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A Novel, Glycan-Dependent Epitope in the V2 Domain of Human Immunodeficiency Virus Type 1 gp120 Is Recognized by a Highly Potent, Neutralizing Chimpanzee Monoclonal Antibody

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An anti-gp120 monoclonal antibody (MAb), C108G (γ1, κ), was isolated from a chimpanzee that had been infected with strain IIIB of human immunodeficiency virus type 1 ($HIV\text{-}1_{IIIB}$) and subsequently immunized with the recombinant glycoprotein $rgp160_{MN}$. This MAb is specific for the IIIB strain of HIV-1 and related clones and exhibits very potent neutralization of these viruses; e.g., 100% neutralization of approximately 8×10^3 infectious units of HXB2 was achieved with 125 ng of C108G per ml. Commensurate with this potent neutralizing activity, the apparent affinity of C108G for $rgp160_{LAI}$ was very high, i.e., approximately 3×10^{10} liters/mol. The C108G epitope was not destroyed by reduction of gp120 disulfide bonds but was profoundly disrupted by removal of N-linked sugars from gp120. Despite the importance of a glycan(s) in forming the C108G epitope, specific binding of C108G to synthetic peptides overlapping in amino acids 162 to 169 of the V2 region was detected, albeit with an affinity approximately 2,000-fold lower than that of C108G's binding to glycosylated envelope protein. This epitope mapping correlated with results of competition assays using MAbs of known epitope specificities. To our knowledge, this is the first description of an anti-V2 MAb raised in response to HIV-1 infection. Its potent neutralizing activity and epitope specificity indicate that the V2 domain of gp120 may be an effective target of the protective immune response and, therefore, potentially an important component of HIV vaccines.

Two major neutralization epitope clusters in gp120 have been identified as targets in human immunodeficiency virus type 1 (HIV-1)-infected individuals: the V3 loop (reviewed in references 27 and 44) and the CD4-binding site (44). Antibodies against the V3 loop inhibit HIV-induced syncytium formation and infectivity, and mutations within this region inhibit the fusogenicity of HIV-1 Env (7, 12, 16, 17, 31, 45) and affect tropism of the virus (3, 16, 37, 49, 50). It has been suggested that at least some antibodies against epitopes overlapping the CD4-binding site may act as competitive inhibitors to block viral attachment to the CD4 receptor (29); however, the neutralization potency of such monoclonal antibodies (MAbs) does not correlate with their ability to be blocked by soluble CD4 (sCD4) in binding to gp120 or to block gp120-CD4 binding (1a, 25), and their mechanism of action has yet to be elucidated.

The V2 region is clearly important in HIV-1 gp120 function, in that the amino acid sequence in this region influences the replication competence of the virus (38) as well as its tropism (21, 50). In addition, single amino acid changes in the V2 domain decrease the association between gp120 and gp41 (38), and the configuration of a hypervariable locus in the V2 domain has recently been correlated with non-syncytium-inducing (SI)-to-SI phenotype conversion (13). Recently, a series of rodent MAbs against the V2 region of HIV-1 gp120 (9, 15, 26, 28, 38) or simian immunodeficiency virus gp130 (2, 19) have identified this region as a neutralization epitope cluster in both viruses. However, it has not been shown that humans or chimpanzees infected with HIV-1 have a significant level of neutralizing antibodies against V2 epitopes. Whereas some fairly potent neutralizing rat MAbs against V2 have been described (26), most of the rodent anti-V2 MAbs characterized to date have weak neutralizing activity, and consequently it has been suggested that the V2 loop represents a weak neutralization epitope cluster (28).

In this report, we present results demonstrating the ability of the V2 region to elicit potent neutralizing antibodies during HIV-1 infection. These results were obtained by isolation and characterization of a potent neutralizing anti-V2 MAb, C108G, from an HIV-1-infected chimpanzee. This chimpanzee, C-087, was experimentally infected with the IIIB strain of HIV-1 as a control animal in vaccine studies (10). Beginning approximately 10 months postinfection, C-087 received three immunizations at monthly intervals with recombinant $gp160_{MN}$ that was prepared by cassette insertion of $gp120_{MN}$ sequences into recombinant vaccinia virus VV-1163 (20). The purified $rgp160_{MN}$ (150 μg per dose) was mixed with Syntex adjuvant formulation (SAF-1) and injected intramuscularly. Approximately 1 month after the third $rgp160_{MN}$ immunization, blood (~50 ml) was taken from C-087 for peripheral blood mononuclear cell isolation and Epstein-Barr virus transformation essentially as described previously (41); from this experiment, MAb C108G was derived.

