Antibodies to the Primary Immunodominant Domain of Human Immunodeficiency Virus Type 1 (HIV-1) Glycoprotein gp41 Enhance HIV-1 Infection In Vitro

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Previous experiments had shown that two human monoclonal antibodies (huMAbs) directed against human immunodeficiency virus type 1 (HIV-1) enhanced HIV-1 infection in vitro (Robinson et al., Proc. Natl. Acad. Sci. USA, 87:3185–3189, 1990). This complement-mediated, antibody-dependent enhancement (C'-ADE) of HIV-1 infection caused 12-fold increases in reverse transcriptase released from MT-2 cells. In the study reported here, it was demonstrated that both of these huMAbs, 86 and V10-9, bound to an immunodominant peptide in gp41 (amino acids 586 to 620). This peptide blocked C'-ADE of HIV-1 infection in vitro regardless of whether huMab 86 or human polyclonal anti-HIV was used as the source of anti-HIV antibody. Blockade of enhanced infections was characterized by decreases in antigen synthesis, cytopathic effect, and reverse transcriptase release. The ability of the huMAbs to enhance infection was determined to be dependent upon specific peptide reactivity and not dependent upon immunoglobulin subclass, complement fixation, or gross antigen reactivity. Since the peptide to which enhancing antibodies bind is immunodominant and does not bind neutralizing antibodies, it may be worthwhile to investigate deletion of this 35-amino-acid peptide from candidate anti-HIV vaccines.

Although the causative agent of the acquired immune deficiency syndrome has been identified to be the human immunodeficiency virus type 1 (HIV-1) (1, 4, 13), the mechanism of immune deficiency has not been fully elucidated. Specific immune responses such as neutralizing antibody, cytotoxic T lymphocytes, T-cell responses, antibody-dependent cell-mediated cytopotoxicity, and natural killer cell responses against HIV-1 have been described, but their roles in immunopathogenesis have yet to be determined. Several recent reports have described an immunological phenomenon called antibody-dependent enhancement (ADE) of HIV-1 infection (8, 10, 23, 24, 31). Two in vitro mechanisms for ADE have been evaluated. The first involves the alternative pathway of complement and antibody to the envelope glycoprotein of HIV-1 (24, 25); the second requires antibody to HIV-1 and cells bearing Fc receptors (8, 10, 31). Complement-mediated ADE (C'-ADE) has been determined to require both CD4 receptors and complement receptor type 2 (21, 26). This complement-dependent mechanism has been called complement-mediated, antibody-dependent enhancement (C'-ADE) of HIV-1 infection, while the latter complement-independent mechanism has been called Fc receptor-mediated, antibody-dependent enhancement (FcR-ADE) of HIV-1 infection (8, 10, 31).

Although no antigenic regions have been described for FcR-ADE, a significant body of evidence suggests that the envelope glycoproteins of HIV-1 are important in C'-ADE of HIV-1 infection. Previous reports have shown that antibodies conferring C'-ADE were bound and eluted from a pENV9 column (25). The pENV9 polypeptide is a nonglycosylated recombinant fragment of the C-terminal 20 amino acids of gp120 and the amino-terminal half of gp41 produced in *Escherichia coli* (18). Three human monoclonal antibodies (huMAbs) against HIV-1 that enhanced HIV-1 infection in vitro via a complement-mediated mechanism were shown to bind to the pENV9 fragment of HIV-1 (20). Two of those huMAbs have been shown to compete for a similar epitope on HIV-1. Those two huMAbs were evaluated further in this study by mapping their binding specificities to a 35-amino-acid immunodominant region of gp41 and demonstrating that this peptide blocked C'-ADE mediated by the two huMAbs and by human polyclonal anti-HIV-1 serum.

**MATERIALS AND METHODS**

**Cells and virus.** A clone of the human T-lymphotropic virus type 1-transformed T-lymphoblastoid cell line MT-2, expressing high levels of both CD4 and complement receptor type 2 (21), was generously donated by D. D. Richman (San Diego, Calif.). HIV cells chronically infected with the HTLV-III B isolate of HIV-1 (HIV3B) were obtained from R. C. Gallo's laboratory (National Institutes of Health, Bethesda, Md.). All cell lines were maintained in RPMI-1640 supplemented with 12% fetal calf serum and containing 50 μg of gentamicin per ml (growth medium). Virus was obtained from culture supernatants clarified of cells by low-speed centrifugation followed by filtration (0.45-μm pore size).

