing new binding regions (Sattentau and Moore 1991). Mutagenic analyses and structural studies point to the role of the gp41 ectodomain in the fusion process of cell membrane with the viral membrane (McDougal, Nicholson et al. 1986; Tan, Liu et al. 1997; Weissenhorn, Dessen et al. 1997). Two potential alpha-helical regions as shown in fig 2, designated as N36 and C34, in the gp41 ectodomain have been shown to form a stable six-helix bundle (Chan, Fass et al. 1997). This bundle, which is believed to represent the final, fusogenic conformation of gp41, consists of three C34 helices packed into the hydrophobic grooves on the outer surface of a trimeric N36 coiled coil.

Figure 2: Three N36 helices form an interior, parallel coiled-coil trimer, while three C34 helices pack in an oblique, antiparallel manner into highly conserved, hydrophobic grooves on the surface of this trimer.

Since, C34-like peptides can efficiently block HIV-1 Env glycoprotein-mediated membrane fusion, a gp41 conformational intermediate in which the grooves in the N36
coiled coil are not occupied by C34 helices has been proposed as shown in fig 3 (Furuta, Wild et al. 1998). The heptad repeats in the envelope protein are important tertiary structure domains involved in trimer formation (Lu and Kim 1997).

Figure 3 : Proposed model for CAP induced formation of the gp41 six-helix bundles within HIV-1 particles. In the native state (1), HIV-1 Env glycoprotein spikes correspond to a trimeric gp120(pink)/gp41(yellow) complex, the fusion peptide from the gp41-N terminus not being shown. The relative positions of the C-helix (yellow) and N-helix in the native envelope spike are not known and only the C-helix is depicted. Following interaction of the gp120 portion of the spikes with cellular receptors or CAP, the gp120/gp41 trimeric complex undergoes a conformational change, in which the fusion peptide (red) becomes exposed and the N-terminal half of gp41 (green) becomes a trimeric coiled coil. In this configuration (2) the C-peptide portion of gp41 (yellow) is not yet associated with the N-peptide coiled coil. This intermediate conformation is converted into a six-helix bundle when the C-peptide region binds to the N-peptide coiled coil region. This structure (3), in the absence of target cell membranes, corresponds to a nonfunctional spike, which cannot initiate anymore fusion with target cells. The position of fusion peptides in the model for "dead-end" spikes is not shown (Neurath et al. BMC Infectious Diseases 2002 2:6).
A general review of the structure and function of the HIV-1 Env glycoprotein, taking into account the x-ray crystallographic data, has been published (Wyatt and Sodroski 1998). The inner domain is believed to interact with the gp41 envelope glycoprotein, while the outer domain, which is quite variable and heavily glycosylated (Figure 2), is believed to be exposed on the assembled envelope glycoprotein trimer (Wyatt, Kwong et al. 1998). The “proximal” side of the gp120 core, which includes the N- and C-termini, is believed to reside near the viral membrane. The “distal” side of gp120 is believed to face the target cell membrane after CD4 binding occurs. The N- and C-termini, the structures related to some of the variable loops, and some of the secondary structural elements are labeled. In Figure 4, the asparagine residues (including side chains) modified by N-linked glycosylation in mammalian cells (Leonard, Spellman et al. 1990) are shown in blue. The concentration of glycosylation on the gp120 surface believed to face outward on the trimeric Env glycoprotein spike.

Fig 4: gp120 core; asparagine residues modified by NLGs shown in blue.
2.3 HIV-1 Env and Apoptosis

Apoptosis, or programmed cell death, is a phenomenon in which dying cells participate actively in their own death and generally do not induce an inflammatory response. Physiologically, apoptosis functions in maintaining tissue homeostasis and is important in removal of lymphocytes after an immune response. This function prevents accumulation of lymphocytes in the blood and is mediated by interaction of the apoptotic receptor, FAS, with FAS ligand. Upon binding FAS ligand, FAS recruits signaling molecules to the death-inducing signaling complex (Ashkenazi and Dixit 1998). The importance of apoptosis in AIDS is controversial although there is abundant in vitro evidence supporting a role for apoptosis in the pathogenesis of HIV-1. The extraordinary rate of turnover of T cells in HIV-1 infected individuals and the apparently high rate of viral production and death are consistent with a large amount of cell death directly due to infection. Apoptosis may be one of the major forms of T cell death in HIV-1 infections. Many apoptotic pathways depend on calcium and therefore would be expected to involve calmodulin.