(A preliminary report on this MAb has been presented [43]).

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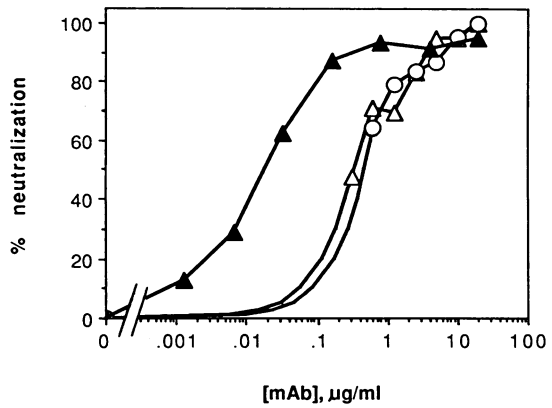

FIG. 1. Neutralization of the IIIB strain of HIV-1 by MAbs 0.5β (△), 1125H (○), and C108G (▲). Approximately 2×10^4 infectious units of virus was used in a 24-h fluorescent focus assay (36, 41, 42, 44).

The C108G (γ1, κ) clone has been in continuous culture for over 2 years and is stable with respect to MAb production. Supernatants of dense cultures (0.5×10^6 to 1×10^6 cells per ml) of C108G cells typically contain approximately 0.5 μg of MAb per ml, which is characteristic of human and chimpanzee Epstein-Barr virus-transformed cell lines (4). The specificity of C108G for an epitope of gp120 lying outside the V3 loop was indicated by its strong reactivity with $rgp160_{LAI}$ and $rgp120_{PV22}$, both of which are produced in hamster cell lines, but not with $V3_{BH10}$ or $V3_{MN}$ peptides in an enzyme-linked immunosorbent assay (ELISA) (data not shown). Furthermore, C108G did not react in ELISA or immunoblot with $rgp160_{MN}$ that is expressed similarly to $rgp160_{LAI}$ (data not shown), indicating that C108G was not likely to be directed to a conserved epitope such as those overlapping the CD4-binding site; most, if not all, MAbs directed against such epitopes recognize envelopes of both the MN and IIIB strains (reviewed in reference 44).

The apparent affinity of C108G for $rgp160_{LAI}$ was determined as described previously (41) and found to be approximately 3×10^{10} liters/mol, which is at least an order of magnitude greater than the apparent affinities previously reported for other neutralizing MAbs against the V2 (26, 28), V3 (11), or CD4-binding (reviewed in reference 44) domain. Furthermore, C108G efficiently neutralized the IIIB strain of HIV-1 at very low concentrations. Figure 1 shows that C108G neutralized 50% of IIIB virus at approximately 15 ng/ml and over 90% of the virus at approximately 300 ng/ml. Higher concentrations of C108G did not neutralize 100% of the IIIB virions, presumably because of the presence of viral clones within this strain that lack the C108G epitope. On the basis of the 50% neutralization titers in this and similar experiments, it is concluded that C108G is approximately 30-fold more potent than the 1125H anti-CD4-binding site human MAb (41) or the 0.5β anti-V3 loop mouse MAb (24) in neutralizing the IIIB strain (Fig. 1). Neutralization of the HXB2 clone of the IIIB strain by C108G was even more efficient: 50% neutralization was achieved with approximately 3 ng of C108G per ml, and 100% neutralization was seen with 125 ng/ml (data not shown). No neutralization of the MN strain was observed with C108G at concentrations of ≤20 μg/ml (data not shown). The latter result correlated with the fact that C108G was unreactive with $rgp160_{MN}$ in ELISA and immunoblot, as discussed above.