**Peptides.** Peptides 140, 110, and 120 were purchased from IAF Biochem (Natick, Mass.). Peptides were confirmed to be 99% pure by reverse-phase high-pressure liquid chromatography and amino acid analysis. The irrelevant HIV-1 gp41 peptide p-peptide was a gift from T. Palker (Duke University, Durham, N.C.).

**HuMAbs.** The huMAbs were obtained by fusion of lymphocytes from an HIV-1 antibody-positive individual with P3X63 AgU1 cells and have been described previously (30).
The HIV antibody-positive immune globulin (HIVIG) was a generous gift of A. Prince (New York, N.Y.).

**Peptide ELISA.** With three commercially available peptides derived from the envelope glycoproteins of HIV3p (IAF Biochem, Natick, Mass.), enzyme-linked immunosorbent assay (ELISA) plates were made by coating Immulon plates with 2 μg of peptide in 0.1 M bicarbonate buffer (pH 9.6) per well. After overnight incubation at 4°C, nonspecific, reactive binding sites were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 2 h at 37°C. The BSA was removed, and the wells were washed three times with PBS containing 1% BSA (PBS-BSA). HuMAbs were added at a concentration of 300 or 30 ng/ml in PBS-BSA. Human sera were added to wells at 1:100 and 1:1,000 dilutions in PBS-BSA and incubated for 2 h at 37°C. Each well was washed three times with PBS containing 0.1% Tween 20 (PBS-Tween), and then horseradish peroxidase-conjugated goat anti-human immunoglobulin G (IgG; Cappe1) diluted 1:200 in PBS-Tween was added to each well and incubated for 1 h at 37°C. Wells were washed three times with PBS-Tween, and then substrate, 0-phenylaminediamine, was added to each well. Reactions were stopped 10 min later by addition of an equal volume of 0.5 M H2SO4 to each well. The A492 was quantitated with a Flow Titter Tek II.

**Assay for blockade of C'-ADE.** In 96-well microdilution plates, twofold dilutions of each peptide were made into growth medium supplemented with either huMAb 86 (60 μg/ml) or HIVIG (1:500 dilution) and 1:20-diluted human complement. Next, HIV-1 was added and incubated at 37°C for 1 h, then 2 × 10^5 MT-2 cells were added to each well, and cells were incubated for 2.5 days at 37°C in sealed modular incubators containing 5% CO2–95% air. Cells were harvested for cytopathic effect as described before (15), and viable cells were quantitated with Fitter neutral red dye. The A530 was measured with a Flow Titter Tek II microcolormeter. Controls were HIV-1 and complement plus MT-2 cells (100% viable) and HIV-1, antibody, complement, and MT-2 cells (0% viable).

**RT release and antigen synthesis.** First, in triplicate wells of a 24-well plate, huMAb 86 was diluted 1:50 (20 μg/ml) in growth medium containing human complement (1:20 dilution) and peptide 140 (20 μg/ml) or an irrelevant HIV-1 peptide (20 μg/ml). After 30 min at 37°C, HIV-1 was added and incubated for 1 h at 37°C. Finally, 1 ml of MT-2 cell suspension was added, and cells were incubated for 12 h at 37°C. Virus and antibody were then removed. Cells were incubated in 2 ml of fresh growth medium at 37°C for 60 h. Cultures were harvested for reverse transcriptase (RT) release by the method of Poiesz et al. (16), and HIV-1 antigen-positive cells were quantitated by immunofluorescence (IF) as described before (22). None of the peptides were growth inhibitory at 20 μg/ml.

