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Review

A vaccine for HIV type 1: The antibody perspective

(HIV envelope/neutralizing antibodies/passive immunization/gp120/gp41)

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ABSTRACT **Antibodies that bind well to the envelope spikes of immunodeficiency viruses such as HIV type 1 (HIV-1) and simian immunodeficiency virus (SIV) can offer protection or benefit if present at appropriate concentrations before viral exposure. The challenge in antibody-based HIV-1 vaccine design is to elicit such antibodies to the viruses involved in transmission in humans (primary viruses). At least two major obstacles exist. The first is that very little of the envelope spike surface of primary viruses appears accessible for antibody binding (low antigenicity), probably because of oligomerization of the constituent proteins and a high degree of glycosylation of one of the proteins. The second is that the mature oligomer constituting the spikes appears to stimulate only weak antibody responses (low immunogenicity). Viral variation is another possible obstacle that appears to present fewer problems than anticipated. Vaccine design should focus on presentation of an intact mature oligomer, increasing the immunogenicity of the oligomer and learning from the antibodies available that potently neutralize primary viruses.**

To have any chance of protecting an individual from HIV type 1 (HIV-1) infection or of modulating the effects of infection, an antibody-based viral vaccine must induce antibodies that bind well to the virus under physiological conditions. The vast majority of antibodies generated during natural HIV-1 infection in humans do not bind well and are probably of limited efficacy in controlling the virus. Viral envelope molecules that have been used to vaccinate humans and animals also have been largely unsuccessful in eliciting antibodies with any evidence for binding to primary viruses, i.e., viruses that have not been passaged in cell lines. This review will discuss some of the likely reasons for these observations and how this knowledge relates to vaccine design. The review focuses entirely on humoral defense. A complete vaccine probably also will aim to elicit vigorous cellular immunity.

The HIV-1 Envelope Spike

HIV-1 is an enveloped virus with up to about 70 envelope spikes per virion (1). The spikes consist of a transmembrane gp41 molecule interacting noncovalently with a gp120 molecule to form an oligomeric structure, which recent physical and crystallographic data suggest is a trimer (2–4), i.e., $(gp41\text{-}gp120)_3$. Oligomerization occurs through gp41-gp41 interactions. Gp41 is postulated to undergo major conformational rearrangements after binding of virus to cells to facilitate fusion of viral and target cell membranes, in the "spring-loaded mechanism" described for influenza hemagglutinin (5). The "sprung" conformation of gp41 (3, 4) is suggested to contain a core formed by an extended triple-stranded α-helical coiled coil. A carboxy-terminal α-helix packs in the reverse direction against the outside of the coiled coil, placing the amino and carboxy-termini near each other at the end of the long rod (Fig. 1). This sprung conformation is believed to be the stable state of gp41. In the mature oligomer on the viral surface, gp120 is believed to constrain gp41 into an "unsprung" metastable state, whose conformation is unknown. Gp120 interacts with the cellular receptor CD4 and one of several possible chemokine receptors (6–9). Macrophage-tropic isolates, important in viral transmission in humans, interact with the CC-chemokine receptor, CCR5. Gp120 is a heavily glycosylated (about 40–50%) protein composed of regions showing differing degrees of sequence variability between different isolates of HIV-1. Five relatively conserved (C1–C5) and five variable (V1–V5) regions exist. The latter regions, with the exception of V5, are bracketed by cysteines forming disulfides and generally are viewed as variable loops. No evidence is available that gp120-gp120 interactions are important in oligomer formation.

The envelope proteins undergo oligomerization and processing before their expression on the infected cell surface (10, 11). The envelope is synthesized first as a monomeric precursor gp160 molecule that oligomerizes for transport from the endoplasmic reticulum to the plasma membrane. During transport gp160 is cleaved into gp120 and gp41 by a cellular endoprotease (12). The mature, processed oligomer then is anchored in the membrane by C-terminal helices of gp41 with most of the gp41 molecule and gp120 expressed extracellularly. Budding of virus particles from the infected cell surface results in incorporation of cell membrane, including envelope oligomer, to become viral membrane. It is generally then assumed that the oligomers presented on infected cells and viral membranes are conformationally identical. The mature oligomer can, under certain conditions, lose or "shed" gp120 molecules. This will generate free monomeric gp120 molecules and gp41 left anchored in the cell/viral membrane.