Competition assays were performed essentially as described

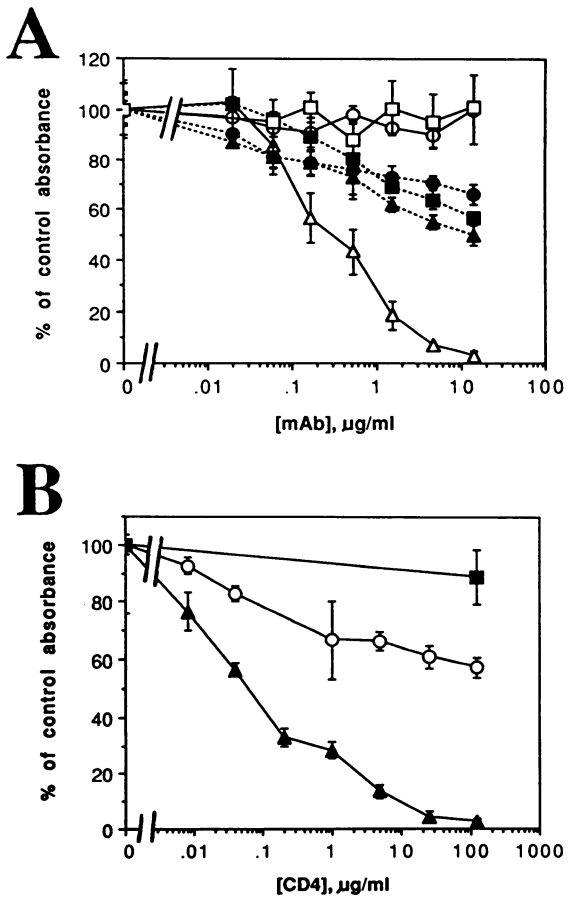

FIG. 2. Results of competition ELISAs assessing the effects of mAbs (A) and sCD4 (B) on binding of C108G to $rgp160_{LAI}$. Error bars represent the standard deviation from the mean of triplicate measurements. (A) Biotinylated C108G was used at a constant concentration of 166 ng/ml. Symbols: △, C108G; ●, G3-136; ○, 1125H; ▲, G3-4; ■, BAT085; □, 0.5β. (B) The MAbs were used at a constant concentration of approximately 100 ng/ml. Symbols: ■, 0.5β; ○, C108G; ▲, 1125H.

previously (32, 41) to determine whether C108G was directed toward an epitope overlapping that of previously mapped neutralization epitopes in gp120. Figure 2A shows that while an anti-CD4-binding site human MAb (1125H) and an anti-V3 loop mouse MAb (0.5β) were unable to inhibit binding of C108G to $rgp160_{LAI}$, three mouse MAbs (G3-4, G3-136, and BAT085) whose epitopes have been mapped to the V2 region of gp120 (9, 15, 28, 38) were able to inhibit binding of C108G to its epitope. This inhibition was partial (~40%) at inhibitor MAb concentrations (~15 μg/ml) equivalent to the concentration of unlabeled C108G required to achieve 100% inhibition of biotinylated C108G binding. Reciprocal competition assays in which each of the MAbs shown in Fig. 2A was biotinylated and tested for competition with unlabeled C108G for binding to $rgp160_{LAI}$ were also performed; the extent of inhibition of each MAb's binding by C108G was similar to that MAb's inhibition of C108G binding at the same unlabeled MAb concentrations (data not shown). These results suggested that C108G binds an epitope in or near the V2 region of gp120 that is distinct from the V3 loop and CD4-binding sites.

Although an anti-CD4-binding site human MAb (1125H)