**Complement activation assay.** HIV3p-infected H9 cells were fixed on glass slides with acetone-methanol (1:1) for 20 min at room temperature. Human monoclonal or polyclonal anti-HIV antibodies were diluted 1:100 in PBS-BSA; added to fixed cells, and incubated for 30 min at 37°C. Slides were washed three times in 0.1 M bicarbonate buffer (pH 7.6) containing 0.1% Tween 20 (wash buffer), and then human complement diluted 1:20 in gelatin-Veronal buffer was added to each slide. Slides were incubated for 30 min at 37°C and then washed three times with wash buffer. Next, fluorescein-conjugated goat anti-human complement component C3 was added, incubated for 30 min at 37°C, and washed three times in wash buffer. Next, cover slips were mounted, and IF-positive cells were enumerated.

**RESULTS AND DISCUSSION**

Enhancing huMAbs bind to epitopes on peptide 140. Two huMAbs against HIV-1, V10-9 and 86, have been shown previously to mediate C'-ADE of HIV-1 infection in vitro (20). The antigen specificities of these two huMAbs were previously shown to be against HIV-1 envelope glycoproteins (20). Peptide ELISA was performed with commercially available synthetic peptides from the HIV-1 envelope glycoprotein. The results of these ELISAs are shown in Table 1. Both V10-9 and 86 bound to peptide 140 ELISA plates giving A492 values of 0.207 and 1.027, respectively, at 30 ng/ml (Table 1). These values are similar to the A492 of four polyclonal anti-HIV sera (0.173 to 0.868). Negative results were obtained for V7-8, an anti-gag huMAb, and HIV-1 antibody-negative serum (0.016 and 0.019, respectively). None of the enhancing huMAbs bound to peptide 110, an immunodominant sequence of gp120, while V10-9 and 86 reacted weakly with peptide 120, a truncated version of peptide 140. All of the huMAbs tested reacted with peptide 140 regardless of whether the disulfide linkage between amino acids 604 and 610 was intact or reduced. The reactivity of V10-9 and 86 to peptide 140 was specific, as peptide 140 but not peptide 110 or peptide 120 blocked binding of the huMAb to peptide 140 by ELISA (data not shown). In other experiments, three other huMAbs, U7-2 (anti-gag) and N2-4 and 13 (both anti-env), were tested at 300 ng/ml (Table 1). At these higher antibody concentrations (300 ng/ml), positive OD values were greater than 0.25. Both of the nonenhancing anti-env huMAbs, 13 and N2-4, and the anti-gag antibody, U7-2, were negative for binding to peptide 140. The sequences of the three peptides and their relationship to the HIV-1 envelope glycoprotein are illustrated in Fig. 1.

**Peptide 140 blocks C'-ADE in vitro.** To determine whether peptides 110, 120, and 140 had any biological effect, enhancing assays in the presence of each of the three peptides were performed as described before (21, 24, 27) with huMAb 86 (Fig. 2A) and an HIV-1 antibody-positive gamma globulin fraction (HIVIG) (Fig. 2B). The HIVIG was tested at dilutions of 1:500 (Fig. 2B) and 1:1,000 (not shown). These concentrations are subneutralizing in the presence of complement, as demonstrated previously (27). As shown in Fig.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Conc (ng/ml)</th>
<th>Peptide 110</th>
<th>Peptide 120</th>
<th>Peptide 140</th>
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</thead>
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<tr>
<td>V10-9</td>
<td>30</td>
<td>0.014</td>
<td>0.042</td>
<td>0.207</td>
</tr>
<tr>
<td>86</td>
<td>30</td>
<td>0.011</td>
<td>0.105</td>
<td>1.027</td>
</tr>
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<td>0.029</td>
<td>0.019</td>
</tr>
<tr>
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<td>0.015</td>
<td>0.016</td>
</tr>
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<td>0.294</td>
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<td>NT</td>
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*HuMAb were tested at approximately 30 ng/ml (86, V10-9, and V7-8 [anti-gag]) or 300 ng/ml (86, 13, N2-4, and U7-2 [anti-gag]). HIV-1 antibody-positive sera 34, 64, 65, and 81 and HIV-1 antibody-negative (Neg) serum were tested at a 1:1,000 dilution in PBS containing 1% BSA. Cut-off values at 30 and 300 ng/ml were less than 0.05 and less than 0.25, respectively.|

*NA, Not applicable; human polyclonal serum.

