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MINIREVIEW

Genetic Subtypes, Humoral Immunity, and Human Immunodeficiency Virus Type 1 Vaccine Development

JOHN P. MOORE,[1]* PAUL W. H. I. PARREN,[2] AND DENNIS R. BURTON[2]*

Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, New York,[1] and Departments of Immunology and Molecular Biology, The Scripps Research Institute, La Jolla, California[2]

Successful vaccination programs, particularly against influenza virus infection, have provided us with an awareness of the need to immunize against the predominant circulating viral strains or genetic subtypes. The lessons and language derived from experience with influenza (and a few other) viruses have often been directly translated to human immunodeficiency virus type 1 (HIV-1) vaccine development. But how appropriate is this? Should an HIV-1 vaccine antigen always be based on the dominant genetic subtype that circulates in the geographical area where a vaccine candidate is to be tested? The answers lie, at least in part, in a consideration of the humoral response to HIV-1 and, in particular, in the relationships between the HIV-1 genetic subtypes and antigenic and neutralization serotypes. Here, we will review what is known about these relationships and seek to clarify confusion that has been created by the use of serological assays that generate misleading, or sometimes artifactual, results. Broadly similar issues are raised when considering the relationship between cellular immune responses and the HIV-1 genetic subtypes, but we will not discuss these here. Instead, we refer the reader to recent articles written by leading cellular immunologists (9, 30, 39, 79). Significantly, a recent study on the cross-clade activity of cytotoxic T-lymphocyte responses in HIV-1-infected Ugandans argued that the use of nonendemic vaccine strains may be initially justified from the perspective of inducing cellular immunity to HIV-1 (15).

HIV-1 GENETIC SUBTYPES

There have been several thorough and recent reviews of this topic, which we recommend for a more detailed picture (17, 63, 93, 127). In summary, 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. Among these, group M viruses are by far the most widespread, being the variants of HIV-1 that are 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 they do other subtypes, across the whole genome (14, 63, 99). Using this definition, there are currently nine circulating genetic subtypes (A through K) within group M. Prototype viruses representing the genetic subtypes E and I have not yet been found. The viruses originally identified as subtype E (the predominant group of viruses involved in heterosexual transmission in Thailand) and I (a small group of viruses from the Mediterranean region) are now considered intersubtype recombinants and have been termed CRF01_AE and CRF04_cpx, respectively (see below). A study of isolates from the Democratic Republic of Congo indicates central Africa as the epicenter of HIV-1 diversity, with a large number of different genetic subtypes and subtype recombinants circulating. Moreover, a number of envelope sequences with novel sequences were identified, suggesting the existence of additional subtypes (120). The prevalence of intersubtype recombinant strains is increasing and creates even more HIV-1 antigenic diversity (43, 64). 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 (63). CRFs have a designation that includes the letters of the parent genetic subtypes (e.g., CRF01_AE), although in CRFs derived by recombination of more than three subtypes, the letters are replaced by cpx (complex), e.g., CRF04_cpx (99). Relevant to this review, recombinant viruses with mosaic envelope sequences generated by multiple intraenvelope crossover events have been described previously (100). All of the M group subtypes, and the CRFs derived from them, can be traced back to a single successful natural transfer of HIV-1 to a human from a chimpanzee infected with simian immunodeficiency virus SIV_{cpz}. This event occurred sometime in the first half of the 20th century, somewhere in central Africa (50)

Globally, subtypes A and C account for most current infections, followed by subtype B and the intersubtype recombinants CRF01_AE and CRF02_AG. Subtype B is dominant in Europe, the Americas, and Australia (which accounts for the emphasis that was placed on this subtype in the early-to-middle years of the AIDS pandemic) (53). Subtype C may be the subtype that currently infects more people worldwide than any other; it is common in southern Africa and India (63). Subtypes A and D infect large numbers of people in central and eastern Africa. The other subtypes infect relatively, but only relatively, small numbers of people in central Africa and South

* Corresponding author. Mailing address for John P. Moore: Joan and Sanford I. Weill Medical College of Cornell University, Department of Microbiology and Immunology, 1300 York Ave., W-805, New York, NY 10021. Phone: (212) 746-4462. Fax: (212) 746-8340. E-mail: jpm2003@med.cornell.edu. Mailing address for Dennis R. Burton: Department of Immunology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., IMM2, La Jolla, CA 92037. Phone: (858) 784-9298. Fax: (858) 784-8360. E-mail: burton@scripps.edu.

