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Conformational Epitope on gp120 Important in CD4 Binding and Human Immunodeficiency Virus Type 1 Neutralization Identified by a Human Monoclonal Antibody

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A human monoclonal antibody designated I5e is reactive with the envelope glycoprotein (gp120) of multiple isolates of human immunodeficiency virus type 1 (HIV-1). Antibody I5e also neutralizes HIV-1 with broad specificity and blocks gp120 binding to CD4. Characterization of the I5e epitope shows that it is conformation dependent and is distinct from previously recognized functional domains of gp120, suggesting that this epitope represents a novel site important for HIV-1 neutralization and CD4 binding. These findings have implications for the development of a vaccine for AIDS.

Identification of functional domains in the envelope glycoproteins (gp120 and gp41) of human immunodeficiency virus type 1 (HIV-1) is important for the development of a vaccine for AIDS. A site in the C-terminal region of gp120 has been shown by Lasky et al. (17) to be critical for virus binding to its receptor, CD4. Additional studies by Cordonnier et al. (4) also showed that several mutations within this region of gp120 resulted in loss of CD4 binding. Interestingly, mouse monoclonal antibodies directed against this putative binding site are either nonneutralizing (17) or only variably neutralizing (30) against HIV-1 in vitro. Several other antibody neutralization domains have been determined (2, 7, 9, 11, 12, 14, 15, 18, 24, 25, 27, 28), among which is the loop structure in the third variable region of gp120 (V3 loop) that is believed to be the principal neutralization domain (9, 14, 15, 18, 24, 25, 27). However, probably related to its sequence heterogeneity among isolates, the V3 loop induces type-specific neutralizing antibodies (9, 14, 15, 18, 24, 25, 27, 28), which do not account for the broad virus-neutralizing activity detected in the sera of most infected persons (1, 12, 33). Such broadly neutralizing antibodies may be directed against a conserved site in gp41 (2, 7) or against conformational epitopes on gp120, as was recently shown by Haigwood et al. (10). We now present data on a human monoclonal antibody which identifies a previously unrecognized, conformation-dependent epitope on gp120 important for both HIV-1 neutralization and CD4 binding.

A human monoclonal antibody, I5e (originally designated N70-1.5e), was derived by Epstein-Barr virus transformation of B cells from an asymptomatic person seropositive for antibodies to HIV-1 (26). This antibody is an immunoglobulin G1 (IgG1) with kappa light chains and is specific for the gp120 of all 11 HIV-1 isolates initially tested by enzyme immunoassays and Western immunoblots (26). In a radioimmunoprecipitation assay described previously (11, 12, 30), I5e was reactive with gp120 and/or its precursor gp160 from HIV-1 isolates IIIB, MN, and Z84 but not with isolates RF and AL (Fig. 1A). I5e did not react with the envelope glycoprotein of the LAV-2_{ROD} strain (3) of HIV-2 by either enzyme immunoassay or radioimmunoprecipitation assay (data not shown).

I5e was also tested for neutralizing activity against multiple laboratory and primary HIV-1 isolates with an assay described previously (11, 12, 30). As shown in Fig. 2A, I5e neutralized laboratory isolates IIIB, Z84, MN, and SA3 with 90% inhibitory doses (dose required to inhibit activity by 90%; ID_{90}) of 0.4, 0.6, 3.5, and 12.0 μg, respectively. In agreement with the immunoprecipitation results, isolates RF and AL were refractory to neutralization by I5e. The neutralizing capacity of I5e against 10 primary HIV-1 isolates from U.S. patients is shown in Fig. 2B. Six primary strains were efficiently neutralized, with an ID_{90} of less than 1.5 μg, while more I5e was required to neutralize three other strains. One isolate was completely resistant. The findings presented in Fig. 2 suggest that I5e is an HIV-1-neutralizing human monoclonal antibody which has broad specificity.