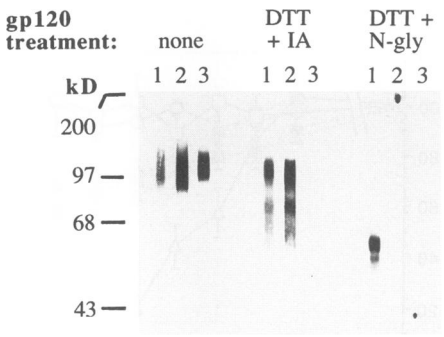

FIG. 3. Immunoblot analysis of $rgp120_{PV22}$ (expressed in CHO cells) after various treatments. Approximately 2 μg of rgp120 was reduced by boiling in 32 μl of 9 mM Tris HCl (pH 7.4)–0.9% sodium dodecyl sulfate (SDS)–0.9% dithiothreitol (DTT). Following the addition of sample buffer (23), boiling, and cooling to room temperature, iodoacetamide (IA) was added to the sample to a final concentration of 100 mM and the mixture was incubated at room temperature for 15 min. The sample was then neutralized with approximately 1/10 volume of 1 M Tris HCl, pH 8.0, prior to loading on the gel. In parallel, rgp120 (~2 μg) was deglycosylated by boiling in 32 μl of 9 mM Tris HCl (pH 7.4)–0.9% Nonidet P-40–0.2% SDS–1.2% DTT and then digested with 8 U of *N*-glycosidase F (N-gly) (Boehringer Mannheim) per ml for 2 h at 37°C. The sample was boiled in sample buffer containing 1% SDS and 1% DTT prior to loading on the gel. An SDS–10% polyacrylamide gel was run and immunoblot strips were prepared essentially as described previously (32). Approximately 0.4 μg of rgp120 was present in each track of the blot. The blot was reacted with either rat antiserum against $rgp160_{LAI}$ from baby hamster kidney cells (lanes 1), purified C108G (20 μg/ml) (lanes 2), or purified 1125H (20 μg/ml) (lanes 3). The molecular weight markers were prestained standards from BRL.

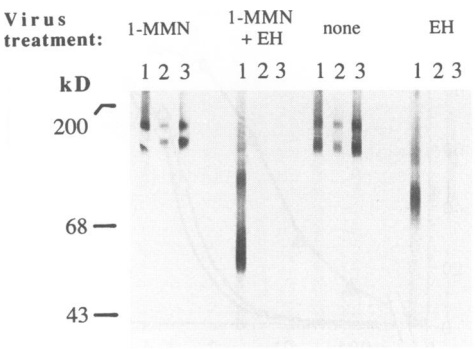

FIG. 4. Immunoblot analyses of lysate from purified HIV-1 after various treatments. Lysate from the IIIB viral strain was prepared from virus-infected cells that had been grown in either the presence or the absence of MMN. A portion of the lysates was then treated with EH, while another portion was not. These procedures were carried out essentially as described previously (41), and the samples were run on an SDS–8% polyacrylamide gel. Blots prepared as described previously (33) from lysates so treated were reacted with rat antiserum against $gp120_{PV22}$ (expressed in CHO cells) (1/100 dilution) (lanes 1), supernatant from C108G cells (~1 μg of MAb per ml) (lanes 2), or purified C108G (15 μg/ml) (lanes 3). The molecular weight markers were prestained standards from BRL.

did not inhibit binding of C108G to its epitope (Fig. 2A), sCD4 did inhibit this interaction. Figure 2B shows that at sCD4 concentrations (~125 μg/ml) required to completely inhibit binding of 1125H to its epitope, binding of C108G to its epitope was partially inhibited (~40%). This phenomenon was also observed for rodent MAbs that bind conformational epitopes overlapping the V2 region (9, 26), where it was interpreted to indicate that binding of sCD4 to gp120 or gp160 results in a conformational change that decreases the accessibility and/or affinity of certain epitopes in or near V2 (26).

The C108G epitope was further characterized by determining the reactivity of C108G with reduced or reduced and deglycosylated gp120. Figure 3 shows that binding of C108G to rgp120 was retained after reduction of disulfide bonds and alkylation. The successful reduction of gp120 disulfide bonds in this experiment was documented by loss of 1125H reactivity, since the epitope of 1125H is dependent on intact disulfide bonds (41). In contrast, removal of N-linked glycans from gp120 with *N*-glycosidase F results in loss of detectable binding of C108G to gp120. Similar results were seen in this experiment with either $rgp160_{LAI}$ expressed in BHK cells or $rgp160_{BH10}$ expressed in insect cells (data not shown).

