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Immunology

The principal neutralization determinant of simian immunodeficiency virus differs from that of human immunodeficiency virus type 1

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ABSTRACT **To identify the principal neutralization determinant (PND) of simian immunodeficiency virus (SIV), antisera were generated using recombinant gp110 [the SIV analog of the human immunodeficiency virus type 1 (HIV-1) external envelope glycoprotein, gp120], gp140, several large recombinant and proteolytic envelope fragments, and synthetic peptides of the SIV_{mac251} isolate. When purified under conditions that retain its native structure, gp110 bound CD4 and elicited antisera that neutralized SIV_{mac251} with high titer. Native gp110 also completely inhibited neutralizing antibody in sera from SIV_{mac251}-infected macaques. In contrast, denatured gp110 and gp140, large envelope fragments, and synthetic peptides (including peptides analogous to the HIV-1 PND) elicited very low or undetectable neutralizing antibody titers and did not inhibit neutralizing antibody in infected macaque sera. Enzymatically deglycosylated gp110 efficiently absorbed neutralizing antibodies from macaque sera, showing that neutralizing antibodies primarily bind the protein backbone. A 45-kDa protease digest product, mapping to the carboxyl-terminal third of gp110, also completely absorbed neutralizing antibodies from infected macaque sera. These results show that the PND(s) of this SIV isolate depends on the native conformation and that linear peptides corresponding to the V3 loop of SIV envelope, in contrast to that of HIV-1, do not elicit neutralizing antibody. This may affect the usefulness of SIV_{mac} for evaluating HIV-1 envelope vaccine approaches that rely on eliciting neutralizing antibody.**

Simian immunodeficiency virus (SIV) causes an AIDS-like disease in several monkey species (for reviews see refs. 1–3), and this virus is being used as a model for human immunodeficiency virus type 1 (HIV-1) pathogenesis, therapy, and vaccine development. Immunization with inactivated SIV confers protection from virus challenge (4–7). This has given encouragement that a vaccine can be developed to protect humans from HIV-1 infection.

Much of the research regarding HIV-1 vaccine development has focused on the external envelope glycoprotein gp120. The principal neutralization determinant (PND) of HIV-1 is within a disulfide crossbridged loop in the third variable domain (the V3 loop) of gp120 (8–11). Immunization with gp120 (12), gp160 (13), or PND-containing peptides (8–10, 14, 15) elicit virus-neutralizing antisera comparable in titer to sera from chimpanzees experimentally infected with HIV-1. Furthermore, serum fractionation has shown that PND peptides bind essentially all of the neutralizing antibodies elicited by gp120 or gp160 (8) and that a substantial fraction of neutralizing antibodies in HIV-1-infected people bind the PND (16). The PND of HIV-1 can therefore be mimicked with synthetic peptides and is not dependent on the tertiary structure of gp120. Recent chimpanzee immunization and challenge experiments have shown a correlation between the presence of PND-binding neutralizing antibody and protection from infection (17–19), and passive immunization of a chimpanzee with a PND-binding monoclonal antibody prevented infection.**

In contrast, neutralization determinants on SIV have not been defined. Immunization with inactivated SIV confers protection against virus challenge (4–7), as does passive immunization of cynomolgus monkeys with high-titer immune sera (20). Thus, although antibody appears to be sufficient to confer protective immunity to SIV, the nature of this antibody-mediated protective immune response is unknown. Further, the role played by neutralizing antibody in protective immunity to SIV has not been elucidated.

To investigate the ability of SIV envelope subunits to elicit virus-neutralizing antibody, we produced envelope proteins and recombinant and synthetic fragments thereof from SIV_{mac251}. These were analyzed for the ability both to elicit neutralizing antibody and to absorb neutralizing antibody from sera of SIV_{mac251}-infected animals. Our results show that the PND of this SIV isolate resides in a 45-kDa fragment from the C-terminal half of the external glycoprotein and that it depends on the tertiary structure of the envelope protein and therefore cannot be mimicked by a synthetic peptide.

MATERIALS AND METHODS

Recombinant Envelope Antigens. Two molecular clones (21), each encoding a portion of the envelope protein, were joined to give the full-length envelope gene. The baculovirus vector used to express gp140 was constructed by creating a *Bam*HI site 25 nucleotides 5′ of the translation initiation codon of the SIV_{mac251} envelope gene and cloning a 2.6-kilobase (kb) *Bam*HI fragment into the vector pVL941 (22). The vector for gp110 was made by introducing two adjacent termination codons immediately 3′ to the codon for amino acid 505. Each of these plasmids was used to create recombinant baculoviruses by standard techniques (22). To purify gp140, baculovirus-infected Sf9 cells were homogenized and centrifuged, and the pellet was extracted with buffer con-

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Abbreviations: HIV, human immunodeficiency virus; PND, principal neutralization determinant; sCD4, soluble CD4; SIV, simian immunodeficiency virus.

