Enhancement of Human Immunodeficiency Virus Type 1 Infection by Antisera to Peptides from the Envelope Glycoproteins gp120/gp41

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Summary

Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins (gp120 and gp41) elicit virus-neutralizing antibodies (VNAB) and also antibodies enhancing HIV-1 infection (EAB). Several epitopes eliciting VNAB have been defined, the principal virus-neutralizing determinant being assigned to the V3 loop of gp120. To provide a background for a rational design of anti-HIV vaccines, it also appears important to define domains eliciting EAB. This was accomplished by screening antisera against synthetic peptides covering almost the entire sequence of gp120/gp41 for their enhancing effects on HIV-1 infection of MT-2 cells, a continuous T cell line. Many (16/30) of the antisera significantly enhanced HIV-1 in the presence of human complement. Antibodies to complement receptor type 2 (CR2) abrogated the antibody-mediated enhancement of HIV-1 infection. Antisera to V3 hypervariable loops of 21 distinct HIV-1 isolates were also tested for their enhancing effects on HIV-1_{IIIB} infection. 11 of these sera contained VNAB and 10 enhanced HIV-1_{IIIB} infection. All antisera with virus-enhancing activity contained antibodies crossreactive with the V3 loop of HIV-1_{IIIB}, and the virus-enhancing activity increased with increasing serological crossreactivity. These results suggest that immunization with antigens encompassing V3 loops may elicit EAB rather than protective antibodies if epitopes on the immunogen and the predominant HIV-1 isolate infecting a population are insufficiently matched, i.e., crossreactive serologically but not at the level of virus neutralization.

It was reported that antibodies mediating enhancement of HIV-1 infection (enhancing antibodies [EAB][1]) could be detected in sera of most HIV-infected individuals (1, 2) and that the occurrence of EAB correlated with severity of disease (3, 4). EAB were detected not only in human sera (1–5), but also in sera of HIV-infected chimpanzees (6, 7), of simian immunodeficiency virus (SIV)-infected rhesus macaques (8), and of guinea pigs immunized with HIV (7). Recently, it was found that some human mAbs also contained EAB (9, 10). However, the epitope specificity of EAB has not been systematically investigated.

The HIV-1 envelope glycoprotein gp160 (gp120 and gp41) contains a number of immunodominant epitopes eliciting both antibody- and cell-mediated immune responses that may contribute to protection against HIV-1 infection and/or to delay of disease symptoms after infection (11–22). Therefore, gp160 has been selected as the first candidate HIV-1 subunit vaccine for phase I trials in healthy volunteers (23) and in patients with early HIV-1 infection (24). The conclusion was reached that gp160 was safe and immunogenic, eliciting both humoral and cellular immunity to gp120/gp41. However, it was also noticed that some sera from healthy subjects immunized with gp160 contained antibodies not neutralizing but rather enhancing HIV-1 infection (23), suggesting that some epitopes on gp160 may elicit virus-neutralizing antibodies while others may elicit EAB. Epitopes on gp160 eliciting virus-neutralizing antibodies have been extensively studied (11–19), but there are only a few reports concerning epitopes that may elicit EAB (9, 10). In the present study rabbit antisera directed against: (*a*) 30 synthetic peptides from the sequence of HIV-1_{IIIB} gp160 and (*b*) V3 hypervariable loops of 21 distinct HIV-1 isolates were tested for their enhancing effects on HIV-1 infection.

Materials and Methods

Cells and Virus. T-lymphoblastoid MT-2 and H9 cells were grown in RPMI 1640 (Gibco Laboratories, Grand Island, NY) with 10% heat-inactivated FCS (Gibco Laboratories) and 100 U of penicillin and 100 μg/ml streptomycin (Gibco Laboratories). HIV-1_{IIIB} was grown in H9 and MT-2 cells. Virus was obtained from pro-

[1] *Abbreviations used in this paper:* EAB, enhancing antibodies; HS, human sera; SIV, simian immunodeficiency virus; TCID, 50% tissue culture infective dose.

ducer cell culture supernatants by low-speed centrifugation followed by filtration through a 0.45-μm filter (Nalgene Co., Rochester, NY). The infectivity of HIV-1_{IIIB} was titrated using MT-2 cells under conditions described by Johnson and Byington (25). The titers of virus stocks were 10^4 to 10^6 50% tissue culture infective doses ($TCID_{50}$).