We next examined I5e for its ability to compete with CD4 for gp120/gp160 binding, as this is one potential mechanism for the HIV-1-neutralizing activity. In the experiment shown in Fig. 1B, increasing doses of soluble CD4 (sCD4) (8) were added to a metabolically labeled lysate of HIV-1 prior to immunoprecipitation. sCD4 competed with I5e in a dose-dependent manner, suggesting that the epitope on gp120 for this human monoclonal antibody may be on the surface, which makes contact with CD4. This observation was confirmed by a quantitative competition enzyme immunoassay described by Moore et al. (20, 21), with recombinant gp120 captured indirectly onto solid-phase and sCD4. As shown in Fig. 3, I5e blocked gp120-sCD4 binding in a dose-dependent manner. In fact, it was more potent in doing so than two mouse anti-gp120 monoclonal antibodies, G3-536 and G3-537 (30), which we have previously mapped to the putative CD4-binding site described by Lasky et al. (17). The amount of I5e, G3-536, and G3-537 required to reduce gp120-sCD4 binding by 50% was 70, 2,500, and 200 ng/ml, respectively.

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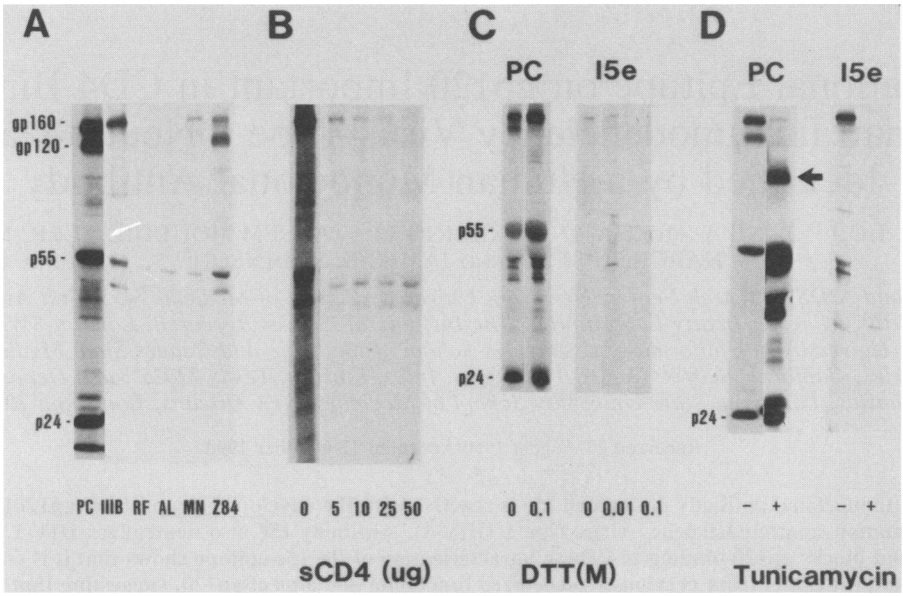

FIG. 1. (A) Reactivity of I5e with diverse laboratory strains of HIV-1 by radioimmunoprecipitation assay (11, 12, 30). PC, Positive control serum reacting with IIIB proteins. (B) Competition radioimmunoprecipitation assay between increasing doses of sCD4 and I5e (10 μg). (C) Reactivity of I5e and a positive control serum with metabolically labeled IIIB lysate with or without reduction by dithiothreitol (DTT). Dithiothreitol was removed by using a Centricon 30 (Amicon, Danvers, Mass.) spin column at 5,000 × *g* for 1 h before the viral lysate was used for immunoprecipitation. (D) Reactivity of I5e and a positive control serum with metabolically labeled IIIB lysate prepared in the presence (+) or absence (−) of tunicamycin (1.5 μg/ml).

The greater ability of I5e in blocking gp120-sCD4 interaction is not due to a greater affinity for gp120, since I5e and G3-536 have similar binding constants for gp120 (50% maximal binding to gp120 was observed at 47 ng/ml for I5e and at 86 ng/ml for G3-536; data not shown).