Calmodulin (CaM), the major intracellular receptor for calcium which is involved in the regulation of diverse cellular functions. Positively charged amphipathic helical segments have been identified as an important structural motif in the recognition of CaM by different CaM-activated enzymes and peptides. The carboxyl-terminal domain of the envelope glycoproteins of HIV-1, HIV-2, and SIV contain regions that can fold into amphipathic helical segments designated as N36 and C34, which closely resemble
the amphipathic segments found in CaM-activated enzymes. Evidence for lack of binding of gp120 to CaM, which does not contain these putative amphipathic helical segments, supports the binding of purified HIV-1 gp160 to CaM. (Srinivas, Srinivas et al. 1993). The HIV-1 Env, gp160, hence, is known to contain two C-terminal calmodulin-binding domains.

Further evidence implicating calmodulin in the cytopathic effect of gp160 is that two calmodulin antagonists, tamoxifen and trifluoperazine, block FAS-mediated apoptosis in gp160-expressing cells. The cytoplasmic domain of the HIV-1 Env protein gp160 could enhance Fas-mediated apoptosis, the major form of apoptosis in lymphocytes (Pan, Radding et al. 1996). Induction of apoptosis was dependent on calmodulin binding to gp160, which was proved since truncations in the C-terminal of gp160 as few as five amino acids did not bind calmodulin and did not show increased apoptosis. The gp160 expression enhances the cellular response to anti-Fas and increases caspase 3 activity and that these events require calmodulin binding to gp160 (Micoli, Pan et al. 2000).

### 2.4 HIV Env and N-linked Glycosylation

Both N- and O-linked glycans are present on the HIV Env glycoprotein. O-linked glycans are present on several unidentified serine or threonine residues in gp120, but little is known about their role in defining the phenotype of HIV or SIV (Pinter and Honnen 1988; Bernstein, Tucker et al. 1994; Chackerian, Rudensey et al. 1997). In contrast, asparagine-linked (N-linked) glycosylation has been more extensively studied,
and it is known that N-linked glycans (NLGs) comprise about 50% of the mass of gp120 (Leonard, Spellman et al. 1990). NLGs are added cotranslationally as the viral Env protein passes through the endoplasmic reticulum and are subsequently modified in the Golgi apparatus, giving rise to three types of glycans, termed high-mannose, hybrid, and complex glycans (Kornfeld and Kornfeld 1985). The addition of NLGs is essential for the correct folding and correct processing of the viral Env protein (Li, Luo et al. 1993; Land and Braakman 2001). While the exact number of NLGs varies among HIV-1 isolates, many sites are highly conserved (Willey, Rutledge et al. 1986; Gao, Morrison et al. 1996).

Carbohydrate-rich regions of glycoproteins are generally poorly immunogenic for a number of reasons. First, carbohydrates exhibit microheterogeneity; therefore, a single protein sequence would be expected to display multiple glycoforms, leading to the dilution of any single antigenic response (Rudd and Dwek 1997). The NLGs covers the potential protein epitopes (Woods, Edge et al. 1994), and thus acts as ‘glycan shield’, which helps the virus to escape from the immune system. The repositioning of glycans on the HIV Env limits its recognition by neutralizing antibodies (NAbs) while maintaining the ability of the Env protein to interact with the CD4 and co receptor molecules on the target cell membrane (Wei, Decker et al. 2003). Several studies have shown that the presence of carbohydrates is especially critical during early steps of Env protein folding and cleavage (Fennie and Lasky 1989; Li, Luo et al. 1993). Effects of glycosylation on viral replication, gp160 cleavage, CD4 binding activity, and coreceptor usage have been documented (Lee, Syu et al. 1992; Nakayama, Shioda et al. 1998).
2.4.1 N-linked glycosylation and gp120

In order to determine which specific NLGs are critical for Env protein function or immune escape, several recent studies have been directed to individual or multiple mutations of these sites. Removal of an NLG in the HIV-1\textsubscript{BRU} Env V1 region made the virus more resistant to neutralization by anti-V3 antibodies (Gram, Hemming et al. 1994). Masking of an immunodominant epitope in the V3 loop with additional NLG, the antibody response was shifted from the V3 epitope to the V1 epitope in an HIV HXB2 strain (Garrity, Rimmelzwaan et al. 1997).