The existence of several conformationally distinct forms of the envelope proteins is a major complicating factor in vaccine design. In particular, many epitopes available on monomeric or unprocessed oligomeric envelope molecules are not available on the mature oligomer. Furthermore, the accessibility of epitopes on primary isolate envelope appears to be generally less than that on the envelope of viruses adapted to grow in T cell lines in the laboratory, so-called T cell line adapted (TCLA) strains of HIV-1 (13–15), on which so much research has been conducted. Primary isolates, which generally have been minimally passaged in peripheral blood mononuclear cells, are expected to most resemble the viruses present in humans. The exposure of epitopes on TCLA viruses may reflect an optimization of the virus-cell interaction, particularly the CD4-gp120 interaction, in the absence of selective pressure provided by serum-neutralizing antibodies. Gradation in epitope accessibility is shown schematically in Fig. 1.

Abbreviations: HIV-1, HIV type 1; SIV, simian immunodeficiency virus; TCLA, T cell line adapted.

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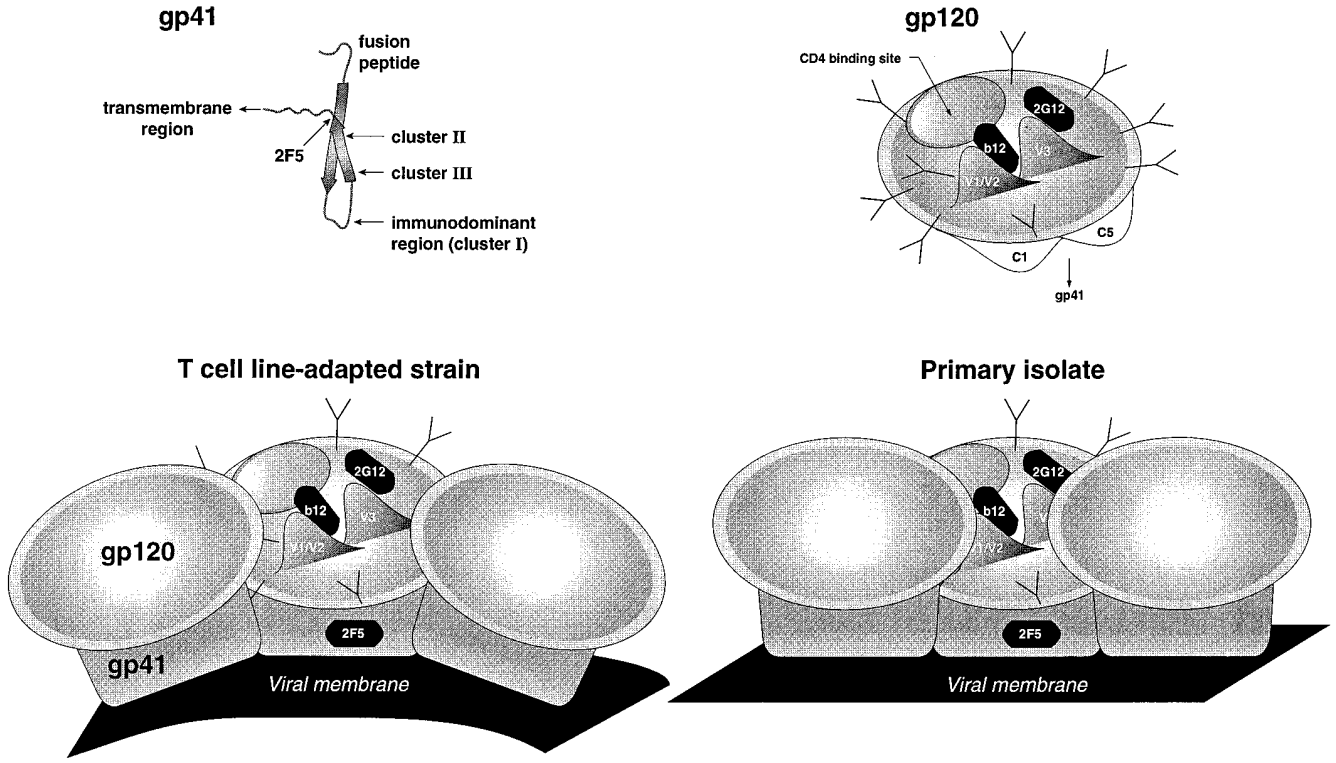