Given I5e's ability to neutralize HIV-1 and to block gp120-sCD4 binding, attempts were made to localize its epitope by peptide enzyme immunoassays. Results from such studies are schematically summarized in Fig. 4. I5e reacted with native gp120 and recombinant gp120 expressed in Chinese hamster ovary (CHO) cells. However, this antibody did not react with unglycosylated gp120 made by *Escherichia coli* or *Saccharomyces cerevisiae*. In addition, I5e did not recognize recombinant envelope fragments (PE3, PB1, and pEnv9) produced in *E. coli*. Furthermore, there was not reactivity with a panel of synthetic peptides representing different regions of gp120, including the second conserved domain (11), the V3 neutralization loop (9, 14, 15, 18, 24, 25, 27, 28), and the putative CD4-binding site defined by Lasky et al. (17). Together, these findings suggest that the epitope of I5e is probably not composed of a linear sequence. Instead, the epitope is likely to be conformation or carbohydrate dependent or both.

The conformational nature of the I5e epitope was con-

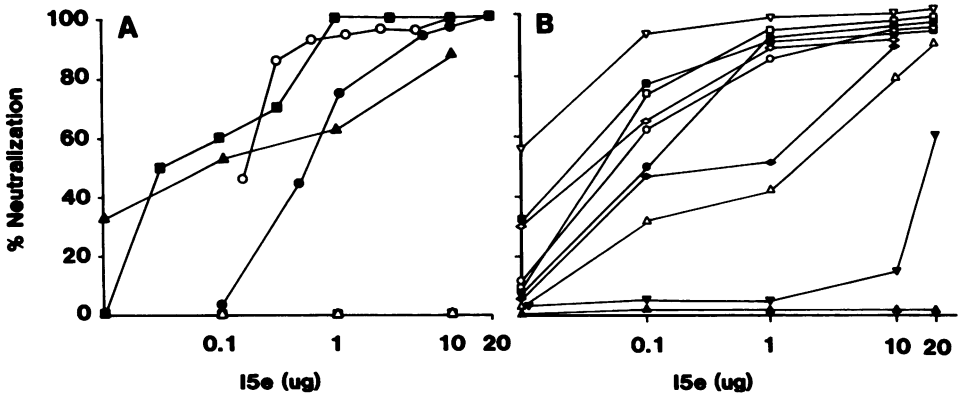

FIG. 2. (A) I5e neutralization of six laboratory strains of HIV-1: IIIB (○), Z84 (■), MN (●), SA3 (26) (▲), RF (△), and AL (11, 12, 30) (□). The neutralization assay was performed as described previously (11, 12, 30), with the p24 antigen concentration in the supernatant used as the indicator of virus infection. (B) I5e neutralization of 10 primary HIV-1 isolates. These viruses were isolated from patients in short-term cultures of normal peripheral blood mononuclear cells. Virus titers were determined by serial dilutions. The virus stains were assayed for susceptibility to I5e neutralization with 50 50% tissue culture-infective doses of virus and a method already described (5). Viruses: AC (●), JRCSF (○), JRFL (△), LS (□), CC (■), JM (▲), RP (▽), TB (▼), EP (◇), and LL (◆).

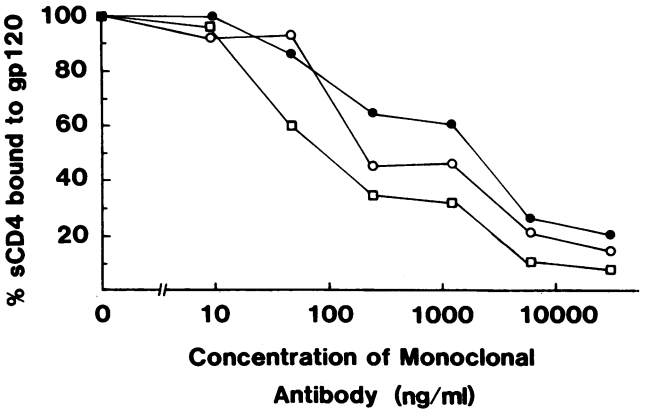

FIG. 3. Inhibition of sCD4-gp120 binding by monoclonal antibodies I5e (□), G3-537 (○), and G3-536 (●). The assays were performed by the procedure of Moore et al. (20, 21). Briefly, recombinant gp120 expressed in CHO cells (Celltech; provided by the Medical Research Council AIDS Directed Reagent Programme) was captured onto solid phase by D7324, a sheep antiserum (Aalto BioReagents) raised against the conserved carboxyl terminus of gp120. Increasing concentrations of the competing antibody were added prior to incubation with sCD4 (50 μl; 1 μg/ml) from Smith Kline Beecham (6). Bound sCD4 was detected by a rabbit anti-CD4 serum (CBL-JA) followed by sheep anti-rabbit IgG conjugated to alkaline phosphatase. Color development was then achieved with the AMPAK enzyme-linked immunosorbent assay (ELISA) amplification system (Novo BioLabs).