Antisera, Normal Sera, and mAb. Rabbit antisera to synthetic peptides were prepared as previously described (17). Briefly, 30 peptides covering almost the entire sequence of HIV-1_{IIIB} gp120 and gp41, and 21 peptides corresponding to full-length V3 hypervariable loops of distinct HIV-1 isolates, were synthesized and purified (17, 18). Two NZW rabbits were immunized with 200 μg of the respective peptides in combination with CFA. Before immunization, the V3 loop peptides were oxidized by exposure to air in PBS overnight to allow the formation of disulfide bonds between the NH_2- and COOH-terminal cysteines. The rabbits were boosted with 200-μg doses of peptides in combination with IFA in biweekly intervals. 2 wk after each immunization, blood samples were taken and analyzed for antibodies by RIA. 10 wk after the initial immunization, the rabbits were killed after collecting blood by cardiac puncture. Before use, all the antisera were inactivated at 56°C for 30 min to destroy complement. Residual cytotoxicity of some antisera after the heat treatment was eliminated by adsorbing them on MT-2 cells (10^7 cells for 1 ml serum) at 4°C for 1 h. Human sera (HS) were collected from healthy individuals, pooled, and kept at −70°C until use. The complement activities in this batch of HS have been determined previously (26, 27). Anticomplement receptor type 2 (CR2, CD21) mAb (OKB7) was purchased from Ortho Diagnostic Systems Inc. (Raritan, NJ).

Double-antibody Radioimmunoassays. The level of antipeptide antibodies in rabbit antisera was determined by double-antibody RIA as described previously (17, 18). In brief, wells of 96-well polystyrene plates (Immulon II; Dynatech Laboratories, Inc., Chantilly, VA) were coated with the respective synthetic peptides (200 μl; 20 μg/ml in 0.1 M Tris, pH 8.8) overnight at 20°C, and postcoated with BSA and gelatin (10 and 2.5 mg/ml, respectively). Serially diluted antipeptide antisera were added to the wells. After overnight incubation at 25°C, the quantity of attached rabbit IgG was determined from subsequent attachment of ^{125}I-labeled goat anti-rabbit IgG. Dilution endpoints were calculated as described by Ritchie et al. (28).

Titration of HIV-1 Infectivity in MT-2 Cells. HIV-1 infectivity was determined by two distinct methods, an ELISA measuring HIV-1 core protein P24 production and a colorimetric assay for HIV-mediated cytopathogenesis, as described previously (29). Briefly, 10^4 MT-2 cells in 96-well plates were infected with dilutions of HIV-1_{IIIB} in 200 μl RPMI 1640 with 10% FCS. After 1 and 24 h, half of the culture medium was changed. On the fourth day after incubation at 37°C, 100 μl of culture supernatant was collected from each well and an equal volume of fresh medium was added to the wells. The collected supernatants were mixed with equal volumes of 5% Triton X-100 and assayed for P24 using a kit from Coulter Immunology (Hialeah, FL). On the sixth day after infection, an indicator XTT tetrazolium dye (1 mg/ml; 50 μl/well; Poly-Sciencies, Inc., Warrington, PA) was added to the cells. After 4 h, intracellular formazan was determined colorimetrically at 450 nm. The percentage of cytotopathogenesis was calculated using the following formula: 100× [(OD_{450} in negative control − OD_{450} in experiment)/(OD_{450} in negative control − OD_{450} in positive control)]. The negative control corresponded to cells mixed with culture medium, instead of HIV, while the positive control represented cells mixed with 100 $TCID_{50}$ HIV-1_{IIIB}, which lysed 100% of the MT-2 cells.

Determination of Antibody-mediated Enhancement of HIV-1 Infection. Antisera were serially diluted in RPMI 1640 containing 10% FCS. Aliquots of the diluted antisera were added to wells of 96-well plates and mixed with an equal volume of HS (final dilution 1:50) and 25 μl of HIV-1_{IIIB} (45 $TCID_{50}$). After incubation at 37°C for 1 h, 10^4 MT-2 cells were added to this mixture. The infectivity of HIV-1 was subsequently determined as described above. The percentages of enhancement of P24 production and of cytopathogenesis were calculated using the following formula: 100× [(E − C)/C]. *E* represents the amount of P24 (ng/ml) and the percent of cytopathogenesis, respectively, in the presence of antisera, and *C* represents those in the absence of antisera.