FIG. 1. Schematic models of the exposed epitopes on isolated gp41, gp120, and the mature oligomer on the virion surface. The schematic model for gp41 structure is adapted from Weissenhorn *et al.* (4). A similar structure has been presented by Chan *et al.* (3). The crystal structures were solved for helical peptides lacking the interhelical region and the fusion peptide. These are included in this schematic representation. The structure corresponds to the "sprung" form of gp41 (see text). The fusion peptide at the N terminus of the protein is linked to an α-helix, which forms a coiled coil in the trimer (only a monomer unit is shown). A disulfide-bridged loop containing the immunodominant epitope links to a C-terminal α-helix, which packs against the core structure. A flexible region links the C-terminal helix to the transmembrane segment. Cluster I-III antibodies recognize three epitope regions as shown. The antibody 2F5 recognizes a region of gp41 close to the transmembrane domain. Further epitopes recognized by single mAbs have been defined. The schematic model for gp120 structure is adapted from Sodroski *et al.* (28), Poignard *et al.* (76), and Burton and Montefiori (15). The molecule is heavily glycosylated as represented by the Y-shapes. Antibody mapping indicates that C1, C5, V1/V2, and V3 regions and the CD4 binding site are at least partially accessible on monomeric gp120. C1 and C5 are on the "back side," nonneutralizing face of this model. The b12 epitope overlaps the CD4 binding site but is also sensitive to V2 loop conformation. The 2G12 epitope appears to involve residues (including possibly carbohydrate structures) from V4 and the base of the V3 loop. Most of the epitopes accessible on isolated gp41 and gp120 molecules are also accessible on unprocessed forms of gp160. For the mature oligomer on the surface of TCLA viruses, many epitopes exposed on the isolated gp41 and gp120 molecules now are occluded by virtue of gp41-gp41 or gp41-gp120 interactions or proximity of monomeric units to one another or the virion surface. In particular on gp41, only the 2F5 epitope is well exposed. On gp120, epitopes on the nonneutralizing face now appear to be buried in the gp41-gp120 interaction. The oligomer is shown as a trimer in line with the oligomerization properties of gp41. For the mature oligomer on the surface of primary viruses, further occlusion of epitopes relative to TCLA viruses results. The only epitopes clearly defined as well exposed on a large fraction of isolates are b12, 2G12 and 2F5. Other epitopes may remain to be discovered.

Epitopes Exposed on the Envelope of Primary Isolates of HIV-1

Gp41 as an isolated recombinant molecule or as part of a recombinant unprocessed gp160 molecule exposes several regions reactive with a range of antibodies arising from natural infection or by immunization with recombinant proteins (16–18). Reactivity with most of these epitopes is lost in the mature oligomer on TCLA viruses, as shown by the inability of the range of antibodies to bind to infected cells (17, 19). It is unclear whether this loss is due to differences in accessibility, differences between unsprung and sprung gp41 conformations, or both (20). As expected, the antibodies do not neutralize TCLA viruses in assays measuring the ability of antibody to inhibit viral infection *in vitro*. The antibodies also fail to neutralize primary isolates, and it is reasonable to assume that this also reflects an inability to bind to mature oligomer. Generally in this review, it is not assumed that neutralization *in vitro* is necessarily the mechanism by which virus is eliminated by antibody *in vivo*. Rather, neutralization is interpreted as a marker for antibody binding to virus in line with studies that show a good correlation between antibody affinity for mature oligomer and virus neutralization (21–23).

One epitope of gp41 that is somewhat exposed on the mature oligomer is located toward the C-terminal part of the extracellular domain and is recognized by the human antibody 2F5 (19, 24, 25). This antibody is potently and broadly neutralizing (24–26). In a recent comparative study, 2F5 was one of three antibodies capable of neutralizing the majority of a panel of typical United States primary isolates (27). The antibody is also highly effective against many isolates from around the world. The epitope recognized by 2F5 is the linear sequence ELDKWA, which is conserved in many isolates of HIV-1.

Gp120 as a monomeric isolated protein or as part of a recombinant unprocessed gp160 molecule displays a wide range of epitopes accessible to antibodies (15). Accessibility to a number of these epitopes is completely lost in the mature oligomer on TCLA strains of the virus (Fig. 1). These epitopes form a surface, the "nonneutralizing face" (28) of the molecule, which interacts with gp41. Two major epitopes exposed on monomeric gp120 and the mature oligomer of TCLA viruses are the V3 loop and the CD4 binding domain. Antibodies reacting with these epitopes generally show moderate to potent neutralization of TCLA viruses. Anti-V3 antibodies in particular are efficient at neutralizing TCLA viruses if the V3

sequence recognized is present (22). The accessibility of the V3 loop in the mature oligomer on primary isolates appears to be greatly reduced relative to TCLA viruses (15, 29), and consequently anti-V3 antibodies generally neutralize primary isolates rather poorly (27). The combination of low accessibility and sequence variation between isolates suggest that the V3 loop is not likely to be a good target for vaccines although it still attracts much effort.