In studies, with SIV, Rhesus monkeys infected with mutants lacking NLGs in the V1 region produced high titers of strain specific NAbs (Reitter, Means et al. 1998). Most importantly, the mutant viruses induced much higher titers of antibody to the wild-type (wt) virus than were induced by the wt itself. Related but less dramatic effects of glycosylation have been observed in the V3 domain of TCLA HIV-1 as HIV IIIB \textit{env} clones lacking an NLG in the V3 loop of Env protein can become more sensitive to virus neutralization (Back, Smit et al. 1994; Schonning, Jansson et al. 1996). Elimination of the V3 N306 glycan of HIV-1 was found to enhance the immunogenicity in mice using a DNA vaccine (Bolmstedt, Hinkula et al. 2001).

Vesicular stomatitis viruses (VSVs) expressing HIV Env V1 and V2 glycosylation mutants appeared more sensitive to neutralization by antibodies raised to mutant or wild-type Env. Moreover the results indicate significant differences between SIV and HIV with regard to the roles of glycans in the V1 and V2 domains (Quinones-Kochs,
Buonocore et al. 2002). Removal of NLGs from V4-V5 region of HIV-1 gp160 was evaluated for induction of NAb response, which resulted in an altered local antigenic conformation but did not uncover hidden neutralization epitopes, broadening the immune response (Bolmstedt, Sjolander et al. 1996).

However, not every potential NLG site is utilized on SIV and HIV, and the roles that individual glycans have in protecting the virus from neutralization most likely differ. It is reported that on the background of the primary CCR5-tropic HIV-1 isolate SF162, the elimination of two NLG sites, from positions 154 and 195, respectively, resulted in increase in the susceptibility of the virus to neutralization by heterologous sera from HIV-infected patients and by certain monoclonal antibodies (MAbs) that bind to the V3 loop and the CD4 binding site (Ly and Stamatatos 2000).

The infectious molecular clone SHIV\textsubscript{SF33A} developed resistance to serum neutralization of SHIV\textsubscript{SF33A} by HIV and SHIV anti-sera by changing the NLGs within the principal neutralization domains. The removal of potential NLGs in the V1 domain or the creation of such a site in the V3 domain did allow the virus to escape serum NAbs that recognized parental SHIV\textsubscript{SF33}. The combination of the V1 and V3 mutations conferred an additive effect on neutralization resistance over that of the single mutations. Taken together, these data suggest that (i) SHIV variants with changes in the Env SU can be selected in vivo primarily by virtue of their ability to escape NAb recognition and (ii) carbohydrates play an important role in conferring neutralization escape, possibly by altering the structure of envelope gp120 or by shielding principal neutralization sites (Cheng-Mayer, Brown et al. 1999).
The V3 loop itself contains epitopes for strain-restricted NAbs, it is a major determinant for viral tropism and coreceptor usage, and its orientation is such that it partially masks the CD4 and chemokine receptor binding sites (Rizzuto, Wyatt et al. 1998). The NLGs within the V3 loop appears to be dispensable for virus replication and yet is highly conserved.

2.4.2 N-linked glycosylation and gp41

In addition to this, the transmembrane gp41 typically contains three or four sites for N-glycan attachment, located within a short stretch (20 to 30 residues) of the C-terminal half of the ectodomain. The significance of each of these sites in relation to the structure and function of the viral envelope glycoprotein was evaluated which indicate that NLG at amino acid position 642 in the glycoprotein is necessary for the correct intracellular processing of gp160 to yield surface-expressed, fusogenic gp41 (Lee, Yu et al. 1992; Dash, McIntosh et al. 1994) and suggested that gp41 individual glycosylation sites are dispensable for the bioactivity and conformation of Env products.

Contradictory reports have been published with removal of the whole gp41 glycan cluster (Fenouillet, Jones et al. 1993). In this report, gp160 synthesis was found to be similar whether cells were infected with control or mutated Env-expressing recombinant vaccinia virus, but about 10-fold less cleaved gp120 and gp41 was produced by the mutated construct than the control construct. The rates of gp120-gp41 cleavage at each of the two potential sites appeared to be comparable in the two constructs. By using a panel of antibodies specific for gp41 and gp120 epitopes, it was
shown that the overall immune reactivities of control and mutated gp41 proteins were similar but that reactivity to epitopes at the C and N termini of gp120, as present on gp160 produced by the mutated construct, was shown to get enhanced.