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taining 8 M urea, 1% sodium deoxycholate, and 50 mM 2-mercaptoethanol. The extract was dialyzed against buffer with 0.25% Triton X-100 and 5 mM 2-mercaptoethanol, and gp140 was purified by lentil lectin (Pharmacia) and Sepharose CL-4B chromatography. gp110 was purified from the Sf9 cell medium by a single affinity step using purified IgG from a SIV_{mac251}-infected macaque. gp110 (0.1 mg) was deglycosylated by incubation with 1 unit of N-Glycanase (Genzyme) in phosphate-buffered saline (0.2 ml) at 37°C for 18 hr. Native gp110 (0.5 mg) was digested with *Staphylococcus aureus* V8 protease (10 μg) in 1 ml of 25 mM Tris (pH 8.3) at 37°C for 18 hr, and the 45-kDa fragment was purified on an HPLC column (Superdex; Pharmacia). Denatured gp110 was prepared by incubation of native gp110 with 8 M urea and 100 mM 2-mercaptoethanol at 37°C for 2 hr, followed by dialysis against phosphate-buffered saline.

Proteins Env7, -2, and -5 were produced in *Escherichia coli* as fusion proteins with 38, 31, and 32 non-SIV amino acids on the N terminus of Env7, -2, and -5, respectively, and 22 amino acids on the C terminus of Env7 and -2. Env7 was extracted from *E. coli* with buffer containing 50 mM 2-mercaptoethanol and purified by DEAE, gel filtration, and Q-Sepharose chromatography. Env2 was extracted from *E. coli* with the extraction buffer used for Env7 but containing 8 M urea. S-Sepharose and gel filtration chromatography were used for purification. Env5 was obtained by first washing and extracting the lysate pellet with extraction buffer containing 8 M urea and was purified directly by elution from an SDS/polyacrylamide gel slice.

Peptide Antigens and "Pepscan" Analysis. Peptides were synthesized and purified as described (23). Peptide RP297 contains cysteines at both N and C termini. Treatment of purified RP297 (0.1 mg/ml) at pH 8.0 in ammonium bicarbonate buffer under oxygen resulted in a single peak in HPLC that did not react with dinitrobenzoic acid. For pepscan analysis, native gp110-immunized guinea pig serum and SIV_{mac251}-infected macaque serum were used at a 1:100 dilution with each of the peptides. The background signal was ≈0.16, and OD values of 0.16 or less are reported as 0.16.

Antisera. Each of the peptides was conjugated to keyhole limpet hemocyanin through a C-terminal cysteine (23). Guinea pigs and goats were immunized as described (8, 15, 23), with Freund's complete adjuvant (first immunization) and incomplete adjuvant (subsequent immunizations).

Neutralization Assays. For the syncytium reduction assay (24), virus was diluted with growth medium to contain ≈100 syncytium-forming units per 30 μl. Twofold serum dilutions (heat-inactivated, 56°C for 30 min) were made in half-area wells of 96-well plates. Virus (30 μl) was added to the wells and the cultures were incubated at 37°C for 30 min. HUT78 target cells (3×10^3 in 30 μl) were then added and syncytia were enumerated 3–4 days later. The neutralization titer is expressed as the reciprocal of the serum dilution that inhibited syncytium formation by 90%. The cell viability assay was done as described (4, 25).

CD4 Binding Assay. Monoclonal antibody OKT4 or OKT4A (5 μg; Ortho Diagnostics) was added to recombinant soluble CD4 (sCD4, 1 μg; American Biotechnologies, Cambridge, MA). After incubation for 10 min, envelope protein (1 μg) was added to give a volume of 100 μl in 50 mM Tris, pH 7.5/0.15 M NaCl. To this was added 100 μl of protein A-Sepharose (Repligen, Cambridge, MA) equilibrated with 10% fetal bovine serum. After 18 hr at 4°C and three washes with the above buffer containing 0.2% Nonidet P-40 and 0.1% SDS, the protein was dissolved in SDS sample buffer. After 9% PAGE, the proteins were transferred to nitrocellulose paper and probed with a mixture of two sera elicited by a 25-amino acid peptide derived from the large disulfide loop of CD4 and by native gp110.