America. In western Africa, an intersubtype recombinant, CRF02_AG (formerly designated as the prototype virus IbnG), is the dominant virus type (64). CRF01_AE (which carries the subtype E envelope sequence) is the most prevalent virus in southeast Asia. In China, intersubtype recombinants between subtypes C and B are becoming common (112). Of course, as HIV-1 continues to spread globally, the geographical restrictions are increasingly breaking down; many European countries, for example, have residents infected with multiple genetic subtypes (21, 26, 46, 84).

It is important to emphasize here that the genetic subtypes or recombinant lineages of HIV-1 are not analogous to classic viral serotypes and they should not be thought of in this way. The HIV-1 genetic diversity currently present in the human population dwarfs anything that has been described for other human viral infections studied. To put the situation into perspective: a few (3, 4) amino acid changes in one of the envelope glycoproteins of influenza virus can be sufficient to trigger a new epidemic; reassortants of influenza virus envelope genes can lead to devastating pandemics (31, 75, 96, 113, 122, 125). Yet, in HIV-1, replicating viruses can differ as much as 10% in sequence even within a single individual (54, 106). Therefore, even within a genetic subtype, the extent of HIV-1 genetic and antigenic diversity is simply enormous when compared to the diversity found for viruses for which effective vaccines have been developed. The degree of genetic, and hence antigenic, diversity therefore is daunting from the perspective of HIV-1 vaccine development. However, the description of a small number of human monoclonal antibodies (MAbs) that do neutralize many different HIV-1 isolates, including ones from different genetic subtypes, suggests that some features of the envelope glycoprotein structure are conserved (see below) (12, 14, 78, 115, 116). It would therefore be desirable to express such conserved structures in vaccine antigens aimed at inducing a broadly reactive humoral immune response. Unfortunately, all attempts to elicit antibodies with the specificities described for these human MAbs have failed to date (13, 89).

NEUTRALIZING ANTIBODIES AND THE HIV-1 ENVELOPE SPIKE

The only HIV-1 gene product known to be relevant to protective humoral immunity is the envelope glycoprotein complex. This is a trimeric structure composed of six individual subunits three gp120s and three gp41s that mediates virion attachment and membrane fusion. This complex is the target for virus-neutralizing antibodies. A series of studies involving epitope mapping with MAbs and site-directed mutagenesis, combined with the X-ray crystallographic solution of the gp120 core, have allowed a global approximation of the antigenic topography of gp120, both in its monomeric, soluble form and in a virion-associated, oligomeric form (references 28, 55, 56, 65, 71–74, and 108; reviewed in references 69, 89, and 131). The CD4 binding site (CD4bs) on gp120 is located within a depression at the interface of the three domains that comprise the gp120 structure (the outer domain, the inner domain, and the bridging sheet). This CD4bs surface is devoid of glycosylation and is relatively well conserved among HIV-1 isolates. The conserved coreceptor binding surface (97) is located at an approximately 90° angle to the CD4bs and is comprised principally of the bridging sheet, with additional contributions from the base of the V2 loop. Additional sequences from the V3 loop probably also contribute to coreceptor binding and are involved in coreceptor specificity (44, 45, 126, 132).