firmed by the studies shown in Fig. 1C. After reduction of a metabolically labeled lysate of HIV-1 by 0.1 M dithiothreitol, the gp120/gp160 reactivity of I5e was lost. This observation was previously made by Robinson et al. (26) in gp120

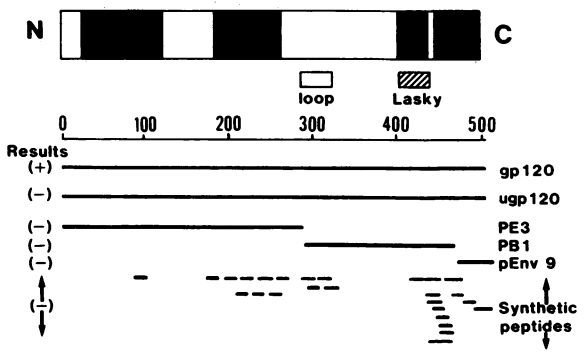

FIG. 4. Epitope mapping of I5e with recombinant and synthetic peptides in enzyme immunoassays. Black boxes show conserved domains of gp120. Loop refers to the V3 neutralization domain (9, 14, 15, 18, 24, 25, 27, 28), and Lasky refers to the putative CD4-binding site (17). The peptide immunoassays were performed as outlined previously (30), and the results are summarized on the left. Native and recombinant CHO gp120 were gifts of G. Robey and N. Haigwood, respectively. ugp120 represents unglycosylated envelope protein expressed in *E. coli* (DuPont) or *S. cerevisiae* (Chiron). PE3, PB1, and pEnv9 are recombinant fragments made in *E. coli* by Putney et al. (25) and provided by S. Petteway. The precise amino acid coordinates of the synthetic peptides are as follows: HXB2, 85 to 100, 174 to 188, 197 to 213, 209 to 223, 219 to 233, 229 to 243, 239 to 253, 249 to 263, 259 to 273, 308 to 322, 413 to 447, 430 to 439, 451 to 477, 466 to 477, 476 to 492, and 489 to 512; and Bru, 430 to 439, 432 to 441, 434 to 443, 436 to 445, 438 to 447, 440 to 449, and 430 to 449.

dot blot assays under reducing and nonreducing conditions. Similarly, the importance of glycosylation to I5e-gp120 reactivity was confirmed by the studies shown in Fig. 1D. Metabolically labeled HIV-1 lysates were prepared in the absence and presence of tunicamycin. The unglycosylated envelope precursor polypeptide of 90 kDa was not recognized by I5e, perhaps because of alterations in the tertiary structure. Alternatively, it is possible that an oligosaccharide moiety contributes directly to form a part of the I5e epitope.

Further attempts to define the I5e epitope were made by testing previously characterized mouse monoclonal antibodies for their ability to compete with labeled I5e for gp120 binding. One antibody (9284) (28) to the V3 neutralization loop, one antibody (G3-136) (8a) to an N-terminal sequence adjacent to a region (amino acids 108 to 116) purported to be important in CD4 binding (31), and two antibodies (G3-536 and G3-537) (30) to the putative CD4-binding site defined by Lasky et al. failed to compete with biotinylated I5e for binding to gp120 (Fig. 5A). In contrast, unlabeled I5e competed well with the labeled antibody. In addition, 36 mouse anti-gp120 monoclonal antibodies from Thiriart et al. (32) also did not compete with I5e for gp120 binding (data not shown). These findings suggest that I5e epitope is not localized to previously defined functional domains of gp120 and is likely to be a novel site important in both CD4 binding and antibody neutralization of HIV-1.