RESULTS

Native gp110, But Not Denatured gp110, Elicits Neutralizing Antibody. Expression vectors were constructed from a molecular clone of SIV_{mac251} (21) to produce gp140 (the entire envelope terminating within the transmembrane protein) and gp110 (the external envelope protein) from baculovirus-infected insect cells (Fig. 1, lane 2 (gp140) and lanes 3 and 7 (gp110); Fig. 2*B*). In addition, we expressed in *E. coli* three fragments (Env7, Env2, and Env5) that collectively spanned nearly all of gp140. Each of these proteins, except gp110 (denoted native gp110), was purified using denaturing conditions. In contrast, gp110 was purified using nondenaturing conditions designed to maintain the native structure and hence the ability to bind CD4. Synthetic peptides were also synthesized that corresponded to several regions of SIV_{mac251} gp140 (Fig. 2*B*). Each of the proteins and selected keyhole limpet hemocyanin-conjugated peptides were used to elicit antisera in goats or guinea pigs.

These antisera and antisera taken from two SIV_{mac251}-infected macaques were assessed by ELISA for binding to either the immunogen or gp140 (Table 1). The ability of these sera to neutralize the SIV_{mac251} isolate was assessed (*i*) by measuring the reduction in infectious virus by antiserum as assessed by the viability of virus-treated cell cultures (cell viability assay) (4, 25) and (*ii*) by measuring the antiserum-mediated reduction in the number of virus-induced syncytia (syncytium reduction assay) (24). Sera from infected macaques neutralized the virus with titers of 45,000 and 800,000 in the syncytium reduction assay, and serum from one of the animals had a titer of 40,960 in the cell viability assay, indicating that sera from different animals can vary significantly in their ability to neutralize virus. Antisera to the peptides (including peptides RP92, -93, and -297, corresponding to the HIV-1 PND), the *E. coli* fragments Env7, -2, and -5, gp140, or native gp110 treated with reducing agents and urea (denoted denatured gp110) did not neutralize the virus or did so with titers >100 times lower than SIV_{mac251}-infected macaque sera. In contrast, antisera to native gp110 neutralized the virus with titers comparable to infected macaque sera.

Native gp110 Absorbs Essentially All Neutralizing Antibody from SIV-Infected Macaque Serum. These findings were confirmed and extended by assessing the ability of these proteins or peptides to inhibit neutralizing antibody in serum from an infected macaque or from a guinea pig immunized with native gp110 (Table 2). Protein or peptides, at the indicated concentrations, were mixed with serum and the neutralization titer (syncytium reduction assay) was determined. Native gp110, at a concentration that has no effect on virus infectivity, reduced the neutralizing titer of anti-gp110 serum by over 98% and of infected macaque serum by 99.8%. This indicates that essentially all of the neutralizing antibodies elicited by either native gp110 or infectious virus bind

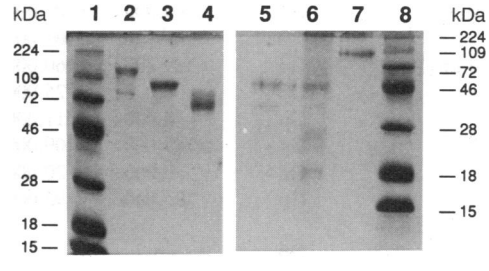

FIG. 1. Gel electrophoresis of recombinant SIV_{mac251} envelope proteins. Lane 1, size markers; lane 2, gp140; lanes 3 and 7, gp110; lane 4, deglycosylated gp110; lane 5, purified 45-kDa fragment; lane 6, V8 protease digest of gp110. Lanes 1–4, 9% denaturing/reducing gel; lanes 5–8, 12% nonreducing gel.