The interactions between gp120 and its receptors are complex and require conformational changes induced by CD4 binding (101, 103, 114). Both the CD4bs and the conserved coreceptor binding site are partially masked by the hypervariable V1V2 loop structure (132). This masking is most prominent in the oligomeric, functional form of gp120, making the relatively conserved receptor binding site surface poorly accessible to antibody. The structure of gp120, and whether it forms intersubunit interactions in the trimeric envelope complex, is not known, although compelling models have been proposed (56). Multivalent attachment between a gp120 trimer and a cluster of CD4 molecules displaces the V1V2 loop and the V3 loop, creating the coreceptor binding site and loosening the association of gp120 with gp41. The CD4 molecule contains flexible segments (129), allowing gp120 to drop down onto the coreceptor, bringing the virus and cell membranes into close proximity. Further conformational changes that activate the fusion machinery of gp41 then take place, leading to virus-cell membrane fusion, as outlined below. The association of gp120 with gp41 is unstable, involving apparently weak, noncovalent interactions. Regions at the N and C termini of gp120 form a discontinuous binding site for gp41 (41, 132). The corresponding binding site on gp41 for gp120 includes a putative N-proximal helical region and a short, intramolecular disulfide-bonded loop (7, 16). The structure of gp41, as it exists in the native envelope glycoprotein complex prior to CD4 and coreceptor binding, is not yet known. Neither is it understood how (or strictly, whether) intersubunit interactions between the different gp41 moieties cause this form of the complex to be trimeric. However, the receptor-triggered events that cause membrane fusion are associated with substantial conformational changes in gp41 that lead to the formation of a highly stable, trimeric coiled-coil structure. This comprises three N-terminal leucine/isoleucine zippers, one from each subunit of the trimer (18, 124). A second, more C-terminally oriented heptad repeat region of gp41 binds into grooves on the exterior surface of the coiled-coil. Hence, the gp41 subunit folds back on itself to form a stable six-helix bundle in which the fusion peptide and the transmembrane domain of gp41 are now positioned at the same end of the molecule (18, 124). In this form of the gp41 protein, the N-terminal fusion peptide points to the target membrane into which it becomes inserted, so that a single gp41 subunit is now attached simultaneously to two membranes: the viral membrane via its transmembrane domain and the cell membrane via the fusion peptide.

It is likely that the stable six-helix bundle represents the terminal conformation of a fusogenic envelope. It has been argued that it is the transition to this six-helix bundle that drives membrane fusion events after the fusion peptide is located in the cell membrane (29, 66). In other words, the six-helix bundle does not itself cause membrane fusion, rather, the dynamic events associated with its formation cause the two membranes to be brought close enough together for fusion to take place. This distinction is important for understanding why antibodies to this six-helix bundle form of gp41, which is highly immunogenic, do not neutralize HIV-1 infectivity; by the time

an antibody is able to react with the six-helix bundle, the fusion events are already over. Although receptor binding is necessary for formation of the six-helix bundle at the right time and place for fusion to occur, it is likely that this form of gp41 will also occur spontaneously, when some of the gp120 moieties naturally dissociate from gp41 during the process known as shedding (104). Because the six-helix bundle form of gp41 is highly stable, it probably persists on the surface of virions and perhaps on virus-infected or envelope-expressing cells.

Most virions, at least in tissue culture, suffer from baldness, or at least a receding hairline, in that they have lost the gp120 components from their full, theoretical complement of about 72 individual functional spikes or they never had them incorporated in the first place (49, 57). The shedding of gp120 will lead to the creation of the six-helix bundle form of gp41 that cannot, itself, mediate virus fusion with the host cell (see above). Thus, a virion can contain a mixture of fusion-competent and dead (postfusion form) spikes. It is not certain how many fusion-competent spikes must exist on a virion for it to retain infectivity, but it may be approximately a dozen (57). Just as the complete loss of gp120 from some spikes does not eliminate the infectivity of the entire virion, the binding of antibodies to some fusion-competent spikes does not do so either. Rather, the evidence suggests that the level of occupancy of binding sites by antibody molecules must exceed an antibody density threshold, after which the entire virion is neutralized (87). On HIV-1, the occupancy of a limited number of fusion-defective or dead (postfusion form) spikes by antibodies likely has minimal effects on virion infectivity, as shown by the absence of neutralization by cluster I and II anti-gp41 antibodies (6). The envelope glycoproteins are also expressed on the surface of naturally infected or envelope-transfected cells. In both cases, the expressed proteins can mediate cell-cell fusion, so functionally active, native complexes are clearly present. But so are defective species of envelope, often in abundance. These include complexes that have lost their gp120 moieties (as occurs on virions) or protein forms that have been improperly processed and so never form a native, fusion-competent complex (see below).

ANTIGENIC SEROTYPES AND GENETIC SUBTYPES

Antigenic serotypes are defined primarily by antibody reactivity with isolated components of the envelope glycoprotein complex. The determination of antigenic serotypes is technically far easier than the determination of neutralization serotypes, but the relevance of antigenic serotypes to vaccine development is extremely limited. For instance, it is well established that antibody reactivity with monomeric gp120 is not predictive of reactivity with envelope spikes or neutralizing ability (2, 34, 61, 68, 88, 98, 102, 117, 119). Thus, it is not unusual to find sera that react with a given isolated monomeric gp120 at titers of 10^5 or above but that have no significant neutralizing titer against the primary virus expressing the corresponding gp120 in trimeric form on its surface.

