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A conserved region at the COOH terminus of human immunodeficiency virus gp120 envelope protein contains an immunodominant epitope

(acquired immunodeficiency syndrome/synthetic peptides/retrovirus/anti-viral antibodies)

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ABSTRACT **A highly immunogenic epitope from a conserved COOH-terminal region of the human immunodeficiency virus (HIV) gp120 envelope protein has been identified with antisera from HIV-seropositive subjects and a synthetic peptide (SP-22) containing 15 amino acids from this region (Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg). Peptide SP-22 absorbed up to 100% of anti-gp120 antibody reactivity from select HIV$^+$ patient sera in immunoblot assays and up to 79% of serum anti-gp120 antibody reactivity in competition RIA. In RIA, 45% of HIV-seropositive subjects had antibodies that bound to peptide SP-22. Human anti-SP-22 antibodies that bound to and were eluted from an SP-22 affinity column reacted with gp120 in RIA and immunoblot assays but did not neutralize HIV or inhibit HIV-induced syncytium formation *in vitro*, even though these antibodies comprised 70% of all anti-gp120 antibodies in the test serum. In contrast, the remaining 30% of SP-22 nonreactive anti-gp120 antibodies did not react with gp120 in immunoblot assays but did react in RIA and neutralized HIV *in vitro*. Thus, ≈50% of HIV-seropositive patients make high titers of nonneutralizing antibodies to an immunodominant antigen on gp120 defined by SP-22. Moreover, the COOH terminus of gp120 contains the major antigen or antigens identified by human anti-gp120 antibodies in immunoblot assays.**

Human T-cell lymphotropic virus (HTLV) type III (1, 2), lymphadenopathy-associated virus (LAV) (3), and acquired immunodeficiency syndrome (AIDS)-associated retrovirus type 2 ARV-2 (4) are isolates of a human retrovirus recently designated as the human immunodeficiency virus (HIV) (5) that preferentially infects the T4+ subset of thymus-derived (T) lymphocytes and is the etiologic agent of AIDS (1–4). Neutralizing epitopes that induce protective immunity to murine (6, 7) and feline (8–10) retroviruses have been found on virus-encoded envelope glycoproteins, and recent evidence suggests that a neutralizing epitope of HIV resides on the 120-kDa external envelope glycoprotein (gp120) (11, 12). Although it is well-documented that HIV$^+$ subjects make antibodies to gp120, the immunogenic sites on this molecule and their corresponding amino acid sequences have not been identified. Moreover, little is known regarding the protective properties of anti-viral antibodies directed to discrete antigenic sites on HIV gp120. To address these questions, we have constructed a synthetic peptide (SP-22) with an amino acid sequence derived from the COOH-terminal hydrophilic region of gp120 that is also highly conserved among HIV isolates (13, 14). In this study, we report on the reactivity of HIV$^+$ patient antibodies to an immunodominant antigen contained by SP-22 and gp120 and on the use of peptide SP-22 as an immunoadsorbent to evaluate the functional importance of the human antibody response to the COOH terminus of gp120.