The carbohydrate dependence of the C108G epitope could indicate either that the epitope contains both peptide and carbohydrate constituents or that it is entirely peptidic but requires one or more glycans for presentation in the appropriate conformation(s) for C108G recognition. C108G specifically reacts with viral gp120 and gp160 but not with viral gp41 (Fig. 4) or cellular glycoproteins in a virus-infected cell lysate (data not shown) or viral lysate (Fig. 4). The strain specificity and exclusivity for gp120 of C108G indicate that the epitope cannot be composed solely of carbohydrate, and thus, it is clearly distinct from those of mouse MAbs targeted to mucin-type carbohydrate epitopes in gp120 described by Hansen et al. (14).

The carbohydrate moieties important for C108G's recognition of its epitope were characterized further. Endoglycosidase H (EH) cleaves high-mannose or hybrid-type glycans but not complex glycans, and EH digestion leaves a single *N*-acetyl-glucosamine at the glycan attachment sites on glycoproteins (39). EH digestion of viral lysate derived from infection of H9 cells eliminated C108G's recognition of its epitope in gp120 and gp160 (Fig. 4). This indicates that (i) in virus grown in H9 cells under the conditions of this experiment, the glycan(s) important for C108G's recognition was in high-mannose and/or hybrid-type form rather than complex form and (ii) a single *N*-acetyl-glucosamine moiety remaining at the glycan attachment sites in gp120 and gp160 following EH digestion was insufficient to maintain the C108G epitope. The ability of high-mannose glycans to maintain the C108G epitope was confirmed by treatment of virus-infected cells with 1-deoxymannojirimycin (MMN) (8); this substance inhibits complex carbohydrate formation, leaving all glycans in the high-mannose form. This treatment had no detectable effect on the binding of C108G to its epitope in gp120 or gp160 (Fig. 4), indicating that presence of a high-mannose glycan(s) is sufficient for C108G to recognize its epitope. Consistent with the results of EH digestion of viral lysate from untreated cells discussed above, cleavage of the MMN-treated viral glycoproteins with EH resulted in loss of C108G recognition of gp160 and gp120 (Fig. 4).

The nature of the glycans important for C108G recognition in recombinant gp120 and gp160 were compared with those of viral gp120 and gp160 by analyzing the ability of C108G to bind to three different untreated and EH-treated recombinant envelope glycoproteins in immunoblots (data not shown). The results showed that C108G recognizes $rgp160_{BH10}$ expressed in insect cells (Repligen), although human MAb 1125H does not recognize this glycoprotein. The 1125H epitope is dependent on disulfide bonds and is discontinuous, involving amino acids from as many as five different constant regions of gp120 (40,

41); therefore, the lack of 1125H recognition of the rgp160 expressed in insect cells is likely to be due to aberrant folding of this recombinant molecule. The fact that C108G recognized this $rgp160_{BH10}$ suggests that it is not as sensitive to the overall conformation of gp120 as 1125H. Insect cell-expressed glycoproteins contain predominately high-mannose and/or hybrid-type glycans and few, if any, complex glycans (22). The sufficiency of these simpler glycans for maintenance of the C108G epitope is consistent with results obtained with viral gp120 and gp160, shown in Fig. 4. Treatment of the rgp160 from insect cells with as little as 0.09 U of EH per ml abolished C108G's reactivity with its epitope. Similar results were seen with $rgp120_{PV22}$ expressed in Chinese hamster ovary cells (Genentech) (data not shown), except that >10-fold-higher concentrations of EH were required to eliminate C108G's reactivity with this rgp120. This may have been due to partial denaturation of the insect protein or could reflect a steric effect of neighboring complex glycans on the high-mannose glycan(s) involved in the C108G epitope in the CHO-expressed protein.

C108G also recognized $rgp160_{LAI}$ expressed in baby hamster kidney cells (Pasteur Merieux) in immunoblots (data not shown), as previously observed in ELISA. Interestingly, treatment of this glycoprotein with excess EH did not result in loss of C108G reactivity. Apparently, the $rgp160_{LAI}$ prepared in BHK cells, though having roughly the same number of high-mannose and/or hybrid-type glycans as $rgp120_{PV22}$ (as determined from relative loss of molecular weight upon limit digestion with EH [data not shown]), has primarily complex glycans attached at the glycan attachment site(s) important for C108G recognition of its epitope. Thus, either a complex glycan or high-mannose glycan can fulfill the carbohydrate requirement of the C108G epitope. These glycans share a core composed of two *N*-acetylglucosamine residues and three mannose residues, but they differ in the terminal sugars attached to this core (22).