Antigenic serotypes can frequently be related to genetic subtypes. Peptides represent the easiest molecules to use for the definition of antigenic serotypes. Peptides corresponding to two linear epitopes are particularly noteworthy. One is the immunodominant V3 loop epitope cluster on gp120; the other is a poorly immunogenic epitope on gp41 defined by the unique human MAb 2F5. Although there is clearly a secondary structure to the V3 loop, its antibody epitopes are often continuous, so they can be represented by peptides and used in simple serology assays with sera from infected people. Here, there is a good correlation between the serotype and the genetic subtype, to the extent that V3 loop peptide immunoassays can be used with some confidence to diagnose the genetic subtype of the infecting strain (1, 19, 47, 59, 76, 80, 92). It should be noted that because of the existence of recombinant viruses, these measurements only serve to define a subtype for the envelope sequence rather than the whole virus. These assays are not perfect, but their simplicity renders them useful whenever absolute precision is a luxury rather than a necessity. Unfortunately, what is learned from V3 peptide assays is of little or no value to vaccine design: the V3 loop is generally only a weak cross-neutralization epitope on primary isolates (89).

The 2F5 epitope is potentially much more important for vaccine development, since this is one of the few sites on the primary virus envelope that represents a real vulnerability from the neutralizing antibody perspective (10, 24, 78, 95, 115). The 2F5 epitope is apparently linear, in that the 2F5 MAb binds to the short peptide ELDKWA (94). Unfortunately, all attempts to present this epitope to the immune system as a peptide, or a peptide fragment within a more complex immunogen, have failed to induce neutralizing antibodies (22, 33, 58, 77). This probably indicates that the true epitope on the native complex has a structure that is significantly affected by other regions of gp41 and/or gp120. Nonetheless, it is possible, to some extent, to pick out viruses that are sensitive or insensitive to the 2F5 MAb by inspection of the primary sequences within the ELDKWA region of gp41 (115). How this relates to genetic subtypes is as yet unclear.

The next level of antigenic complexity following peptides that is used to define antigenic serotypes is monomeric gp120. This protein contains several epitope clusters, many of which are discontinuous in nature (73). Some of these are strong neutralization epitopes for T-cell line-adapted viruses, including the V3 loop and the CD4bs-related epitopes. When MAbs are used to probe the topology of gp120s from multiple subtypes, some patterns are observed that reveal a subtype dependency to the antibody-recognition pattern (70, 134). Most of the MAbs used in this sort of study were raised against subtype B gp120s, either during natural infection of humans or after gp120 immunization of animals. MAbs to the variable regions of gp120 usually, but not invariably, recognize subtype B gp120s more efficiently than they do gp120s from other subtypes. Those that recognize more conserved regions of gp120 show a stronger degree of cross-subtype reactivity, in some cases virtual panreactivity (70, 82) Unfortunately, these MAbs almost never strongly neutralize multiple primary isolates, even from within the same genetic subtype. This is because epitope exposure on the native envelope glycoprotein complex is much less than is found on the dissociated gp120 subunits under the immunoassay conditions discussed above (68, 90, 98).

In serological studies using monomeric gp120, there can be reasonable concordance between the genetic subtype of the infecting virus and serum reactivity. Thus, sera from people

infected with subtype A viruses tend to react better with subtype A gp120s than they do with gp120s from subtypes B, C, D, etc. (59, 67, 118). This phenomenon probably reflects the immunodominance of the V3 loop epitope cluster in gp120-binding assays. This immunodominance, it is important to note, is not seen in primary virus neutralization assays (59).