Role of NLGs in the specific fusion activity of TM is suggested through studies of Env expression of the mutant with the most severe defect which demonstrated no significant effects on Env protein synthesis, conformation, processing, multimerization, or release into the culture medium. In addition, these site-specific mutants did not eliminate the ability of the virus to infect and replicate in CD4+ cells, but infectivity was reduced with all of these mutants, and syncytia induction was attenuated with two of these mutants (Dedera, Gu et al. 1992).

Transfection of various cell lines with the mutants derived from SHIV-KB9, (simian immunodeficiency virus/HIV chimera with an env sequence that originated from a primary HIV-1 isolate) showed that the NLG sites are largely dispensable for viral replication. The four single mutants, six double mutants and three of the four triple mutants replicated well in cell types tested. One triple mutant and the quadruple mutant did not replicate in any cell line tested. The quadruple mutant envelope protein was nonfunctional in a quantitative cell-cell fusion assay. The synthesis and processing of the quadruple mutant envelope protein appeared similar in transient assays to those of the parental SHIV-KB9 envelope (Johnson, Sauvron et al. 2001).

Additionally, due to the potential role of these NLGs from HIV Env, recently, these have been targeted for anti-HIV therapy (Witvrouw, Fikkert et al. 2005). Even then, a fact that all the studies conducted for studying the role of NLGs were either
using lab adapted strains or SIV and most of them belonging to HIV-1 subtype B cannot be ignored.

### 2.5 Cytoplasmic Tail of HIV Env

HIV-1 Env is proteolytically cleaved by a cellular proprotein convertase into gp120 and gp41. The gp41 subunit is composed of cytoplasmic, transmembrane, and ectodomain segments. The role of the ectodomain in membrane fusion, particularly its hydrophobic glycine-rich fusion peptide, is well established. Two regions with heptad coiled-coil repeats in the ectodomain of gp41 are involved in viral fusion (Weissenhorn, Wharton et al. 1996). After fusion, these two alpha-helices, connected via a disulfide-stabilized loop (Sattentau, Zolla-Pazner et al. 1995; Kent and Robinson 1996), presumably undergo a transient conformational change to a fusion active state. These changes allow the formation of a six-member helical hairpin intermediate structure that presumably exposes the fusion peptide at the NH$_2$ terminus of gp41, allowing fusion to the target cell membrane (Binley and Moore 1997; LaCasse, Follis et al. 1999). The transmembrane subunit of Env is a multifunctional protein and known to be involved in various functions like- Env incorporation into the Virion (Freed and Martin 1996; Murakami and Freed 2000), for virus infectivity and pathogenicity (Dubay, Roberts et al. 1992).

Truncation in the CT, affects the biological functions of the Env glycoprotein. The role of the CT of Env glycoproteins in virus replication was investigated. Deletion of residues 840 to 856 at the carboxyl terminus of gp41 reduced the efficiency of virus
entry during an early step in the virus life cycle between CD4 binding and formation of the DNA provirus without affecting Env glycoprotein synthesis, processing, or syncytium-forming ability (Gabuzda, Lever et al. 1992). Effects of deletion in the CT of Env on the process of membrane fusion were investigated. Full-length Env (wild type) and Env with its CT truncated (DeltaCT) were expressed on cell surfaces. These cells were fused to target cells, and the inhibition of fusion by peptides that prevent Env from folding into a six-helix bundle conformation was measured. For both X4-tropic and R5-tropic Env proteins, DeltaCT induced faster fusion kinetics than did the WT, and peptides were less effective at inhibiting DeltaCT-induced fusion. (Abrahamyan, Mkrtchyan et al. 2005).

The CT of Env is known to alter the biochemical and immunologic properties of the Env ectodomain, and also increases neutralization sensitivity of virus as proved by many researchers (Spies, Ritter et al. 1994; Edwards, Hoffman et al. 2001; Edwards, Wyss et al. 2002). It has been suggested that HIV Env CT may play important roles in virus infection and pathogenesis by modulating its immunogenicity. Analysis of immune responses induced by DNA immunization of mice showed that the DNA construct for the mutant Env exhibiting enhanced surface stability induced significantly higher levels of antibody responses against the HIV Env protein (Ye, Bu et al. 2004).