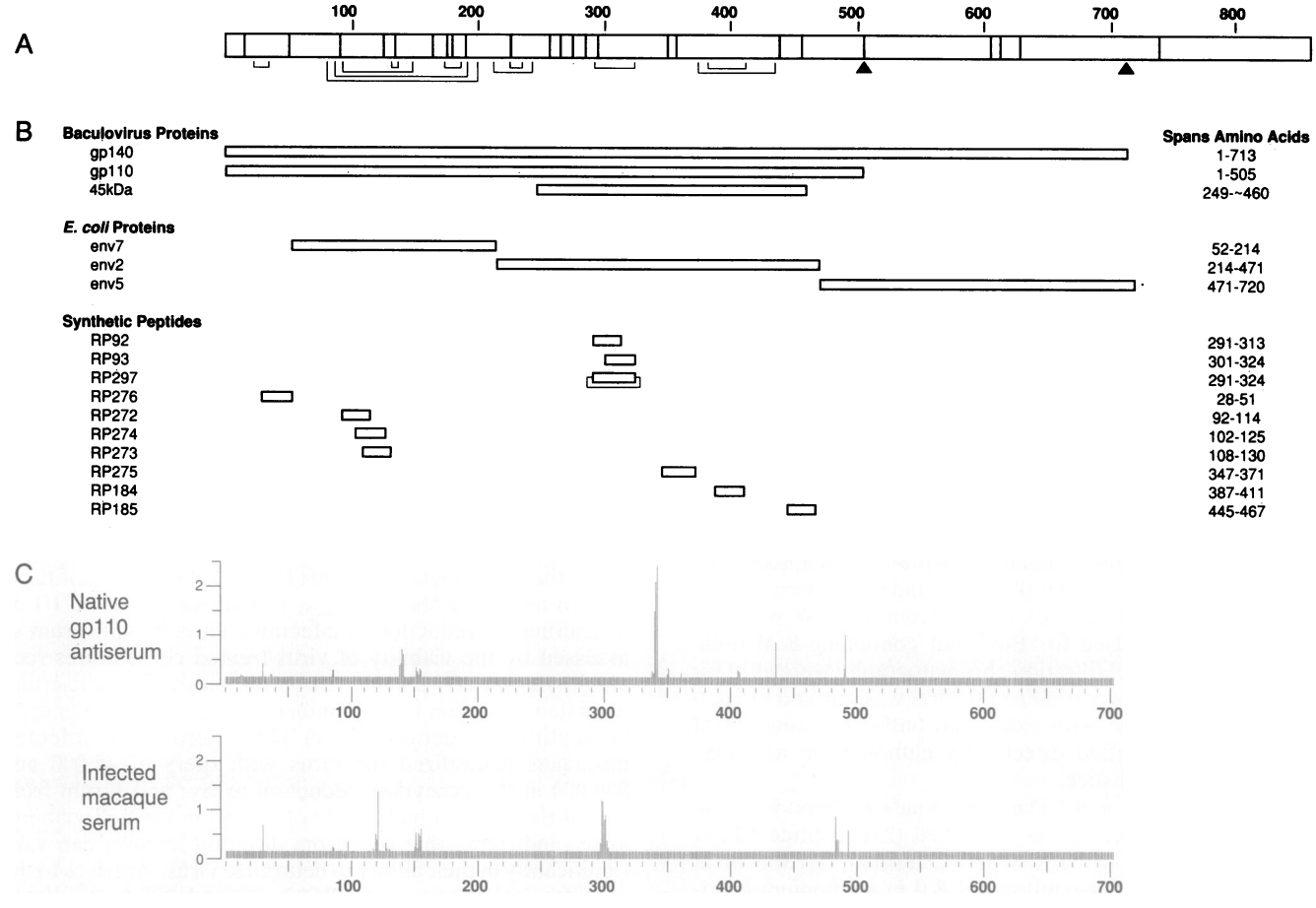

FIG. 2. (*A*) Map of SIV_{mac251} envelope protein. (*B*) Location of recombinant envelope proteins and synthetic peptides. (*C*) Pepscan analysis. Amino acid numbering (21) starts with the first amino acid of the protein after cleavage of the secretion signal. The coding sequence contains a termination codon after amino acid 713, and cleavage between amino acids 505 and 506 generates gp110. Vertical lines within the envelope map indicate potential N-linked glycosylation sites, and each of the disulfide crossbridges are shown below the map linking the corresponding cysteines as predicted for HIV-2 (26).

native gp110. Treatment of the envelope protein with 8 M urea and 100 mM 2-mercaptoethanol (denatured gp110) essentially eliminated the ability to bind neutralizing antibody. In addition, neither gp140 nor any of the recombinant fragments or synthetic peptides (including the peptides corresponding to the HIV-1 PND) significantly reduced the neutralization titer of either serum.

Pepscan Analysis. To identify the linear determinants recognized by antibodies in the native gp110 and infected macaque sera, pepscan analysis (27, 28) was performed using

Table 1. ELISA and SIV_{mac251} neutralization titers of immune sera

Immunogen	Animals	ELISA titer			Titer in syncytium reduction assay			Titer in cell viability assay		
		Serum 1	Serum 2	Serum 3	Serum 1	Serum 2	Serum 3	Serum 1	Serum 2	Serum 3
—	Macaque*	7,000	9,000		45,000	800,000		ND	40,960	
Denatured gp140	Gp	60,000	≥109,000	≥109,000	42	≤20	≤20	80	160	40
	Goat	≥109,000	≥109,000		28	120		ND	160	
Native gp110	Gp	≥109,000	≥109,000	≥109,000	12,500	15,500	37,000	ND	5,120	5,120
	Goat	≥109,000	≥109,000		46,000	80,000		ND	ND	
Denatured gp110	Gp	≥109,000	60,000	97,000	≤10	≤10	≤10	ND	ND	
Env7	Gp	30,000	25,000	60,000	≤20	≤20	≤20	≤20	≤20	≤20
Env2	Gp	70,000	11,000	35,000	≤20	≤20	≤20	≤20	≤20	≤20
Env5	Gp	≥109,000	≥109,000	≥109,000	≤20	≤20	≤20	≤20	≤20	≤20
RP92	Gp	40,000	20,000	4,500	≤20	≤20	≤20	≤20	≤20	≤20
RP93	Gp	95,000	95,000	55,000	≤20	≤20	≤20	≤20	≤20	≤20
RP297, -184, -185, -272, -273, or -274	Gp				≤20	≤20	≤20	ND	ND	ND