The above assays are reliable in that they generate readily interpretable and reproducible results, even if those results have little direct relevance to vaccine immunogen design. Greater problems arise, however, with attempts to study more complex forms of the HIV-1 envelope glycoprotein complexes, notably those that are presented on the surfaces of virions of virus-infected cells. Here, artifacts are a major concern, which is why the results of such assays stand to cause significant confusion within the HIV-1 vaccine field. The artifacts also have an impact on any attempts to relate the results of cell- and virion-antibody binding assays to neutralization assays. The principal problem affecting the performance and interpretation of assays that attempt to measure antibody binding to the native HIV-1 envelope glycoprotein complex is the heterogeneity of the spike structures on the virion and cell surfaces. Thus, antibody binding to defective spikes does not affect HIV-1 infectivity, yet antibody binding sites on defective spikes can be dominant in the overall assay signal, as will be shown below. The first example of difficulties in estimating neutralizing Ab by a direct binding assay arose from attempts to measure MAb binding to envelope glycoprotein complexes on the surface of primary, $CD4^+$ T cells infected with HIV-1 primary isolates (128, 134). Unfortunately, gp120 monomers dissociate from the native complexes, or are otherwise secreted, and bind to the CD4 antigen on the surface of the same or another cell. These complexes of gp120 monomers with CD4 are good substrates for antibody binding, because several immunodominant epitopes are nicely exposed on the gp120-CD4 complex (73). These include the V3 loop and the C5 region of gp120, both epitope clusters that are substantially or completely sequestered on the native envelope glycoprotein complex (8, 55, 56). Conversely, the CD4bs epitopes are occluded (by CD4) and so MAbs to this site do not register in the assay, a feature that is diagnostic of the problem and the underlying artifact (128, 134). This type of assay has no more practical value than a simple capture enzyme-linked immunosorbent assay using a gp120-CD4 complex as the test antigen. The assay is still being used, for example, to evaluate the properties of vaccinee sera where, in our view, it is suggested inappropriately that it measures antibody responses to native envelope glycoprotein complexes (38).

As a second example of estimating neutralizing Ab by direct binding, assays are being employed that rely on antibody binding to envelope species on cell lines transfected with an envelope gene, with the suggestion that the signal derives from native envelope glycoprotein complexes (37, 86). These assays may, however, be compromised by the presence on the surface of transfected cells of misfolded or improperly processed envelope glycoprotein complexes, on which at least some epitopes are inappropriately expressed. Such defective complexes are probably functionally inert, but they are not inert in antibody-binding assays. Consequently, attempts to correlate the two parameters of antibody binding and fusion inhibition are fraught with difficulty; one can simply never be sure whether the immunoassay signal is or is not derived from a functional envelope glycoprotein complex. The most common, but probably not the only, source of improperly constituted envelope is the incomplete cleavage of gp160 into its gp120 and gp41 components, an event mediated by the cellular protease furin or related enzymes (27, 62, 111). Incomplete cleavage of Env occurs to a much greater extent in Env-transfected cells than in naturally infected cells. In a recent study employing this method it was indeed shown that up to 75% of envelope on the transfected cell surface existed in the form of immature gp160 (133). This is because the cellular proteases become saturated when Env is overexpressed by virtue of the use of strong promoters or more effective signal sequences, something which to a degree can be overcome by cotransfection of additional furin (7). Furthermore, the presence of Gag proteins affects the organization of Env on the surface of transfected cells. In infected cells and cells cotransfected with Env and Gag, the Env glycoproteins are clustered at the sites of Gag assembly, but in the absence of Gag, the Env glycoproteins are diffusely scattered across the cell surface (42). Whether the absence of Gag affects the structure of the envelope glycoprotein complexes is not known, but there is evidence that the intracytoplasmic, Gag-interactive domain of gp41 has an influence on the topology of the extracellular gp120-gp41 complex (109, 110).

More reliable results can be obtained from assays in which a cell line is infected with HIV-1, provided that CD4 is downregulated at the time of the assay (105). Here, the envelope glycoprotein complexes are apparently mostly present in the form of assembling or budding virions (35, 85). Assays of this type have been used to show that antibody binding to the infected cell surface (read serotype) strongly correlates with neutralization. Unfortunately, this assay has only been successfully used for cell line-adapted viruses (88, 102) and not yet for primary isolates.

Assays that attempt to measure antibody binding to functional envelope spikes by virion capture are also problematic and form a third example of the difficulties in this area (37, 81). Here again, a positive immunoassay signal does not necessarily mean that an antibody has reacted with a functional envelope complex capable of mediating infection. It may instead have emanated from an antibody complex with a defective spike. For instance, most gp41 epitopes are sequestered on a native complex, but they are exposed when gp120 has dissociated (105). This creates a fusion-defective spike with immunodominant, yet nonneutralizing gp41 epitopes available for antibody binding. Likewise, the virion reactivity of MAbs to nonneutralizing C5 epitopes on gp120 likely involves defective complexes; there is ample evidence that this region of gp120 is involved in gp41 binding and is thus substantially or completely inaccessible on the native complex (41, 130). Antibodies will certainly bind to virions via defective spikes, but this is a result of little practical value. Of course, some of the other spikes on the same virion will probably still be fusion competent, so it can legitimately be claimed that the gp41 epitopes are accessible on infectious virions (81). But this is beside the point. The nonneutralizing gp41 epitopes are not exposed on a native complex, and it is to native complexes that a vaccine-induced antibody must bind if a neutralizing antibody-based vaccine is to be effective. Defective spikes on the virion or infected cell