The long CT of HIV Env is known to play important role in modulating surface expression of the Env protein. The deletion in the CT generates the membrane anchored gp150 protein which retained on the cell surface, as that of gp160 (Berlioz-Torrent, Shacklett et al. 1999). Deletion in the CT has been associated with the exposure of the
some epitopes from the ectodomain of the HIV Env, and also shown to render neutralization sensitivity by HIV positive immune sera and the monoclonal antibodies (Edwards, Wyss et al. 2002).

The protein, gp160 forms trimers in vivo and the domain required for trimer formation resides in the ectodomain of the gp41. The soluble gp140, lacking the cytoplasmic tail forms monomers, dimmers or oligomers and exhibited an exposure of gp120 and gp41 elements consistent with that expected for the functional HIV-1 envelope glycoprotein spike (Hallenberger, Tucker et al. 1993; Earl, Broder et al. 1994). The oligomerization profile of gp140s from different isolates belonging to clades A, B, and E were very similar. Analysis of oligomers revealed that dimeric and trimeric fractions of all the gp140s studied contained mixtures of oligomers possibly stabilized by interchain disulfide bonds and non-covalently linked oligomers (Staropoli, Chanel et al. 2000). The gp140 of YU2 strain showed that the hydrophobic groove on the N36 trimer was either not formed or not accessible on the soluble gp140 trimers. The soluble gp140 trimers also appeared to assemble in a manner such that the receptor-binding regions and neutralizing antibody epitopes are exposed (Yang, Farzan et al. 2000).

2.6 HIV-1 Env and Immune Responses

Important considerations in the development of an HIV-1 vaccine are to determine the antigen composition of the vaccine and the contributions of each component to vaccine efficacy. Studies indicate that both neutralizing antibodies (NAbs) to the Env protein and cytolytic T cells (CTLs) to internal proteins most notably
gag, are required to limit infections with HIV-1 or SIV. Internal viral antigens, such as Gag and Pol, have been demonstrated to be potent vaccine components for eliciting T-cell responses (Barouch and Letvin 2000; Barouch, Santra et al. 2000; Amara and Robinson 2002; Casimiro, Tang et al. 2002; Shiver, Fu et al. 2002). The value of the HIV Env in a CTL-based vaccine is less well defined. Several studies have previously shown that inclusion of Env in vectored multicomponent vaccine was able to confer protection against SHIV challenge (Ourmanov, Brown et al. 2000; Amara, Smith et al. 2002). However, since these studies employed Env antigens homologous to the challenge virus, the contribution of Env-induced CTL responses to the vaccine efficacy could not be differentiated from the potential contribution of homologous neutralizing antibody responses (Liang, Casimiro et al. 2005).

There is a consensus that a broadly neutralizing humoral response is an essential component of a protective HIV vaccine. Unfortunately, current vaccine approaches have not been able to produce such NAb responses against primary HIV isolates despite induction of high titers of antibodies, including antibodies capable of neutralizing specific test strains (Beddows, Schulke et al. 2005). The only relevant HIV-1 antigens for induction of NAbs are the Env glycoproteins encoded by the viral env gene. The Env glycoprotein complex present on the virion surface, a trimer of gp120 and gp41 is responsible for elicitation of NAb response. The structure of Env molecule has multiple properties that account for development of heterogeneous NAb response. Hence a number of novel strategies are actively pursued for identifying, designing and generating new Env immunogen that are able to accomplish effective NAb response.
Various forms or subcomponents of the HIV-1 Env complex have been tested as vaccine immunogens in animals and humans over the past 20 years (McMichael and Hanke 2003). The most studied proteins have been individual, monomeric gp120 subunits, expressed as recombinant proteins from mammalian cells. These gp120 monomers induced antibodies capable of recognizing the immunizing antigen and of neutralizing atypically sensitive HIV-1 strains, including those that have been adapted to replication in vitro (Parren, Moore et al. 1999).