Selected immunogens (Fig. 1) were used to elicit antisera in either three guinea pigs (Gp) or two goats and assayed by ELISA for reactivity to the immunogen or, for infected macaque and Env7, -2, and -5 sera, to gp140. ELISAs were performed as described (23) and the neutralization assays as described in *Materials and Methods*. Three guinea pigs were immunized with each peptide conjugate. ELISA data of peptide sera not shown here were of high titers. ND, not done.
*Two SIV_{mac251}-infected macaques.

Table 2. Inhibition of neutralization of anti-native gp110 serum and infected macaque serum by native gp110 and gp110-derived proteins

Addition	Conc., μM	% inhibition	
		gp110 serum	Macaque serum
Native gp110	0.07	99.4	99.8
Denatured gp110	0.07	0	62
Denatured gp140	0.05	18	50
Deglycosylated gp110	0.07	87	98.2
V8 digest of gp110	0.07	96.4	99.5
45-kDa fragment	0.20	93.2	98.2
RP297	1.5	0	0
RP297, -272, -273, -184, -185, -274, -275, or -276	3	0	0

The syncytium reduction assay (Table 1) was performed by mixing the diluted serum with the protein or peptide at the indicated concentration in the well prior to addition of the virus.

702 nonapeptides, each staggered by one amino acid over the length of gp140. Reactivity of antibodies in the two sera with each peptide was determined (Fig. 2*C*). Both sera reacted with few determinants, all of which were within gp110, and there were only two regions (surrounding amino acids 150 and 490, which are within Env7 and Env5, respectively) that reacted with antibodies in both sera. The peptides corresponding to the HIV-1 PND reacted with the infected macaque serum but not with the gp110 antiserum. The fact that peptides corresponding to this region did not elicit or absorb neutralizing antibody from either of these sera shows that this determinant, at least as a peptide independent of other determinants on the envelope, is not a neutralization determinant. This was true even when this peptide was synthesized (RP297) (Table 2) in its entirety with an intramolecular disulfide crossbridge to attempt to mimic this domain as it exists in HIV gp120 and that is proposed to exist in SIV and HIV-2 gp120 (26).

The PND Resides in a 45-kDa Fragment. To localize the binding site of neutralizing antibodies on gp110, native gp110 was treated either with N-Glycanase, which hydrolyzes N-linked oligosaccharides, or with V8 protease. N-Glycanase reduced the apparent size of gp110 to 70 kDa (Fig. 1, lane 4). This 70-kDa protein reduced the neutralization titer of anti-gp110 serum or infected macaque serum by about 87% and 98%, respectively (Table 2). This indicates that gp110 deglycosylated in this manner is recognized by neutralizing antibodies, although less efficiently than is native gp110, but that the majority of the neutralizing antibodies bind to deglycosylated gp110. It is possible that removal of carbohydrate alters the tertiary structure of native gp110, thereby making it less efficiently recognized by neutralizing antibody.

Treatment of native gp110 with V8 protease, under conditions designed to maintain the native structure of the digestion products, resulted in fragments of 45, 25 and 20 kDa visible by Coomassie blue staining after nonreducing SDS/PAGE (Fig. 1, lane 6). The V8 digestion products reduced the neutralization titer of both the anti-gp110 and infected macaque sera comparably to native gp110. Native gel electrophoresis, followed by Western blotting and probing with either of these antisera, revealed that primarily the 45-kDa fragment was immunoreactive (data not shown), suggesting that it contains the PND(s). The 45-kDa fragment was therefore purified by gel filtration chromatography (Fig. 1, lane 5). Amino acid sequencing showed the N terminus to be at amino acid 249 (Fig. 2). Based on the apparent molecular mass, and assuming that the mass contributed by the carbohydrate is equally distributed over the length of gp110, we estimate that the C terminus of the 45 kDa fragment is near amino acid 460.