MATERIALS AND METHODS

Synthetic Peptides. Synthetic peptides SP-22 and SP-1, containing amino acid sequences derived from COOH- and NH_2-terminal regions of HIV gp120, respectively, and SP-70A from the gp45 envelope protein of HTLV-I were synthesized on an Applied Biosystems 430A peptide synthesizer using chemical and program cycles supplied by the manufacturer. The amino acid sequences of these peptides are as follows: SP-22, Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-(Cys) [envelope amino acids 504–518 as described in Ratner *et al.* (15)]; and SP-1, Ala-Cys-Val-Pro-Thr-Asp-Pro-Asn-Pro-Gln-Glu-Val-(Tyr) (envelope amino acids 80–91). Amino acid residues in parentheses were added to facilitate iodination of the peptide (Tyr) and coupling to carrier protein (Cys). The COOH-terminal arginine of SP-22 is situated at the proteolytic cleavage site between gp120 and gp41 (15). Additional control peptides included SP-70A [(Tyr)-Pro-Pro-Phe-Ser-Leu-Ser-Pro-Val-Pro-Thr-Leu-Gly-Ser-Arg-(Cys)] from the COOH terminus of HTLV-I gp45 and SP-73 [Tyr-Leu-Phe-Pro-His-Trp-Thr-Lys-Lys-Pro-Asn-Arg-Asn-Gly-(Cys)] from the NH_2-terminal region of HTLV-I gp45 (16), the latter obtained from S. Oroszlan (Frederick Cancer Research Institute, Frederick, MD). Peptides were conjugated to bovine serum albumin (BSA) (peptide/BSA molar ratio, 30:1) with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) according to Green *et al.* (17). Conjugation of peptides to BSA was monitored by subjecting peptide–BSA conjugates to $NaDodSO_4$/PAGE on 10% gels under nonreducing conditions. After the gel was stained with Coomassie blue R-250 and destained, the average number of synthetic peptide molecules bound to BSA was estimated by comparing the molecular weights of peptide–BSA conjugates to those of molecular weight standards and to BSA. The average numbers of peptide molecules bound to BSA were as follows: SP-73–BSA, 4; SP-70-A–BSA, 9; SP-22–BSA, 7; and SP-1–BSA, 7. Conjugation of peptides to BSA was also monitored by the addition of ^{125}I-labeled synthetic peptides (5×10^5 cpm) to the coupling reaction. Peptide SP-22, lacking tyrosine, was trace-labeled

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Abbreviations: RIP, radioimmunoprecipitation; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; HTLV-III, human T-cell lymphotropic virus type III; LAV, lymphadenopathy-associated virus; MBS, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester; BSA, bovine serum albumin.

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with ^{125}I-Bolton–Hunter reagent for this purpose. After coupling, peptide–BSA conjugates were dialyzed against 4 liters of phosphate-buffered saline (PBS) (pH 7.4) for 3 days at 4°C with three changes, and the amount of nondialyzable radioactivity was evaluated on a γ counter. The percentage of peptides bound to BSA was evaluated for the following peptides: SP-22–BSA, 27%; SP-1-BSA, 29%; and SP-73–BSA, 14%.

Recombinant Proteins and Antisera. Recombinant protein env-9 (18) containing the COOH-terminal region of gp120 and the NH_2 terminus of gp41 [*Bgl* II–*Bam*HI sites (15)] and goat antisera to env-9 were obtained from S. R. Petteway (DuPont de Nemours, Experimental Station, Wilmington, DE). Recombinant protein E3 containing an NH_2-terminal region of gp120 (*Kpn* I–*Pvu* II sites) was obtained from N. Chang (Centecor, Malvern, PA). Sera from asymptomatic seropositive and HIV$^+$ symptomatic hemophiliac patients were obtained from G. White (University of North Carolina Hemophilia Center, Chapel Hill, NC). Sera from AIDS patients were obtained from B. Safai (Memorial Sloan–Kettering Institute, New York).

HIV-Infected Cells. H-9 T cells (2, 19–21) infected with HTLV-III$_B$ (2), HTLV-III$_{MN}$ (2, 19–21), and HTLV-III$_{RF}$ (2, 19–21) were obtained from R. Gallo (National Cancer Institute, Bethesda, MD). Cells were maintained in RPMI 1640 medium (GIBCO) supplemented with 20% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml).