surface may have some relevance as targets for complement-mediated virolysis or antibody-dependent cellular cytotoxicity, but it is far from obvious that these immune mechanisms are at the forefront of HIV-1 vaccine design.

NEUTRALIZATION SEROTYPES AND GENETIC SUBTYPES

Multiple studies have been performed to investigate whether genetic subtypes correspond to neutralization serotypes (51, 52, 60, 67, 83, 123). These involved testing panels of primary HIV-1 isolates from multiple subtypes (usually A through E; subtype E was represented by CRF01_AE viruses which have an E envelope sequence) for the ability to be neutralized by heterologous sera from people infected with viruses from defined subtypes (again, usually A through E). Most commonly, checkerboard analyses were carried out to see whether there was any subtype-dependency to the patterns of neutralization that were observed. These analyses are limited by the likelihood that many viruses are circulating recombinant forms (see above), something that was not understood at the time the studies were performed. Nevertheless, the studies all concluded that there was little or no relationship between the genetic subtypes and what was observed in neutralization assays. Some sera had cross-subtype-neutralizing activity (usually weak); some isolates were fairly easily neutralized; others were resistant, but this was not subtype dependent. There is no consistent evidence, for example, that sera from people infected with subtype A viruses preferentially neutralized subtype A viruses (3, 51, 52, 67, 83, 123). One report did find that subtypes B and E formed discrete neutralization serotypes when compared directly against each other (60). The envelope glycoproteins from subtypes B and E are at opposite ends of the antigenic diversity spectrum, so if there was ever going to be a subtype dependency to the outcome of neutralization assays, it would probably be seen with these two subtypes. However, no consistent discrimination between subtypes B and E has been observed in several other studies (3, 51, 52, 59, 67, 83, 123). Overall, neutralization serotypes, in the conventional sense of the phrase, are not apparent in these various studies.

The lack of correlation between genetic subtypes and neutralization may seem intuitively surprising. It implies that the sequence similarities that are sufficient to enable organization of isolates into genetic subtypes are not important in defining neutralization epitopes common to different isolates. Is then the concept of neutralization serotypes just not useful for HIV-1 because of the enormous sequence diversity of different isolates? Put another way, are there so many serotypes that they render facile any classification attempts? Highly potent neutralizing responses that are essentially unique to a particular isolate have been described (references 20 and 107; see below). The most persuasive evidence that some grouping of primary isolates into neutralization serotypes may be possible comes from the description of the few MAbs (e.g., b12, 2F5, and 2G12) that are able to neutralize a sizeable proportion of isolates (32, 89). Of note is the fact that these antibodies, broadly speaking, do not significantly discriminate between genetic subtypes. They are directed to relatively conserved features of the envelope that are largely retained in an approximately similar proportion of isolates from a given genetic subtype (48, 91, 115). An exception exists for the broadly cross-neutralizing human MAb 2G12. This does not recognize isolates with subtype E envelopes because of an unusual structural feature (an additional disulfide bond) in the V4 loop region that appears to be unique to the subtype E gp120 protein (115). It should be emphasized that these conserved antigenic features appear to be very poorly immunogenic. Thus, neutralizing antibodies against these epitopes do not represent a significant fraction, if any, of the typical antibody response against HIV-1 envelope in infected persons or people immunized with experimental Env vaccines developed thus far (11).