Despite the dependence of C108G on a glycan(s) in gp120 for optimal recognition of its epitope, we reasoned that, because of the very high apparent affinity of C108G for native gp120 and gp160 ($\sim 3 \times 10^{10}$ liters/mol), we might be able to detect binding with a significantly lower affinity to an appropriate synthetic peptide if it was provided in sufficient concentration. We therefore tested the reactivity of C108G with a series of 20-mer synthetic peptides with 14-amino-acid overlaps from the V1/V2 region of the HXB2 clone by ELISA. Figure 5A shows that C108G reacted specifically with three overlapping peptides (peptides 6 through 8) in this analysis; its reactivity with the other nine V1/V2 peptides tested was not significantly above background reactivity with bovine serum albumin (BSA) (data for peptides 1 through 4 and 10 through 12 not shown). Analysis of the half-maximal binding of C108G to peptide 7 revealed that its apparent affinity for this peptide is approximately 2×10^7 liters/mol (Fig. 5C), or approximately 2,000-fold lower than for glycosylated envelope. Figure 5B shows that peptides 6 through 8 overlap in the sequence STSIRGKV corresponding to amino acids 162 through 169 (Los Alamos numbering) at the N terminus of the V2 region. This sequence apparently represents the minimal peptide portion of the C108G epitope.

Since the epitope mapping results indicated that amino acids 162 through 169 of gp120 are the major determinant of HIV-1 strain specificity of C108G, the envelope sequences in this region of different HIV-1 strains should correlate with C108G's recognition of those strains. The latter was determined by ELISA, immunoblot analysis, immunofluorescence assay (41), and/or neutralization assay. Strains recognized by C108G, i.e., HXB2, LAI, PV22, and BH10 (data not shown),