Immunoassays with HIV$^+$ Patient Sera. To evaluate the binding of antibodies from HIV-seropositive subjects to SP-22–BSA and SP-1–BSA, peptide-conjugates (10 μg) were first iodinated using Iodogen (Pierce) and 0.5 mCi of ^{125}I (1 Ci = 37 GBq). ^{125}I-labeled peptide conjugates (5×10^5 cpm) in 0.5 ml of radioimmunoprecipitation (RIP) buffer (10 mM sodium phosphate, pH 7.4/300 mM NaCl/0.2% Triton X-100/0.01% Tween 80/0.1% gelatin/0.1% BSA/0.02% sodium azide) were precleared by sequential 20-min incubations with 5 μl of normal human serum and 100 μl of 10% fixed *Staphylococcus aureus* (Pansorbin, Calbiochem). After centrifugation, precleared supernatants were incubated for 1 hr at 23°C with 10 μl of serum from HIV$^+$ patients (n = 42) as well as control normal human sera (n = 10) or sera from patients with systemic lupus erythematosus (n = 19). After immune complexes were precipitated by incubation with fixed *S. aureus* for 30 min at 4°C, samples were centrifuged and pellets were washed three times in RIP buffer. Samples were then evaluated for bound radioactivity in a γ counter. Assays were performed twice on separate days and the mean Δcpm values for two experiments were calculated by subtracting the mean background value obtained with normal sera from cpm values obtained with test sera.

Immunoblotting assays with purified gp120 and with 1% Triton X-100 lysates of H-9 cells infected with HTLV-III$_B$ were performed as described (22). HIV gp120 envelope protein was purified from lysates of H-9 cells infected with HTLV-III$_B$ as described (23). Competition immunoblot assays were performed by incubating peptide–BSA conjugates or BSA treated with MBS (100 μl of 1-mg/ml solution) with 50–100 μl of HIV$^+$ patient serum for 1 hr at 23°C prior to testing in immunoblot assays. Competition RIP assays were performed by incubating HIV$^+$ patient sera with dilutions of peptide–BSA conjugate, purified gp120, Friend leukemia virus gp71, or recombinant proteins in RIP buffer prior to incubation with ^{125}I-labeled gp120 or peptide–BSA conjugate (5×10^5 cpm). Immune complexes were then precipitated with fixed *S. aureus*, washed three times in RIP buffer, and evaluated for bound radioactive antigen on a γ counter.

Affinity Chromatography of HIV$^+$ Patient Sera. Peptide–BSA conjugates SP-22–BSA or SP-70A–BSA (2 mg) were each conjugated to 1 g of CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's specifications and used to prepare affinity columns. Coupling efficiencies of both peptide–BSA conjugates to CNBr-activated Sepharose 4B were >95%, as determined with ^{125}I-labeled conjugates. An affinity column containing purified HTLV-III$_B$ gp120 was prepared as described (12).

After HIV$^+$ patient serum or normal human serum (0.5 ml) was passed over the SP-22 Sepharose column, the column was washed with 40 bed vol of PBS (10 mM sodium phosphate, pH 7.4/150 mM NaCl) and further eluted with 4 M $MgCl_2$. Bound and unbound fractions were collected, pooled, dialyzed against 0.1× PBS (1 mM sodium phosphate, pH 7.4/15 mM NaCl), lyophilized, and then reconstituted with distilled water to the original volume. Reconstituted samples were further dialyzed against isotonic PBS (pH 7.4) prior to testing in syncytium and neutralization assays. For negative controls, separate serum samples were similarly treated with an affinity column consisting of a HTLV-I-derived peptide (SP-70A) bound to BSA and coupled to Sepharose.

Neutralization Assay. HIV neutralization assays were performed as described (12) by incubating 1000 infectious units of HTLV-III$_B$ virus with antibody samples for 30 min at 37°C prior to incubation of virus with H9 cells (1×10^5 cells per well). After infected H9 cells were grown in culture for 10 days, supernatants were tested for reverse transcriptase activity using poly(rA) and poly(dT)$_{12-18}$ as template and primer (2). Tissue culture supernatants were also tested for the presence of HIV p24 core protein in a competition RIA (developed by DuPont).