Blocking Complement Receptor Type 2 (CR2) with mAb. MT-2 cells (4×10^5) were pretreated with 25 μg of OKB7 mAb at 37°C for 1 h and washed twice in RPMI 1640. The cells were resuspended in culture medium and placed in 96-well plates. HIV-1_{IIIB} (45 $TCID_{50}$), 1:20 diluted rabbit antisera directed against the V3 loop of HIV-1-NY-5 (which was shown to contain EAB in preliminary experiments), and HS (1:50 diluted in RPMI 1640) were added to MT-2 cells. The cells were subsequently infected with HIV. Untreated infected cells were used as controls. Virus enhancement was calculated as described above.

Results

Enhancement of HIV-1_{IIIB} Infection by Rabbit Antisera Directed Against Synthetic Peptides from HIV-1_{IIIB} gp120/gp41. Rabbit antisera directed against 30 synthetic peptides from gp120 and gp41 were screened for their enhancing activity on HIV-1 infection of MT-2 cells. Without adding HS, none of the antisera significantly enhanced either P24 production or cytopathogenesis mediated by HIV-1 infection (data not shown). However, after adding HS, 16 of the 30 antisera significantly enhanced (>100%) P24 production (Fig. 1 *A*) and 15 antisera markedly (>60%) enhanced HIV-mediated cytopathogenesis (1 *B*). Although the enhancement of P24 production by these antisera is higher than that of cytopathogenesis, both are closely correlated ($r = 0.869$). Epitopes eliciting the highest levels of EAB appeared clustered mainly in regions located NH_2 terminally from the V3 loop of gp120 and on segments of gp41. The only antiserum significantly inhibiting virus replication was that directed against the V3 loop peptide.

Enhancement of HIV-1 infection mediated by the rabbit antisera is dose dependent (Fig. 2), decreasing gradually with increased antiserum dilution from 1:10 to $1:10^6$.

Enhancement of HIV-1_{IIIB} Infection by Rabbit Antisera to Synthetic Peptides from V3 Hypervariable Loops of 21 Distinct HIV-1 Isolates. 21 rabbit antisera directed against V3 hypervariable loops of gp120 from distinct HIV-1 isolates were also tested for their enhancing effects on HIV-1_{IIIB} infection. The results are shown in Fig. 3. In the presence of HS, 11 antisera neutralized the infectivity of HIV-1_{IIIB} at least partially, while 10 antisera enhanced P24 production. Similar results were obtained by measuring HIV-mediated cytopathogenesis (data not shown). Antisera to peptides most closely related immunologically to the BH10 V3 peptide (18) neutralized the infectivity of HIV-1_{IIIB}. Interestingly, the enhancement of HIV-1 infection mediated by the 10 non-neutralizing antisera was correlated with their less-close crossreactivity with the