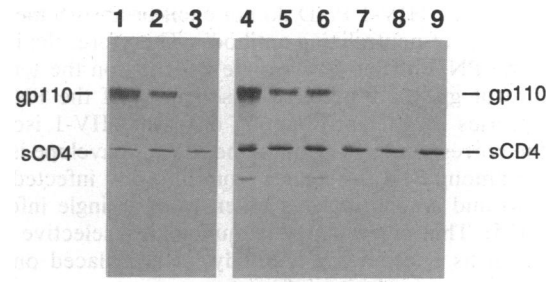

FIG. 3. Native gp110, but not denatured gp140 or gp110, deglycosylated gp110, or the 45-kDa fragment, is immunoprecipitated by OKT4 after binding to sCD4. The indicated envelope proteins were incubated with sCD4 and OKT4 (lanes 2 and 5–9) or with OKT4A (lane 3), and the antibody and sCD4 were precipitated with protein A-Sepharose. The precipitate was electrophoresed, transferred to nitrocellulose and probed with antisera to both gp110 and CD4. If envelope proteins bound sCD4 and were hence coprecipitated, they appear as a band in addition to that of sCD4. Lanes 1 and 4, gp110 and sCD4 proteins directly electrophoresed without immunoprecipitation; lanes 2 and 5, gp110 and sCD4 precipitated with OKT4; lane 3, gp110, sCD4, and OKT4A; lane 6, gp110 (treated under deglycosylation conditions without N-Glycanase), sCD4, and OKT4; lane 7, denatured gp110, sCD4, and OKT4; lane 8, 45-kDa fragment, sCD4, and OKT4; lane 9, deglycosylated gp110, sCD4, and OKT4.

The 45-kDa fragment, therefore, contains the six C-terminal cysteines in gp110 and encompasses the analogous HIV-1 PND and CD4-binding domain.

The purified 45-kDa fragment reduced the neutralization titer of anti-gp110 serum and infected macaque serum by 15- and 55-fold, respectively, indicating that the large majority of neutralizing antibodies in these sera bind to the 45-kDa fragment. Consistent with our previous results, when the 45-kDa fragment was denatured, it no longer reduced the neutralization titer of either sera. This shows that the PND is contained in this fragment and that the ability to bind neutralizing antibody depends on its tertiary structure.

Native gp110, But Not Denatured gp110, Binds CD4. To determine whether native gp110 has the tertiary structure to allow it to bind to CD4, we tested whether it could prevent virus infection in the syncytium reduction assay and whether it bound CD4 in an immunoprecipitation assay. Native gp110 prevented syncytium formation ($IC_{50} \approx 0.2$ μM; data not shown), suggesting that it can bind CD4 and prevent virus attachment. In contrast, denatured gp110, gp140, deglycosylated gp110, and the 45-kDa fragment, even at 0.5 μM, did not reduce the number of syncytium. This suggests that these proteins do not bind CD4. The CD4-binding ability of these proteins was tested directly in an immunoprecipitation assay in which the protein was incubated with human sCD4, the sCD4 was precipitated with OKT4 antibody and protein A-Sepharose, and the precipitate was analyzed by gel electrophoresis and Western blotting for both sCD4 and the envelope protein. Whereas native gp110 was precipitated by virtue of its ability to bind sCD4 (Fig. 3, lanes 2, 5, and 6), the denatured (lane 7) or deglycosylated (lane 9) gp110 and the 45-kDa fragment (lane 8) were not. gp110 was not precipitated by sCD4 and OKT4A (lane 3). These results parallel those obtained with HIV-1 gp120, which when denatured or fragmented shows a large reduction in the ability to bind CD4 (29–33).

DISCUSSION

The data show that the PND of HIV-1 differs from that of SIV_{mac251}. In contrast to HIV-1, the native form of SIV_{mac251} gp110 is necessary to elicit appreciable titers of neutralizing antibody, and denatured gp110, gp140, large envelope fragments, or synthetic peptides containing the region corre-

sponding to the HIV-1 PND do not elicit or absorb measurable amounts of neutralizing antibody. Therefore, the integrity of the PND of this SIV isolate depends on the tertiary structure of gp110. Whereas the sequence of the PND of HIV-1 varies significantly among different HIV-1 isolates (11), the corresponding region in the SIV_{mac} envelope is less variable among SIV_{mac} isolates from different infected animals (34) and among isolates taken from a single infected animal (35). This suggests that immunological selective pressure, such as neutralizing antibody, is not placed on this envelope sequence during infection.