One approach to eliciting NAb more efficiently is to try to make and then modify proteins that mimic the native trimeric Env structures that exist on the virion surface (Burton, Desrosiers et al. 2004). There are two major obstacles to the success of a NAb-based vaccine: HIV-1 sequence variation and the defenses that the Env complex has evolved to thwart the humoral immune system. The defenses of Env against Ab have two principal but interlocking functions: to limit the induction of NAb in the first place and then reduce the probability that any elicited Ab can bind to the functional Env complex on the virion surface and thereby neutralize infectivity. Components of the defenses include the extensive glycosylation of both the gp120 and gp41 subunits, particularly gp120 since half its molecular weight is attributable to N-linked sugars; the structural flexibility of the CD4 and co receptor binding sites on gp120; the limited availability of T-helper epitopes on Env; the creation of the coreceptor binding site on gp120 only after CD4 binding, combined with its inaccessibility after the Env complex has attached to cell surface CD4; and the limited exposure of conserved gp41 structural
elements that are formed only during the fusion process itself (Johnson and Desrosiers 2002; Burton, Desrosiers et al. 2004; Grundner, Pancera et al. 2004).

### 2.7 Use of Ad-5 in HIV Vaccine Research

An effective Env-based vaccine must mimic the antigenic structure of the trimeric complex from the native virion. However, despite some encouraging results (Gao, Weaver et al. 2005; Grundner, Li et al. 2005; Lian, Srivastava et al. 2005; Wang, Arthos et al. 2005), oligomeric immunogens failed to elicit potent broadly reactive neutralizing antibodies against primary isolates. Several explanations for this failure could be hypothesized: (i) the complex 3D structure of gp120 and the hetero-oligomerization status of Env spike, (ii) the high degree of genetic variation and (iii) the extensive glycosylation of gp120 about 50% of its weight, continuously restructured by the changing antibody repertoire (Wei, Decker et al. 2003).

To overcome these difficulties, themes that have emerged include the need for alternative, more effective in vivo expression systems and for selection or manipulation of env genes or proteins to enhance immunogenicity of epitopes that are not commonly immunogenic on soluble HIV-1 Env proteins. Among the numerous approaches that have been used for in vivo expression of HIV or SIV gene products in experimental vaccines, promising results have been obtained by using a vector derived from recombinant adenoviruses, which offer the practical advantages over DNA vaccination in a highly efficient and immunogenic vector system (Peng, Wang et al. 2005; Gomez-Roman, Florese et al. 2006). Replication-defective adenoviruses encoding an antigen of...
interest can be rapidly produced in high titers (Graham and Prevec 1995). Adenoviruses efficiently infect a variety of target cell types in vitro or in vivo, leading to high levels of protein production in situ. Vaccination with recombinant adenoviruses has been shown to induce potent humoral (Juillard, Villefroy et al. 1995; Xiang, Yang et al. 1996; Flanagan, Pringle et al. 1997) immune responses to transgene encoded products.

In case of HIV, the Env have been expressed in actively replicating adenovirus vectors (Dewar, Natarajan et al. 1989; Chanda, Natuk et al. 1990; Natuk, Chanda et al. 1992; Natuk, Lubeck et al. 1993; Lubeck, Natuk et al. 1994)) and shown to induce neutralizing antibodies in animal models (Dewar, Natarajan et al. 1989; Chanda, Natuk et al. 1990; Natuk, Chanda et al. 1992; Natuk, Lubeck et al. 1993; Lubeck, Natuk et al. 1994). However, replication-deficient adenovirus vectors have attracted increasing attention because of their improved safety and containment characteristics and their ability to limit expression in the target cell to the gene encoding the vaccine antigen.

In a study to evaluate the efficacy of a DNA vector and two viral vectors, modified vaccinia virus Ankara (MVA) and replication-defective adenovirus serotype 5 that expressed a simian immunodeficiency virus (SIV) Gag protein to protect monkeys against simian-human immunodeficiency virus (SHIV) challenge was carried out which indicated that Ad-5 vector proved to be the most immunogenic for elicitation of a cellular immune response that effectively mitigated the pathogenic immunodeficiency virus challenge (Shiver, Fu et al. 2002).

A non-replicating adenoviral vector, have been shown to induce both humoral and CTL response to HIV-1 envelope in murine model (Bruce, Akrigg et al. 1999).
Further, a multigenic Ad-SIV recombinant priming envelope subunit boosting approach elicited potent protection in 39% of vaccinated macaques against the virulent SIV\textsubscript{mac251} virus (Patterson, Malkevitch et al. 2004). Durable protection in 73% of these protected animals was subsequently demonstrated (Malkevitch, Patterson et al. 2006). These results have provided the basis for moving the replication-competent Ad-HIV recombinant approach into phase I human trials.