Do HIV-1-infected persons have I5e-like antibodies? If so, when? To address these questions, serum samples were obtained from patients at different stages of HIV-1 infection and tested for their ability to compete with biotinylated I5e for gp120 binding in an enzyme immunoassay. First, serum samples from nine patients with AIDS were studied. Two such sera and a normal human serum did not have appreciable I5e-competing activity, whereas seven other sera from AIDS patients contained antibodies that competed with I5e (Fig. 5B). Interestingly, the latter serum samples had cross-neutralizing antibodies against the IIIB laboratory isolate, with titers ranging from 1:8 to 1:64. In contrast, the two sera from AIDS cases which did not compete with I5e failed to neutralize IIIB even at a 1:4 dilution (data not shown).

We next tested sequential serum samples from seven patients in the first few months following initial HIV-1 infection and seroconversion (29; unpublished data). As shown in Fig. 5C, in the first 2 months after seroconversion, patients lack antibodies which compete with I5e, although all had anti-gp120 antibodies as determined by radioimmunoprecipitation assays (11, 12, 30). In one patient, there was evidence that I5e-competing antibodies began to appear at 6 months. This led us to study serum samples from three patients from whom multiple sequential blood samples were obtained in the first 2 years following seroconversion (13). Each serum sample was assayed for its anti-gp120 antibody reactivity, ability to compete with I5e for gp120 binding, IIIB neutralizing titer, and activity in blocking HIV-1 virion binding to $CD4^+$ cells (Fig. 6). Anti-gp120 antibodies reached maximum levels between 7 and 10 months after seroconversion, whereas the I5e-competing activity was substantially delayed, only reaching peak levels at 12 to 15 months. More importantly, the time course for the development of I5e-competing antibodies paralleled the time course for the development of antibodies which neutralize the IIIB isolate or block virion binding to $CD4^+$ cells. These findings suggest, but do not prove, that the I5e epitope may be responsible for the slow induction in vivo of antibodies which broadly neutralize HIV-1 and block virus attachment.