Although we have not determined precisely the determinant(s) to which the SIV-neutralizing antibodies bind, because its integrity depends on the native tertiary structure of gp110, the determinant probably consists of amino acids adjacent in the tertiary structure but distant in the primary sequence. Polio-, rhino-, and foot-and-mouth-disease viruses, for example, contain such major nonlinear neutralization determinants (36–38). In addition to the PND, HIV-1 elicits neutralizing antibodies that bind to other determinants (16, 39–42). For example, fractionation of neutralizing antisera from selected HIV-1-infected humans with PND peptides indicates that a significant fraction of neutralizing antibodies in such sera do not bind the PND (16). Furthermore, monoclonal antibodies (39, 40, 42) that block binding of gp120 to CD4, derived from HIV-1-infected individuals, bind to native but not to denatured gp120 and thus recognize a determinant dependent on the tertiary structure.

Immunization with inactivated SIV confers protection against virus challenge (4–7). It remains to be determined whether native gp110 induces neutralizing antibody that will confer protection from SIV challenge. Whether the properties of the neutralizing determinants of other SIV isolates will be similar to those reported here and how useful SIV will be for assisting the development of a vaccine for HIV-1 remain to be determined.

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1. Desrosiers, R. & Letvin, N. (1987) *Rev. Infect. Dis.* **9**, 438–446.
2. Gardner, M. & Luciw, P. (1989) *FASEB J.* **3**, 2593–2606.
3. Desrosiers, R. C. (1990) *Annu. Rev. Immunol.* **8**, 557–578.
4. Desrosiers, R., Wyand, M. & Kodama, T. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6353–6357.
5. Murphey-Corb, M., Martin, L., Davison-Fairburn, B., Montelaro, R., Miller, M., West, M., Ohkawa, S., Baskin, G., Zhang, J.-Y., Putney, S., Allison, A. & Eppstein, D. (1989) *Science* **246**, 1293–1297.
6. Sutjipto, S., Pedersen, N. & Miller, C. (1990) *J. Virol.* **65**, 2290–2297.
7. Gardner, M. (1990) *AIDS Res. Hum. Retroviruses* **6**, 835–845.
8. Rusche, J. R., Javaherian, K., McDanal, C., Petro, J., Lynn, D. L., Grimaila, R., Langlois, A., Gallo, R. C., Arthur, L. O., Fischinger, P. J., Bolognesi, D. P., Putney, S. D. & Matthews, T. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3198–3202.
9. Goudsmit, J., Debouck, C., Meloen, R. H., Smit, L., Bakker, M., Asher, D. M., Wolff, A. V., Gibbs, C. J., Jr., & Gajdusek, D. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 4478–4482.
10. Palker, T. J., Clark, M. E., Langlois, A. J., Matthews, T. J., Weinhold, K. J., Randall, R. R., Bolognesi, D. P. & Haynes, B. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1932–1936.
11. LaRosa, G. J., Davide, J. P., Weinhold, K., Waterbury, J. A., Profy, A. T., Lewis, J. A., Langlois, A. J., Dreesman, G. R., Boswell, R. N., Shadduck, P., Holley, L. H., Karplus, M., Bolognesi, D. P., Matthews, T. J., Emini, E. A. & Putney, S. D. (1990) *Science* **249**, 932–935.
12. Robey, W. G., Arthur, L. O., Matthews, T. J., Langlois, A., Copeland, T. D., Lerche, N. W., Oroszlan, S., Bolognesi, D. P., Gilden, R. V. & Fischinger, P. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7023–7027.
13. Rusche, J. R., Lynn, D. L., Robert-Guroff, M., Langlois, A. J., Lyerly, H. K., Carson, H., Krohn, K., Ranki, A., Gallo, R. C., Bolognesi, D. P., Putney, S. D. & Matthews, T. K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6924–6928.
14. Putney, S. D., Matthews, T. J., Robey, W. G., Lynn, D. L., Robert-Guroff, M., Mueller, W. T., Langlois, A. J., Ghrayeb, J., Petteway, S. R., Jr., Wienhold, K. J., Fischinger, P. J., Wong-Staal, F., Gallo, R. C. & Bolognesi, D. P. (1986) *Science* **234**, 1392–1395.
15. Javaherian, K., Langlois, A., LaRosa, G., Profy, A., Bolognesi, D., Herlihy, W., Putney, S. & Matthews, T. (1990) *Science* **250**, 590–593.