The CD4 binding domain is well exposed on gp120 and unprocessed gp160, with typically higher antibody affinity for the latter molecule (30). Exposure is decreased on the mature oligomer of TCLA viruses as indicated by an approximately 10-fold decrease in antibody affinity relative to monomeric gp120 (21, 22). This is reflected in rather poor neutralization efficacy for these antibodies. For primary isolates, it is likely that accessibility is reduced still further because neutralization efficacy is typically reduced by one or two orders of magnitude relative to TCLA viruses. This lowered neutralization efficacy is below that likely to be physiologically useful.

Only two gp120 epitopes have been established as clearly accessible on a range of primary isolates. These epitopes are defined by the human mAbs b12 and 2G12 whose accessibility is inferred from neutralization properties and more recently from direct binding studies (23). The recombinant antibody b12 (21, 31–33) defines an epitope overlapping the CD4 binding domain and influenced by the V2 loop. The neutralizing potency against the majority of a wide range of primary isolates is in a physiologically achievable concentration range (27, 33–35). For example, at 25 μg/ml in an infectivity reduction assay, b12 efficiently neutralized 35 of 35 primary isolates, including isolates from a range of geographical locations (36). 2G12 defines an epitope containing residues near the base of the V3 loop (C2 and C3) and the V4 region (37). Antibody binding is sensitive to changes that are at N-glycosylation sites or are part of the signal sequence for N-glycosylation. It is not clear whether the 2G12 epitope is entirely peptidic but influenced by the presence of N-linked carbohydrates, or whether it involves carbohydrates directly. 2G12 is capable of broad, potent neutralization of primary isolates (27, 35, 37). Other epitopes on gp120 are inferred to have limited accessibility on some primary isolates through the neutralization properties of the relevant mAbs. Such epitopes are not likely to play a major role in vaccine design and will not be discussed further.

In summary, very few generally accessible epitopes on the envelope of primary viruses have been found. Only three epitopes have been well documented. The oligomeric nature of the envelope proteins together with the extraordinarily high glycosylation of gp120 probably contribute to this paucity. Thus far the focus has been on antigenicity of envelope, i.e., epitopes that are accessible to antibody. Vaccine design must consider immunogenicity, i.e., epitopes that are accessible should stimulate a good antibody response in many different individuals. In this context, the human antibody response to HIV-1 will be reviewed.

The Immunogenicity of HIV-1 Envelope

It is well known that generally a vigorous response to HIV-1 envelope antigens occurs during natural infection. Most of the response is directed to conformational epitopes (38). The response to gp41 is predominantly to the epitope clusters mentioned above with the greatest response being to an immunodominant epitope contained on a disulfide bridged loop (39). None of these gp41 epitopes appear to be exposed on mature oligomer (19). These epitopes are relatively conserved between different isolates. The response to gp120 is directed predominantly to the CD4 binding site, the V3 loop, the V2 loop, and epitopes containing residues from the N- and C-terminal regions of the protein (C1 and C5) (15). These latter epitopes are not accessible on mature oligomer, being involved in gp120-gp41 interaction, and the others are only poorly accessible (Fig. 1). Antibodies to the b12, 2G12, and 2F5 epitopes have been isolated only from single infected donors, indicating that they may be poorly immunogenic.

Therefore, the humoral response to natural infection is directed to many different envelope epitopes, but a very small fraction of this response is directed to epitopes well presented on virions. The resolution to this paradox has not been established. We believe that the humoral response in natural infection is directed not to the virus but to viral debris, i.e., not to the mature envelope oligomer but to other conformations of the envelope proteins and in particular unprocessed gp160 (40). We have been drawn to this interpretation by consideration of the binding affinities of panels of human antibodies from HIV-1-infected individuals for various forms of HIV-1 envelope (40, 41). The highest affinities of a selection of human Fabs reactive with the variable loops and the CD4 binding site of gp120 and with gp41 are found for a recombinant truncated form of gp160. Lower affinities are found for monomeric gp120 and even lower for binding to mature envelope on infected cells. The most extreme example is the overwhelming majority of anti-gp41 antibodies, which do not bind significantly to mature oligomer on infected cells. The most straightforward explanation of these data is that the antibodies described were elicited by, and affinity matured against unprocessed gp160. Varying degrees of crossreactivity with mature oligomeric envelope then occur. Crossreactivity is greatest with the envelope of TCLA viruses, which are effectively neutralized, and least with primary viruses, which are relatively refractory to antibody neutralization.