*NT, Not tested.
FIG. 1. Synthetic peptides derived from HIV-1 envelope glycoproteins. The HIV-1 envelope glycoprotein from HIV-1 is illustrated, including the gp120-gp41 cleavage site. Peptide 110, amino acids 495 to 517; peptide 120, amino acids 604 to 620; peptide 140, amino acids 586 to 620; and irrelevant p-peptide, amino acids 651 to 670. Amino acid sequences and numbers are those of Ratner et al. (19).

2A, only peptide 140 blocked C'-ADE by huMAb 86. Complete blockade of C'-ADE is indicated by 100% cell viability. Therefore, peptide 140 blocked C'-ADE at concentrations greater than 1 μg/ml, while neither peptide 110 nor peptide 120 had any effect. Peptide 140 also blocked C'-ADE mediated by polyclonal HIV-1 antibody-positive serum. In this experiment, 100% viability indicates complete (100%) protection from C'-ADE. No protection from C'-ADE is indicated by 0% viability. As shown in Fig. 2B, peptide 140 gave >50% protection at concentrations greater than 1 μg/ml, while neither peptide 120 nor peptide 110 gave greater than 30% protection from C'-ADE. Therefore, one domain to which antibodies that cause C'-ADE of HIV-1 infection bind is encoded by the peptide 140. This peptide corresponds to amino acids 586 to 620 of HIV-3B (19). This peptide has been demonstrated to be an immunodominant portion of gp41 (3, 5, 11, 23, 29) and lies within the pENV9 polypeptide (18). Because this peptide significantly blocked C'-ADE mediated by HIVIG, it seems likely that this peptide binds a significant portion of the enhancing antibodies present in HIV-1 antibody-positive serum. Furthermore, it is likely that only a small number of enhancing domains exist, since one peptide significantly reduced C'-ADE of HIV-1 infection. Efforts to map the actual epitopes to which V10-9 and 86 bind are under way.

Blockade of C'-ADE confirmed by RT release and antigen synthesis. The ability of peptide 140 to block antibody-dependent enhancement of HIV-1 infection mediated by huMAb 86 was confirmed by RT release and IF-positive cells (Table 2). Cells challenged with HIV-1 in the absence of enhancing antibody were only 25% positive for HIV-1 antigens, and no RT activity could be detected 72 h after virus challenge. Cells challenged with HIV-1 and enhancing huMAb 86 were 100% positive for antigen, with RT activity of almost 300,000 cpm of 3H-dTTP incorporated per milliliter of culture supernatant. Peptide 140 completely blocked enhancement mediated by huMAb 86, while an irrelevant peptide had no activity. Neither peptide inhibited antigen synthesis or RT release in the absence of C'-ADE.

Complement activation by huMAb is not sufficient for C'-ADE. Because enhancement of HIV-1 infection mediated by huMAb was dependent on complement, it was necessary to rule out different abilities of the huMAbs to activate complement rather than different epitopes to which the huMAbs bind as the cause of enhancement. As indicated in