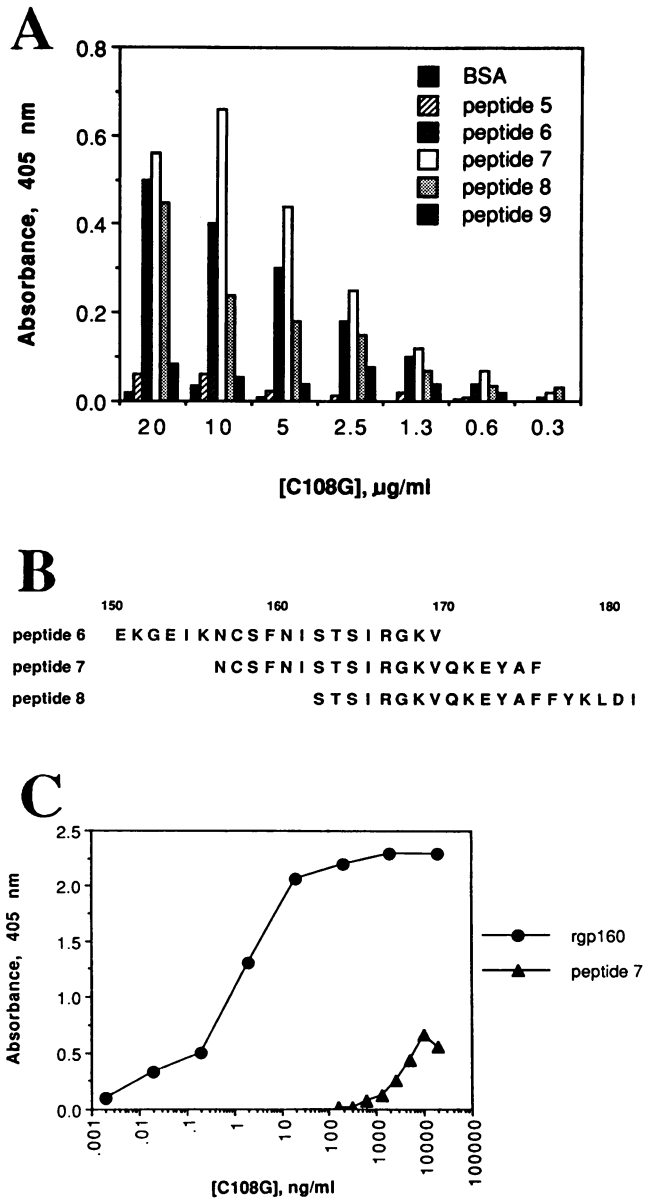

FIG. 5. Epitope mapping of C108G on synthetic peptides. Peptides were used to coat PVC ELISA plates at 200 ng per well in PBS, and various concentrations of C108G were incubated with each peptide. Diluted sera from seropositive individuals were also separately incubated with the peptides on plates and served as positive controls to ensure that each peptide had attached to the plate. A standard ELISA protocol was used to detect bound chimpanzee and human immunoglobulin G antibodies (41). (A) Data are averages of duplicate measurements for C108G binding to selected peptides versus BSA (negative control). (B) Sequences of three overlapping peptides (peptides 6 through 8) specifically recognized by C108G as shown in panel A. (C) Comparison of C108G reactivity with $rgp160_{LAI}$ expressed in BHK cells to that with synthetic peptide 7 over a broad range of C108G concentrations. Both of the antigens were used at 200 ng per well, and each point represents the average of duplicate measurements. From the concentration of C108G at which half-maximal binding occurred in this experiment, apparent affinities (*K*) of 2×10^7 liters/mol for peptide 7 and 5.5×10^{10} liters/mole for $rgp160_{LAI}$ were calculated as described previously (47). The average apparent affinity (*K*) of C108G for $rgp160_{LAI}$ determined from results of several such experiments was found to be 3×10^{10} liters/mol.

have identical sequences in amino acids 150 through 182, corresponding to the region covered by peptides 6 through 8 in Fig. 5. Interestingly, C108G does not recognize the IIIB-related clone NL4-3 (data not shown); this clone has a single amino acid substitution (G changed to D) at position 167 that lies within the minimal epitope defined by peptide reactivity. The MN, SF-2, and RF strains are not recognized by C108G (data not shown), and each has the G-to-D substitution at position 167; however, these strains have additional differences from HXB2 within the minimal C108G epitope and elsewhere in V1/V2 that may contribute to their lack of expression of the epitope. In order to see whether the single (G-to-D) substitution at position 167 was sufficient to abrogate C108G's recognition of the peptidic portion of its epitope, a synthetic peptide identical to peptide 7 seen in Fig. 5B except that it had the substitution mentioned above, called peptide 7 (G/D), was tested in ELISA. Despite specific binding to this peptide by selected seropositive sera from humans, C108G had no reactivity above the background level with peptide 7 (G/D), while it bound peptide 7 efficiently in the same experiment (data not shown). This provides further confirmation that residue 167 is an important part of the C108G epitope.

This paper describes the first isolation and characterization of a chimpanzee MAb against HIV and of an anti-V2 MAb that arose in response to infection by HIV-1. C108G was clearly elicited by virus infection since it is IIIB strain specific and therefore could not have been raised de novo following immunizations of chimpanzee C-087 with $rgp160_{MN}$. However, it is not possible to rule out that the IIIB-specific antibody response represented by C108G was boosted by the $rgp160_{MN}$ immunizations; such "original antigenic sin" responses have been observed for the V3 loop of HIV-1 (30) as well as for other viral, bacterial, and nonviral antigens (reviewed in reference 30).

The epitope of C108G appears to be unique compared with those of the rodent anti-V2 MAbs previously described (9, 15, 26, 28, 38). The rodent MAbs recognize either linear epitopes in V2 that are independent of disulfide bonds or glycosylation, or they recognize conformational epitopes. Binding of the latter MAbs was sensitive to mutation of amino acids in the C-terminal half of the V2 domain, i.e., residues 183 and 184 and residues 191 through 193, but generally not to mutation in residues 166 (K to L) and 168 (R to L) that are present in the C108G epitope (26, 28). It was shown that binding of two of these rodent MAbs recognizing conformational epitopes in V2 was partially diminished by removal of peripheral monosaccharides present on complex glycans, i.e., sialic acid and galactose residues (26). This effect appears distinct from the marked dependence of the C108G epitope on N-linked glycans, and this conclusion is confirmed by recent studies demonstrating that these two rodent MAbs, but not C108G, retain reactivity with a correctly folded fusion protein that contains a deglycosylated V1/V2 domain (51).