A relatively strong response to gp160 is consistent with the kinetics of envelope processing and rapid turnover of infected cells. Only a small fraction of uncleaved gp160 is processed into mature gp120 in infected cells, whereas the remaining fraction is retained and recycled intracellularly (10). In the case of rapid cell turnover, relatively large amounts of gp160 can be expected to be released to challenge the immune system. Furthermore, a strong initial response to gp160 may suppress the response to lower concentrations of mature envelope expressing crossreactive epitopes according to the mechanism operating in the phenomenon of original antigenic sin (42–45). This phenomenon, originally described in influenza vaccination of humans, also is found in hapten immunization of mice (46, 47). In essence, it appears that immunization with antigen 1 (here unprocessed gp160) can establish a population of memory B cells such that subsequent challenge with related antigen 2 (here mature oligomer) stimulates a response of high affinity to antigen 1 but more moderate affinity for antigen 2.

Immunization of animals, in which the virus does not replicate, with whole virus could give information on the immunogenicity of viral envelope. Surprisingly few such studies have been reported. Interestingly, immunization of macaques with a fixed inactivated primary isolate of HIV-1 produced essentially no antibody response to envelope (48), suggesting poor immunogenicity.

Evidence for Antibody Protection Against HIV-1 and Simian Immunodeficiency Virus (SIV) *in Vivo*

From the above it appears that very few epitopes on primary isolate envelope are accessible to antibody and the immunogenicity of the mature oligomer is low. Therefore it may be difficult to elicit antibodies binding efficiently to primary isolate envelope. If such antibodies could be elicited, what is the evidence that they would offer any benefit? The most direct evidence comes from passive immunization studies using monoclonal or polyclonal antibodies. Antibodies to the V3 loop and the CD4 binding domain of gp120 have been shown to completely protect chimpanzees and severe combined im-

munodeficiency mice populated with human peripheral blood lymphocytes (hu-PBL-SCID mice) from infection with TCLA viruses (49–51). More importantly, the b12 antibody has been shown to completely protect hu-PBL-SCID mice against challenge with two primary isolates of HIV-1 (52) (M. C. Gauduin, P. W. H. I. Parren, R. Weir, C. F. Barbas, D.R.B., and R. A. Koup, unpublished work). This protection was apparent even if the antibody was given several hours postviral challenge. A major cautionary note to be attached to the latter studies is the high dose of antibody (50 mg/kg, corresponding to a serum concentration of about 500 μg/ml) required for complete protection. Another theme, which is apparent in all protection studies (53), is that the level of antibody required to protect depends markedly on the challenge virus. The ability of the potent anti-gp41 antibody 2F5 to protect chimpanzees against challenge with a chimpanzee-adapted primary virus has been investigated (54, 55). Protection was not observed, but seroconversion was delayed and the peak of measurable virus-specific serum RNA either was delayed or did not reach levels comparable to control animals through 1 year of follow-up.

Passive transfer of pooled Ig from HIV-1 seropositive donors (HIVIG) to chronically infected humans has failed to produce convincing evidence of therapeutic benefit. In the most recent and best controlled study, no changes in viral load were observed (56). Other studies relying on clinical benefit (57, 58) may have measured the effects of passively transferred Ig specific for opportunistic pathogens and not specific effects on HIV-1. HIVIG also fails to protect hu-PBL-SCID mice from primary isolate infection under conditions where the b12 mAb is protective (M.C., unpublished work). Generally HIVIG preparations are found to neutralize primary isolates poorly (e.g., ref. 33). Passively transferred anti-SIV polyclonal antibodies have been reported to confer protection or benefit in some studies (59–61) but not in others (62, 63). One of the great problems in interpreting many of the SIV studies is that neutralization or binding assays frequently are not carried out with the same virus as used for challenge. A claim that "neutralizing" antibody is affecting or failing to affect the course of infection requires that neutralization is carried out with the challenge virus grown and assayed under as similar physiological conditions as possible (64). Most interestingly, protection against SIV in macaques has been consistently observed by passive transfer of antibodies to host cell components (56, 65, 66). Briefly, if SIV is grown in human cells then human cell surface molecules are incorporated into the virion envelope, and macaques make a vigorous antibody response to these molecules when infected with virus. Passive transfer of these antibodies to naive animals now offers protection against SIV infection or reduced viral titers. If SIV is grown in macaque cells and the same protocol followed, no effect is observed. These studies suggest that antibody can protect against or modulate SIV infection, but that the envelope spikes are not behaving equivalently to other molecules at the virion surface. The possible reasons for this are many, but one is that, because of their low antigenicity and immunogenicity, the spikes fail to elicit antibodies of sufficient affinity and concentration to coat the virus to a level required for inhibition of infectivity. Potent neutralizing antibodies to SIV [or to HIV-1 in the SHIV model (in essence SIV with an HIV-1 envelope)] could answer many questions in this area.