FIG. 2. Peptide 140 inhibits C'-ADE of HIV-1 infection mediated by HIVIG or huMAb 86. In 96-well plates, twofold dilutions of peptide 140 (circles), peptide 110 (squares), and peptide 120 (triangles) were made in growth medium supplemented with either 20 μg of huMAb 86 (A) or a 1:500 dilution of HIVIG (B) per ml and a 1:20 dilution of human complement. An equal volume (50 μl) of HIVIG/ was added to each well. After 1 h at 37°C, 100 μl of MT-2 cell suspension (5 × 10⁶ cells per ml) was added. Cytopathic effect of HIVIG was measured 2.5 days later. Cells plus HIVIG were 100% viable; cells plus antibody plus HIVIG were 0% viable; therefore, percent protection refers to the ability of the peptide to block C'-ADE relative to infection of cells by HIV-1 in the absence of C'-ADE. Error bars are ±1 standard deviation.
Table 3, two huMAbs to HIV-1 (V7-8 and N2-4) could activate complement but did not bind to peptide 140 and could not enhance HIV-1 infection in vitro. The enhancing huMAbs V10-9 and 86 as well as polyclonal anti-HIV HIVIG and antibody 202 bound to peptide 140, activated complement, and enhanced HIV-1 infection in vitro. Therefore, C’-ADE of HIV-1 infection was epitope specific. There appeared to be no correlation between IgG subclass or Western immunoblot specificity and C’-ADE. Some anti-env antibodies, like N2-4, did not react with peptide 140 and did not enhance HIV-1 infection. Also, some IgG1s did not enhance HIV-1 infection. Because peptide 140 blocked C’-ADE mediated by both huMAb and polyclonal serum, it is possible that peptide 140 interacted with the complement receptor type 2 and not the antibody. However, C’-ADE mediated by another huMAb recognizing a separate domain on the gp41 was not blocked by peptide 140 (unpublished data), demonstrating that such a block was mediated by a specific antibody-peptide interaction rather than a peptide-HIV or peptide-complement receptor type 2 interaction.

**Peptide 140 domain and vaccine development.** Two huMAbs which enhanced HIV-1 infection in vitro were mapped by ELISA to the peptide 140 domain of the HIV-1 gp41. Although the specificities of the huMAbs are illustrated at only one concentration (Table 1), the huMAbs were tested at multiple concentrations from 3 μg/ml to 1 ng/ml. Only huMAbs 86 and V10-9 bound to peptide 140 and only at concentrations greater than 3 ng/ml. These two huMAbs did not react with other peptides. In addition, these two huMAbs were mapped to a larger envelope fragment containing the same polypeptide (amino acids 560 to 640) by another laboratory with recombinant polypeptides from the gp160 envelope protein (data not shown). Previously, these two huMAbs were shown to bind to the pEnv9 polypeptide, which contains peptide 140 (20). It had been demonstrated earlier that these two enhancing huMAbs, 86 and V10-9, reacted specifically with HIV-1 by IF, Western immunoblot, and whole-cell ELISA and that these antibodies recognized gp41 by Western blot. These results, combined with the ability of peptide 140 but not peptide 120 or 110 to block binding of huMAb 86 to ELISA plates, demonstrate that the amino acid sequence encoded by the peptide 140 domain is the domain to which these two enhancing huMAbs bind.

Furthermore, the ability of peptide 140 but not peptide 110 or 120 to block C’-ADE of HIV-1 infection in vitro suggests that this specific interaction is required for C’-ADE to occur. Although the in vivo biological effect of C’-ADE in HIV-1 immunopathogenesis has yet to be determined, the presence of antibodies to HIV-1 which enhance HIV-1 infection in vitro is disturbing and suggests that current vaccine development strategies should proceed with caution. Current vaccine attempts have focused in this regard on envelope (2, 9, 17), and antibodies mediating C’-ADE of HIV-1 infection have been found in serum from HIV-1-infected chimpanzees (27). Such laboratory findings at least support the hypothesis that FcR-ADE and C’-ADE are important in HIV-1 pathogenesis. A recent report by Homsy et al. (6) suggests that, as with dengue virus (12), FcR-ADE may play a role in disease progression. Such results for C’-ADE of HIV-1 infection are lacking, but a recent report by Montefiori et al. suggests a possible role for C’-ADE in the progression of simian immunodeficiency virus infection (14).

The results reported here demonstrate that one domain of the gp41 molecule, amino acids 586 to 620, contains an epitope(s) that induces the production in vivo of antibodies that enhance HIV-1 infection in vitro. Since such a region is immunodominant (3, 5, 11, 28, 29) and has no neutralizing activity, it may be of benefit to delete such a region from candidate anti-HIV-1 vaccines. The identification of huMAbs that enhance HIV-1 infection in vitro and domains of the HIV-1 gp41 that induce the production of such enhancing antibodies offers a major first step in the search for in vivo relevance of C’-ADE of HIV-1 infection and could improve the ability to formulate a successful anti-HIV vaccine.

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**LITERATURE CITED**


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