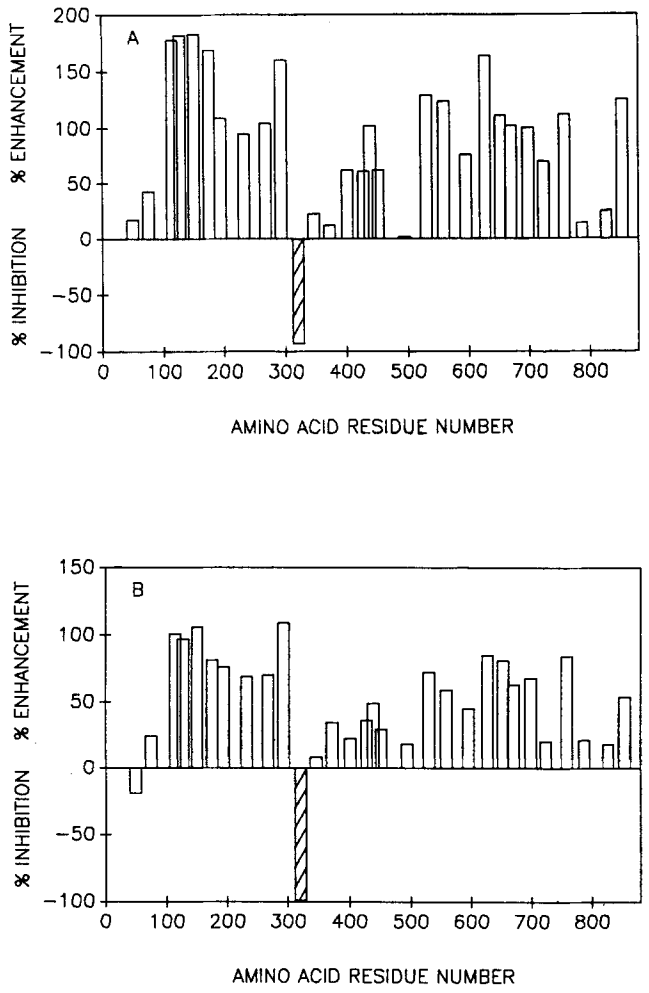

Figure 1. Enhancement of HIV-1_{IIIB} infection by rabbit antibodies directed against synthetic peptides from gp120/gp41 of HIV-1_{IIIB}. Enhancement (or inhibition) was measured by production of P24 gag antigen (*A*) or by HIV-mediated cytopathogenesis (*B*) (see Materials and Methods). Positive results represent enhancement, while negative results indicate inhibition (i.e., virus neutralization). (▨) Peptide corresponding to the V3 loop; (□) all other peptides.

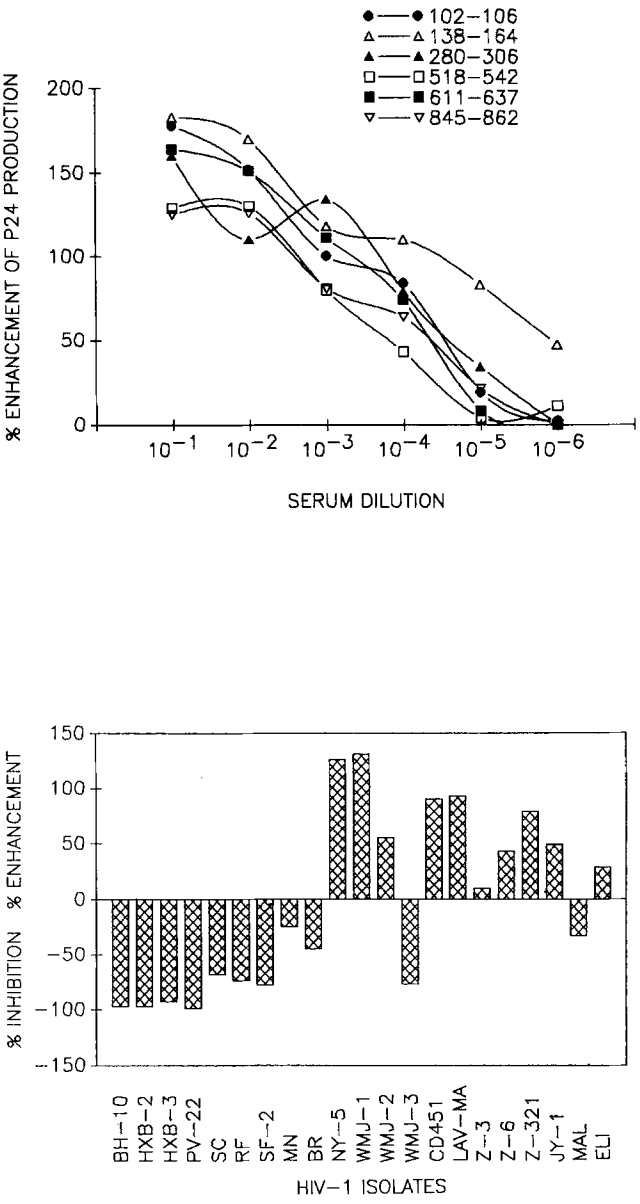

Figure 3. Effect of rabbit antisera directed against V3 hypervariable loop peptides from 21 distinct HIV-1 isolates on replication of HIV-1_{IIIB}. Rabbit antisera directed against synthetic peptides from HIV-1 isolates, indicated on the abscissa, were tested for enhancement or inhibition of HIV-1_{IIIB} infection of MT-2 cells in the presence of HS. Experimental conditions and calculations are the same as for Fig. 1.

V3 loop peptide of HIV-1_{IIIB} as detected by RIA (18) (Fig. 4). These results suggest that subtype-specific antibodies directed against V3 loops, if not sufficiently matched with the HIV-1 strains used for infection of cells, may not neutralize but rather enhance HIV-1 infection and that higher titers of non-neutralizing antibodies may result in higher enhancing activity.