Vaccine studies are less direct than passive immunization in evaluating the benefit of antibodies as they are largely correlative. Generally serum-neutralizing antibodies appear to be a good correlate of protection when the challenge virus is neutralization-sensitive, e.g., TCLA HIV-1 in chimpanzees (reviewed in ref. 67) and TCLA SHIV in macaques (reviewed in ref. 15). Vaccine protocols with a number of envelope presentations, including live and inactivated virus and recombinant envelope proteins, have not been particularly informative with regard to the ability of antibody to protect against challenge with primary viruses because none have clearly elicited strong neutralizing responses to primary isolates of HIV-1 or SIV (reviewed in ref. 68). The strong protection observed with live attenuated vaccine against challenge with SHIV in the absence of a neutralizing antibody response to the challenge virus (69) does suggest, however, that mechanisms other than antibody can confer protection.

Candidate Vaccines

The strategies under investigation can be conveniently placed in five groups. The first is to immunize with oligomer in a mature conformation by vaccination with virus in an attenuated or inactivated form. An attenuated virus is attractive in that although most virions are noninfectious, typical of retrovirus populations, it appears that they predominantly display mature oligomer (30). The disadvantages are the low apparent immunogenicity of viral oligomer and safety issues that have been extensively discussed (53, 70). Inactivated virus has lesser safety concerns, but inactivation may be difficult without perturbing oligomer conformation. The second strategy is to immunize with oligomer expressed on a suitable vector such as vaccinia virus or Semliki Forest virus. Here major problems may be to ensure efficient processing of gp160 and low oligomer immunogenicity. The third strategy is DNA immunization where major obstacles are the relatively poor antibody responses elicited to HIV-1 envelope in primates thus far by this route (71–73) and the necessity to ensure efficient processing of gp160. The fourth strategy is to prepare and immunize with a recombinant mature oligomeric molecule. Attempts thus far have failed to reproduce critical features of the mature conformation (30) although these difficulties eventually may be surmounted. The fifth strategy is to prepare and immunize with epitope mimics of the potent neutralizing antibodies described above. The potential advantage of this approach is that a highly focused response could be elicited, possibly circumventing the immunogenicity problem. The difficulty is in producing appropriate epitope mimics. This is liable to be particularly problematic for discontinuous epitopes, and even immunization with mimics of the continuous epitope recognized by the antibody 2F5 has failed to generate a response that neutralizes primary isolates (74, 75).

In summary, evidence exists that antibodies can protect against HIV-1 infection or modulate disease if they bind well to the challenge virus. In human infection, challenge is presumed to be by a macrophage-tropic virus akin to the primary isolates grown *in vitro*. Antibodies produced in natural infection or typical vaccination protocols bind weakly to primary isolates and may not offer decisive benefit. At least two problems exist. First, very few epitopes on the primary isolate envelope are accessible for antibody binding. Second, the immunogenicity of the envelope is apparently low, especially in relation to other nonnative forms of the envelope proteins produced in infection (and termed here "viral debris"). The response to these forms may even hinder the development of effective responses to mature envelope on the viral surface. It is suggested that some priority should be given to (*i*) molecular definition of the epitopes recognized by the antibodies that do interact with primary isolate envelopes, (*ii*) approaches to maximize the expression of mature oligomeric structures, and (*iii*) approaches to enhance the immunogenicity of mature oligomer. All of these endeavors may be crucial in a rational approach to maximizing the useful antibody response elicited by potential vaccines.

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