Apart from the triad of broadly cross-reactive neutralizing antibodies described above, HIV-1 primary isolates can sometimes be neutralized by highly type-specific antibody preparations, such as autologous sera or MAbs against the variable loops on gp120 (2, 23, 36, 121). An example of a highly potent but isolate-specific neutralizing antibody response has been studied in detail (20, 107). A serum sample from an HIV-1-infected chimpanzee was shown to potently neutralize the autologous virus but no other viruses against which it was tested. In passive antibody transfer experiments, this serum could protect macaques from infection with a simian-human immunodeficiency virus that expressed the corresponding envelope (107). The dominant epitopes targeted by the serum were highly conformational and involved elements from all the hypervariable loops of gp120 (20). Such an epitope specificity explains the inability of the serum to cross-neutralize any other primary isolates. Some MAbs against the V3 loop of HIV-1 have been shown to neutralize limited subsets of isolates within a genetic subtype (2, 23). We suggest that it is this type of cross-reactivity that would usually define a neutralization serotype for a less variable virus. This line of thought suggests that each genetic lineage of HIV-1 then consists of scores of distinct neutralization serotypes. Although these types of neutralizing antibody responses can protect against challenge with a primary isolate (107), they have little practical value for the development of a vaccine. This is the case even if that vaccine were aimed at only a single genetic subtype or lineage of HIV-1 circulating in a single geographical area. The extent of HIV-1 diversity forces vaccine development to focus on the very highly conserved HIV-1 epitopes that have thus far been shown to be retained across subtypes. For the humoral response, a genetic subtype-targeted approach to vaccine design therefore seems currently unnecessary and without scientific foundation.

In the absence of any truly useful information on neutralization serotypes, what we should do? Are there any arguments for a vaccine antigen that is closely matched to the locally circulating strains? At present, we believe that concerns about creating vaccines closely matched to local circulating HIV-1 strains are overstated from the standpoint of humoral immunity, as implied above. For example, vaccines based on monomeric gp120 are not likely to become significantly more effective when formulated as a multivalent vaccine (derived from multiple isolates or genetic subtypes) than they are as a monovalent formulation (40). Phase III vaccine efficacy trials are still under way, but initial analyses of phase II trials with (monovalent) monomeric gp120 vaccines (prepared from SF2 and MN strains) have indicated no obvious benefit in persons who

experienced breakthrough infections. The distribution of infected individuals in vaccinated and control groups furthermore was similar (40). Another analysis performed on a subset of the same cases has suggested that the frequency of certain signature sequences, particularly the V3 loop, in viruses derived from breakthrough infections differ from historic virus sequences from the same genetic subtype (4, 5). This divergence, however, was not significant if all breakthrough cases were considered (25). Nevertheless, it was suggested that skewing of V3 loop sequences in selected viruses indicates the presence of immune pressure on the challenge virus, allowing only more variable viruses to break through, and thus monomeric gp120 vaccines should be combined in bivalent or multivalent formulations to block a broader range of viruses (4). That analysis is controversial, as great emphasis is put on sequences which do not appear to constitute a strong cross-neutralizing epitope for primary isolates of HIV-1. It has indeed been clearly established that immunization with simple gp120 or gp160 subunit vaccines does not induce antibodies against broadly conserved neutralizing epitopes, not even at low levels (40, 89). Antibodies against more variable and exposed epitopes that are elicited by vaccines of this type may neutralize the autologous virus and even a few closely envelope sequence-related isolates. Such antibodies, however, are unlikely to make an impact on the HIV-1 epidemic because of the enormous issue of virus diversity (even within genetic subtypes). A multivalent vaccine that induced neutralizing antibodies only to variable epitopes could only be effective if it included perhaps thousands of different subunit components, and each individual component would have to be able to deal with a measurable fraction of circulating strains: this seems implausible, based on current knowledge.

In summary, the primary goal in this area should be to design an immunogen that can be shown to elicit neutralizing antibodies against a significant proportion of primary isolates from any geographical area. If such an immunogen is developed, the corresponding sera should then be evaluated against isolates from many geographical areas, including the target area. This will reveal whether the immunogen could benefit from some engineering to optimize neutralizing responses to viruses from the target area; clinical trials can assist in vaccine design, in an iterative process, if and when meaningful results on virus neutralization are obtained. Our collective hope must be that any real success at generating primary isolate-neutralizing antibodies with a practical immunogen could be exploited rapidly, to generate variants of that immunogen able to broaden the immune response. That seems to us to be more important than worrying whether a subtype B protein could be tested in Africa, a subtype A in Asia, etc. After all, the genetic subtypes were not designated based on the antigenic or immunogenic properties of the envelope glycoproteins and they do not correspond to neutralization serotypes. It is to be hoped that regional and national political considerations are not permitted to override sound scientific arguments in the development of an HIV-1 vaccine.

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