Heat Treatment of HS Decreases Enhancement of EAB-mediated HIV-1 Infection. It was reported that human complement was involved in human, chimpanzee, and rhesus macaque EAB-mediated enhancement of HIV and SIV infection, respectively, via CR2 (2, 4, 6, 8, 30, 31), and that human complement could directly enhance HIV-1 infection via the alternative pathway (32, 33). In this study, it was found that rabbit antisera enhanced HIV-1 infection of MT-2 cells only in the presence of HS. To establish whether or not human complement is the essential factor in normal HS required by rabbit EAB for enhancing HIV-1 infection, HS was incubated at 56°C for 30 min and used in virus-enhancing assays. Without adding EAB (rabbit antiserum against the V3 loop peptide from HIV-1 isolate NY-5, a preselected HIV-1-enhancing antiserum), HS itself could significantly enhance P24 production (>100%) (Fig. 5). However, when heated HS was added to the virus, no significant enhancement could be observed, suggesting that human complement itself is able to enhance HIV-1 infection, probably via the alternative pathway, as sug-

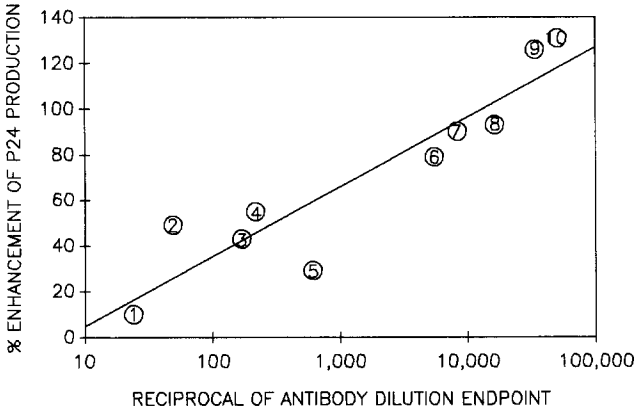

Figure 4. Correlation between enhancement of HIV-1_{IIIB} replication by rabbit antisera directed against V3 peptides from distinct HIV-1 isolates and the reactivities of these antisera with the BH10 V3 peptide. Ordinate, percentage of enhancement of P24 production mediated by rabbit antisera directed against synthetic peptides from V3 loops of HIV-1 isolates: *1*, Z-3; *2*, ELI; *3*, Z-6; *4*, JY-1; *5*, WMJ-2; *6*, Z-321; 7, CD451; *8*, LAV-MA; *9*, NY-5; *10*, WMJ-1. Abscissa, reciprocal dilution endpoints of the antisera as measured by RIA using wells coated with the BH10 V3 peptide (results from reference 18).

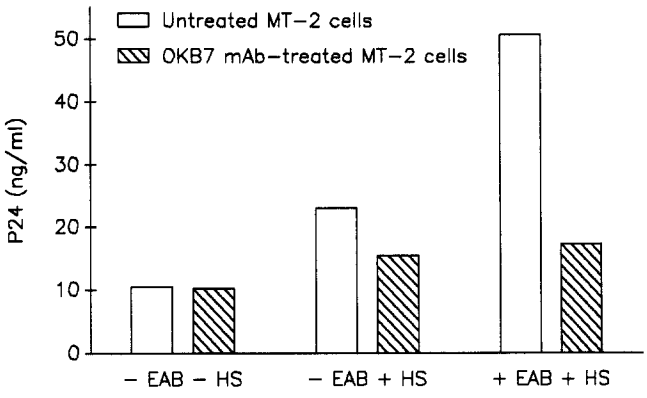

Figure 6. Anti-CR2 mAb blocking of complement-dependent, rabbit EAB-mediated enhancement of HIV-1 infection of MT-2 cells. MT-2 cells were treated with OKB7 (anti-CR2 mAb) or untreated before they were mixed with HS, rabbit EAB, and HIV-1_{IIIB} for virus-enhancing assays.

gested by Boyer et al. (32). When EAB and HS were added to the virus at the same time, P24 production was further increased (~400%). This increase was not observed when heated HS, instead of untreated HS, was added. These results suggest that rabbit EAB-mediated HIV-1 infection of MT-2 cells is dependent on the presence of human complement.