16. Profy, A. T., Salinas, P. A., Eckler, L. I., Dunlop, N. M., Nara, P. L. & Putney, S. D. (1990) *J. Immunol.* **144**, 4641–4647.
17. Emini, E., Nara, P. L., Schleif, W. A., Lewis, J., Davide, J., Lee, D., Kessler, J., Conley, A., Matsushita, S., Putney, S., Gerety, R. & Eichberg, J. (1990) *J. Virol.* **64**, 3674–3678.
18. Berman, P., Gregory, T., Riddle, L., Nakamura, G., Champe, M., Porter, J., Wurm, F., Hershberg, R., Cobb, E. & Eichberg, J. (1990) *Nature (London)* **345**, 622–628.
19. Girard, M., Kieny, M.-P., Pinter, A., Barre-Sinoussi, F., Nara, P., Kolbe, H., Kusumi, K., Chaput, A., Reinhart, T., Muchmore, E., Ronco, J., Kaczorek, M., Gomard, E., Gluckman, J.-C. & Fultz, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 542–546.
20. Putknonen, P., Thorstensson, R., Ghavamzadeh, L., Albert, J., Hild, K., Biberfeld, G. & Norrby, E. (1991) *Nature (London)* **352**, 436–438.
21. Franchini, G., Gurgo, C., Guo, H.-G., Gallo, R., Collalti, E., Fargnoli, K., Hall, L., Wong-Staal, F. & Reitz, M. (1987) *Nature (London)* **328**, 539–542.
22. Luckow, V. & Summers, M. (1989) *Virology* **170**, 31–39.
23. Javaherian, K., Langlois, A. J., McDanal, C., Ross, K. L., Eckler, L. I., Jellis, C. L., Profy, A. T., Rusche, J. R., Bolognesi, D. P., Putney, S. D. & Matthews, T. J. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6768–6772.
24. Langlois, A., Weinhold, K., Matthews, T. & Bolognesi, D. (1991) *AIDS Res. Hum. Retroviruses* **7**, 713–720.
25. Daniel, M., Letvin, N., Sehgal, P., Hunsmann, G., Schmidt, D., King, N. & Desrosiers, R. (1987) *J. Gen. Virol.* **68**, 3183–3189.
26. Gregory, T., Hoxie, J., Watanabe, C. & Spellman, M. (1991) in *Advances in Experimental Medicine and Biology*, Proceedings of the International Symposium on Immunobiology of Proteins and Peptides, ed. Atassi, M. (Plenum, New York), pp. 1–14.
27. Geysen, H. M., Meloen, R. H. & Barteling, S. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3998–4002.
28. Geysen, H., Barteling, S. & Meloen, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 178–182.
29. McDougal, J. S., Nicholson, K. A., Cross, G. D., Cort, S. A., Kennedy, M. S. & Mawle, A. C. (1986) *J. Immunol.* **137**, 2937–2944.
30. McDougal, J. S., Nicholson, J. K. A., Cosand, W. L., Cross, G. D., Cort, S. P., Kennedy, M. S. & Mawle, A. C. (1988) *Hum. Retroviruses Cancer AIDS*, 269–281.
31. Fennie, C. & Lasky, L. A. (1989) *J. Virol.* **63**, 639–646.
32. Matthews, T. J., Weinhold, K. J., Lyerly, H. K., Langlois, A. J., Wigzell, H. & Bolognesi, D. P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5424–5428.
33. Dowbenko, D., Nakamura, G., Fennie, C., Shimasaki, C., Riddle, L., Harris, R., Gregory, T. & Lasky, L. (1988) *J. Virol.* **62**, 4703–4711.
34. Myers, G., Korber, B., Berzofsky, J., Smith, R. & Pavlakis, G., eds. (1991) *Human Retroviruses and AIDS Database* (Los Alamos National Lab., Los Alamos, NM), pp. II-91.
35. Burns, D. & Desrosiers, R. (1991) *J. Virol.* **65**, 1843–1854.
36. Hogle, J., Chow, M. & Filman, D. (1985) *Science* **229**, 1358–1365.
37. Rossman, M., Arnold, E., Erickson, J., Frankenberger, E., Griffith, J., Hecht, H.-J., Johnson, J., Kamer, G., Luo, M., Mosser, A., Ruekert, R., Sherry, B. & Vriend, G. (1985) *Nature (London)* **317**, 145–153.
38. Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D. & Brown, F. (1989) *Nature (London)* **337**, 709–716.
39. Ho, D., McKeating, J., Li, X., Moudgil, T., Daar, E., Sun, N. & Robinson, J. (1991) *J. Virol.* **65**, 489.
40. Posner, M., Hideshima, T., Cannon, T., Mukherjee, M., Mayer, K. & Byrn, R. (1991) *J. Immunol.* **146**, 4325–4332.
41. Haigwood, N., Shuster, J., Moore, G., Lee, H., Skiles, P., Higgins, K., Barr, P., George-Nascimento & Steimer, K. (1990) *AIDS Res. Hum. Retroviruses* **6**, 855.
42. Tilly, S., Honnen, W., Racho, M., Hilgartner, M. & Pinter, A. (1991) *Res. Virol.* **142**, 247–251.