In conclusion, we have found that the epitope on gp120

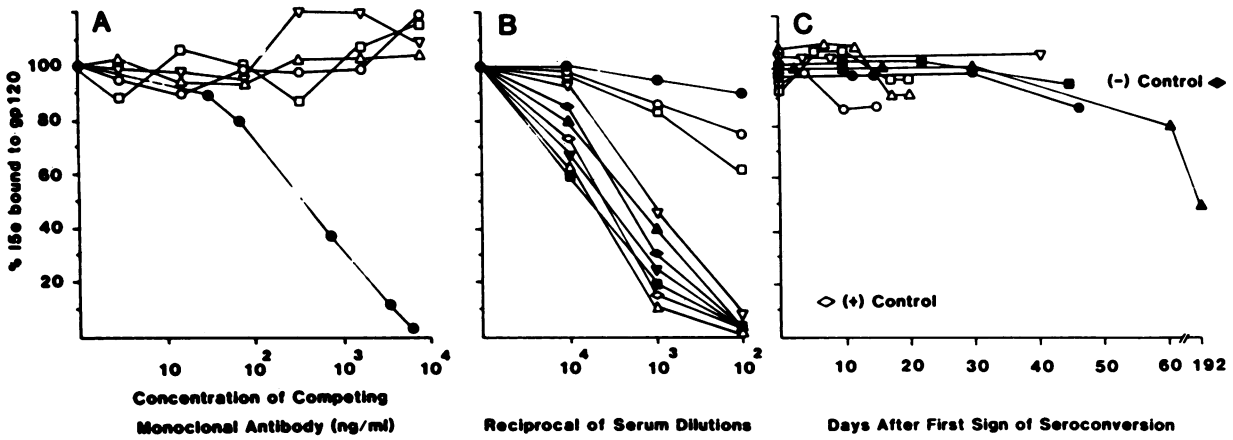

FIG. 5. Competition with biotinylated I5e for gp120 binding by (A) four mouse anti-gp120 monoclonal antibodies and by sera from (B) patients with AIDS and (C) patients who had recently seroconverted. The assays were carried out by the method of Moore et al. (22). Briefly, recombinant CHO gp120 (Celltech) was captured on solid phase by using antibody D7324. I5e was biotinylated by the standard protocol, and the concentration required for half-maximal binding was used, along with increasing concentrations of the competing antibody. Detection of bound I5e was achieved by adding streptavadin-alkaline phosphatase, followed by the use of the AMPAK ELISA amplification system. (A) Symbols: ●, unlabeled I5e; ▽, monoclonal antibody 9284; ○, G3-536; □, G3-537; △, G3-136. (B) ●, Seronegative control; the other nine sera were obtained from patients with AIDS. (C) Each symbol represents a seroconvertor or a positive or negative control. The serum samples were all tested at a dilution of 1:500.

identified by I5e is a conformation-dependent (Fig. 1C and 4), previously unrecognized site important in CD4 binding (Fig. 1B and 3) and virus neutralization (Fig. 2). It is distinct from the putative binding site defined by several groups (4, 17, 30). Kowalski et al. (16) had previously shown that several distinct regions of the C-terminal half of gp120 contribute to CD4 binding. More recently, Olshevsky et al. (23) have found that the CD4-binding site may be formed by the juxtaposition of the third and fourth conserved domains of gp120. It will be important to examine the possibility that I5e is directed against such a conformational determinant.

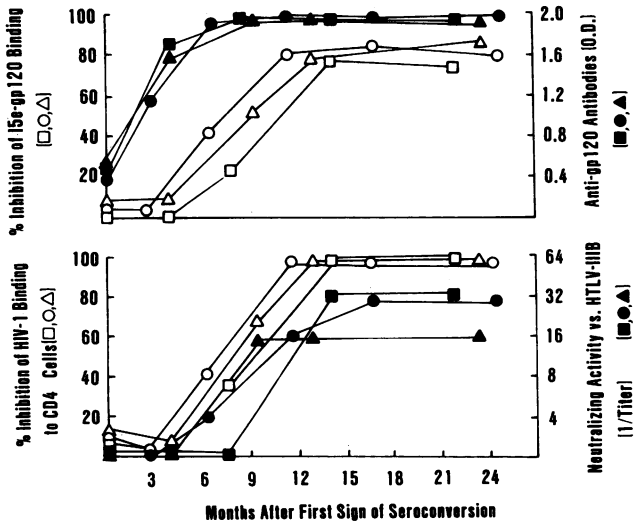

FIG. 6. Results of multiple studies on sequential serum samples from three seroconvertors. Each patient is represented by a different symbol. Inhibition of I5e-gp120 binding was determined as described in the Fig. 5 legend; anti-gp120 antibodies were determined by an enzyme immunoassay (30) with serum samples used at a dilution of 1:100; inhibition of HIV-1 virion binding to $CD4^+$ cells was determined by our published method (11, 30), which was based on that of McDougal et al. (19); neutralizing titer against the IIIB isolate was measured by our standard assay (11, 12, 30). Similar kinetics for anti-gp120 antibody development were obtained at serum dilutions of 1:1,000, which was not saturating in the assay.

I5e neutralized multiple laboratory and primary HIV-1 isolates in vitro with variable potency (Fig. 2). It is as yet unclear whether the differential sensitivity to I5e neutralization is the result of differential affinity of the antibody for various gp120 molecules or, alternatively, of differential affinity of the gp120 molecules for the CD4 receptor. Although 3 of 16 isolates tested were completely refractory to neutralization by I5e (up to 20 μg), the epitope identified by this human monoclonal antibody appears to be reasonably well conserved. Attempts should be made to preserve this conformational epitope in candidate gp120 or gp160 subunit vaccines. Given the slow development of I5e-like antibodies in HIV-1-infected persons (Fig. 5C and 6), prolonged immunization may be necessary to induce antibodies to the I5e epitope, unless the immunogenicity of this domain can be enhanced. Finally, if the concept of passive immunotherapy is proven efficacious in patients, I5e will be an excellent candidate for clinical use.

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