Complement-dependent Rabbit EAB-mediated Enhancement of HIV-1 Infection of MT-2 Cells Is Blocked by Anti-CR2 mAb It was reported that CR2 played an important role in complement-dependent human EAB-mediated enhancement of HIV-1 infection (2, 4, 30–32, 34). MT-2 cells express a high level of CR2 (2), which were effectively blocked by an anti-CR2 mouse mAb OKB7 (34). To determine whether or not CR2 plays a role in rabbit EAB-mediated, human complement–dependent enhancement of HIV-1 infection of MT-2 cells, the effect of mouse anti-CR2 mAb, OKB7, on enhancement was studied. As shown in Fig. 6, P24 production by HIV-1-infected OKB7-treated and untreated MT-2 cells did not differ significantly when human complement and rabbit EAB were not added. When human complement, but no rabbit EAB, was added to HIV-1, P24 production in OKB7-treated MT-2 cells was lower than that in untreated MT-2 cells, confirming that human complement–mediated enhancement of HIV-1 infection occurred, and was partially blocked by anti-CR2 antibody. With untreated MT-2 cells and rabbit EAB, human complement significantly increased P24 production, as already shown in Fig. 5. However, when MT-2 cells were pretreated with OBK7 mAb, the enhancement of P24 production by human complement and rabbit EAB was abolished. These results suggest that human CR2 indeed plays an important role in rabbit EAB-mediated, human complement–dependent enhancement of HIV-1 infection.

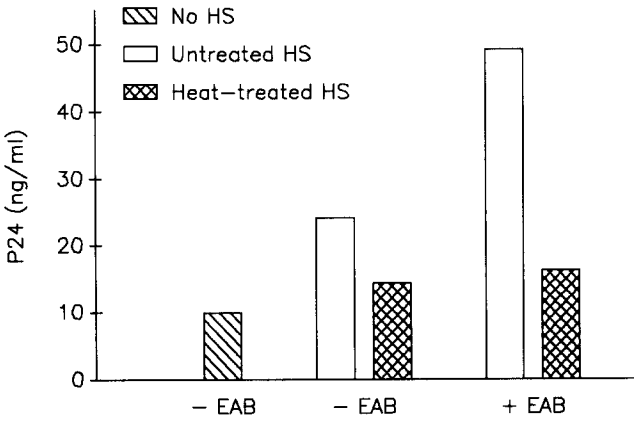

Figure 5. Comparative effects of untreated and heat-treated HS on rabbit EAB-mediated enhancement of HIV-1 infection of MT-2 cells.

Discussion

The following major conclusions emanate from the results presented here: (*a*) antisera to several peptides from HIV-1 gp120/gp41 enhance HIV-1 infection, suggesting the presence of a mosaic of contiguous epitopes on the virion surface and that attachment of antibodies to many, if not all, of these epitopes facilitates HIV-infection; (*b*) EAB-mediated enhancement of HIV-1 infection of MT-2 cells is dependent on human complement and CR2; (*c*) V3 hypervariable loops from one HIV-1 isolate may elicit antibodies enhancing infection by another HIV-1 isolate.

It has been well established that sera from HIV-1-infected humans (1–5) and chimpanzees (6, 7), from SIV-infected rhesus macaques (8), and from HIV-immunized guinea pigs (7) contain antibodies that could enhance HIV-1 or SIV infections. For the design of immunogens for vaccination against HIV-1, it seems important to establish the epitope specificity of EAB. The availability in our laboratory of a multitude of antisera to peptides from gp120/gp41 (17, 18) offered an opportunity to accomplish this by screening these sera for EAB. It was found that many epitopes on gp120/gp41 can elicit EAB.