The epitope mapping data for C108G indicated that the minimal peptide portion of its epitope corresponds to amino acids 162 through 169 on the N-terminal side of the V2 domain of gp120. McKeating et al. (26) have described rat MAbs against linear V2 epitopes mapping to this region (amino acids 162 through 171) that neutralize the HXB10 strain of HIV-1. C108G differs from these antibodies both in its carbohydrate dependence and in its high apparent affinity ($\sim 3 \times 10^{10}$ liters/mol) and remarkable potency in neutralization of IIIB and HXB2. The concentration of C108G required for 50% neutralization of IIIB in our fluorescent focus assay was approximately 15 ng/ml (Fig. 1); under the same assay conditions, the rat anti-V2 MAbs described by McKeating et al. (26) required at least 800 ng/ml, and G3-4, a previously characterized mouse MAb against a conformational V2 epitope (15), required more than 80 μg/ml to achieve this level of IIIB neutralization (our unpublished data). We have also observed that C108G binds efficiently to the surfaces of IIIB-infected cells by flow cytometry (1), indicating that its epitope is exposed on native, oligomeric Env proteins. Taken together, these results indicate that the N-terminal side of the V2 domain is a highly exposed region in native virions that contains strong neutralization epitopes.

The C108G epitope may be either a glycopeptide or an entirely peptidic epitope whose conformation is best bound by C108G when a glycan(s) is attached nearby. We believe that most, if not all, of the glycan(s) required for C108G's recognition of its epitope lies in or near the V2 domain, since a fusion glycoprotein comprising the glycosylated V2 region and limited flanking regions of HIV-1 gp120 engrafted onto gp70 from murine leukemia virus essentially as described before (18) is efficiently immunoprecipitated by C108G (34, 51). The glycan requirement of the C108G epitope can be satisfied by either a high-mannose or a complex glycan(s) but not by a single *N*-acetylglucosamine residue. Thus, there is flexibility in the peripheral carbohydrate residues tolerated in glycans forming the C108G epitope. Further studies are required to determine the minimal glycan requirement for this epitope.

In conclusion, C108G recognizes a unique, glycan-dependent epitope in the V2 region of HIV-1 gp120 that is exposed on the surface of infectious viral particles and that plays an important role in the infectivity process. Native V2 epitopes that are more highly conserved across HIV-1 isolates and/or are present in a greater percentage of primary isolates than that of C108G could be important components of an HIV vaccine. Type-specific antibodies reactive with V2 peptides have recently been detected in human sera (18, 26, 28), and broadly reactive antibodies against glycan- and/or conformation-dependent epitopes in the V1/V2 domain have also been detected (18). Although the neutralizing activities of these antibodies have not been determined, their presence in human sera supports the feasibility of seeking anti-V2 responses in vaccine recipients. Furthermore, we have observed that C108G synergistically neutralizes the IIIB strain of HIV-1 when combined with either anti-CD4-binding-site or anti-V3-loop MAbs (48). This indicates that certain anti-V2 MAbs may enhance the neutralization potency and/or breadth of strain specificity of neutralizing antibodies against the other two major neutralization epitope clusters previously characterized.

Finally, the neutralization potency and unique epitope of C108G demonstrate the utility of the chimpanzee system for isolation and characterization of novel and potentially therapeutic MAbs against higher-primate viruses such as HIV. Chimpanzees are experimental animals that may be infected and/or immunized to obtain optimal neutralizing antibody titers and/or other immune responses, and therefore they represent a valuable source of MAbs that can identify epitopes likely to be functionally important in humans. Furthermore, chimpanzee immunoglobulins are ~98% homologous to those of humans (5, 35, 46), so that MAbs from these animals may be useful for passive immunotherapy (5, 6).

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