Robinson et al. (9) reported that two human mAbs, which bound to an immunodominant region in gp41 (amino acid residues 586–620), enhanced HIV-1 infection. Enhancement mediated by these two mAbs and by human polyclonal antisera to HIV-1 could be blocked by the synthetic peptide (586–620). More recently, Robinson et al. (10) reported another four human mAbs with HIV-1-enhancing activity, three of which recognized the same peptide and one of which recognized a peptide (651–670; numbering system as in references 9 and 17) from gp41. They concluded that these two domains on gp41 could stimulate the in vivo formation of EAB and that there are only a few epitopes on HIV-1 gp120/gp41 that could stimulate the production of EAB. Our results confirmed that these two regions in gp41 elicit EAB (Fig. 1). However, our results indicate that many other regions in gp120/gp41 are involved in apparent virus infectivity enhancement after binding antibodies. Regions of gp120/gp41 involved in binding of antipeptide EAB appear to be exposed on the surface of virions (our unpublished data), as one would expect. Therefore, it seems possible that any antibodies binding complement and attaching to the surface of HIV-1 particles will function as EAB, unless they are virus neutralizing. This conclusion is supported by the enhancing activity of some antibodies directed against HIV-1 gp120 V3 loop (Fig. 3, see below). The discrepancy between these conclusions and the findings of Robinson et al. (9, 10) is apparent rather than factual for the following reasons: the region (579–611) of gp41 is immunodominant and antibodies against this domain are the most prevalent in individuals infected with HIV-1 (24, 35–37). Therefore, B lymphocyte clones that produce mAb against the aforementioned domain and having enhancing activity on HIV-infection would be preferentially selected from peripheral B cells of HIV-1-infected individuals. It is also not surprising that most of EAB were removed from human anti-HIV-1 sera by adsorption on a synthetic peptide from this immunodominant region (9, 10).

The V3 hypervariable loop of gp120 encompasses the principal neutralizing determinant (18, 38–40) and is involved in the process of virion–cell membrane fusion (41, 42). Because of the biologically important role of the V3 loop in the life cycle of HIV-1, the peptide from this region may be considered as a candidate component for a vaccine against HIV-1 infection. Indeed, antibodies to the V3 loop rendered $HIV\text{-}1_{IIIB}$ virions noninfectious for chimpanzees (43). Recently, it was demonstrated that high levels of virus-neutralizing antibodies were elicited by boosting chimpanzees with synthetic peptides from the V3 loop after priming with gp160 and that the antibodies were protective against HIV-1 challenge (44, 45). Neutralizing antibodies elicited by the V3 loop are HIV-1 subtype (isolate) specific (39, 40), suggesting the need to incorporate a multitude of V3 loop peptides into a vaccine expected to be broadly effective (18). The serological crossreactivity, as measured by RIA, between V3 loops of distinct HIV-1 isolates is related to the extent of their amino acid sequence homology (18). However, the impact of serological crossreactivity on biological properties of anti-V3 loop antisera, i.e., virus neutralization or enhancement, has not been systematically investigated before. Results presented here show that V3 hypervariable loops from one HIV-1 isolate may elicit antibodies enhancing infection by another HIV-1 isolate and that the enhancement mediated by the antibodies, if they had no neutralizing effect on the HIV-1 isolate tested, correlated with serological crossreactivity. Therefore, immunization with HIV-1 antigens encompassing the V3 loop or with synthetic peptides from the V3 loop may be potentially harmful rather than protective if epitopes on the immunogen and the predominant HIV-1 isolate infecting a population are insufficiently matched, i.e., crossreactive serologically but not at the level of virus neutralization.

The generation in vivo of HIV-1 escape mutants that cannot be neutralized by anti-V3 loop-specific antibodies generated in the course of infection has been well documented (46, 47). Considering our results, it seems possible that these antibodies may have an enhancing effect on the replication of the emerging variant(s) and may thus contribute to persistence and pathogenesis of the infection.

It has been established that human complement and CR2 play important roles in human EAB-mediated enhancement of HIV-1 infection of $CD4^+$ T lymphocytes (2, 8, 30, 31). Our results demonstrated that rabbit EAB also require human complement to enhance HIV-1 infection. CR2 is also critical in the rabbit EAB-mediated, complement-dependent enhancement of HIV-1 infection, since this type of enhancement of HIV-1 infection could be blocked by anti-CR2 antibody. Most antibodies directed against synthetic peptides from the sequence of HIV-1 gp120/gp41 bind onto the surface of HIV-1 virions (our unpublished data). If the respective epitopes are located on functional regions critical for virus replication and transmission, the bound antibodies may neutralize HIV-1. If not, the virion-antibody complexes may activate complement and via CR2 enter the target cells, resulting in enhancement of HIV-1 infection. Thus, any antibodies specific for epitopes on gp120/gp41, if not protective, may have virus-enhancing effects and be potentially harmful. How to minimize the generation of potentially harmful antibodies and to maximize the production of protective antibodies is a critical question in designing vaccines against HIV-1.

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