Syncytium Inhibition Assay. Syncytium inhibition assays were performed in 96 one-half well microtiter plates (Costar, A/2 cluster 96 wells) by mixing 7.5×10^4 CEM cells with 5×10^3 chronically infected CEM/HTLV-III$_B$ cells either in the presence or absence of anti-gp120 antibodies (T.J.M. and D.P.B., unpublished data). Plates were centrifuged at 400 × g for 5 min and incubated in a 5% CO_2/95% air humidified chamber at 37°C for 24 hr. The number of giant cells in each well was determined by microscopic examination at ×40 magnification.

RESULTS

Reactivity of HIV$^+$ Patient Sera to Synthetic Peptide SP-22 from HIV gp120. To evaluate the reactivity of HIV$^+$ patient antibody to synthetic peptide SP-22, RIP assays were performed using ^{125}I-labeled conjugates of SP-22 coupled to BSA and sera from 42 HIV$^+$ subjects in various stages of HIV-associated disease [asymptomatic seropositive (n = 14), lymphadenopathy syndrome (n = 10), or AIDS (n = 18)] (Fig. 1). Forty-five percent (19 of 42) of HIV$^+$ patient sera contained antibodies that reacted with SP-22 by greater than twice the background cpm value obtained with 10 normal serum controls. Antibody reactivity to SP-22 did not appear to correlate with the stage of HIV-associated disease. In contrast, none of the sera from patients with systemic lupus erythematosus had reactivity to SP-22. Also, in the same assay, HIV$^+$ patient sera had negligible reactivity to synthetic peptide SP-1 derived from the NH_2-terminal region of HIV gp120 (not shown). In competition RIP assays, the percentage of total anti-gp120 antibody reactivity that could be absorbed by SP-22 was determined for each of those sera that reacted with SP-22 by greater than twice background values. Mean values for the percentage of total anti-gp120 antibody reactivity absorbed by SP-22 in RIP assay for each disease classification were as follows: asymptomatic seropositive = 42.5% (ranging from 12% to 79%), lymphadenopathy syndrome = 46% (ranging from 10% to 66%), and AIDS = 31% (ranging from 14% to 71%). To evaluate further the specificity of patient antibody reactivity to SP-22, competition immunoblot RIP and assays were performed with synthetic peptide–BSA conjugates and three sera (nos. 1–3) were

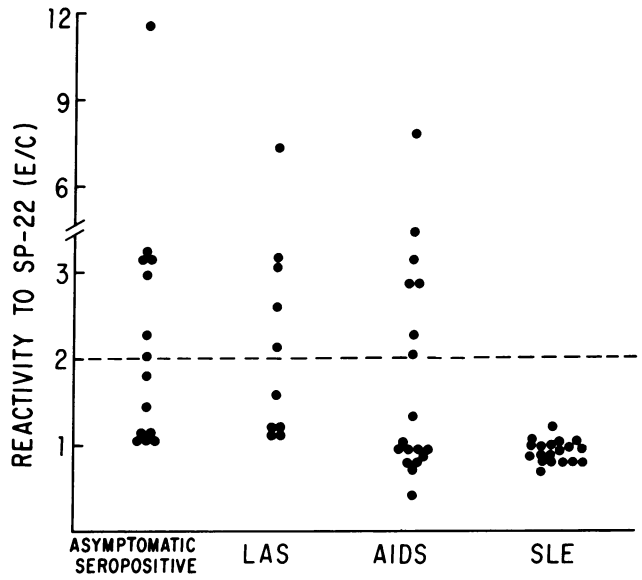

FIG. 1. Reactivity to SP-22 of antibodies from asymptomatic HIV$^+$-seropositive patients (n = 14), patients with lymphadenopathy syndrome (LAS, n = 10), AIDS (n = 18), and systemic lupus erythematosus (SLE, n = 19). HIV$^+$ patient sera were tested for reactivity to ^{125}I-labeled SP-22–BSA conjugate in RIA as described, and results are expressed as a ratio of cpm precipitated by experimental sera and the mean cpm value obtained with 10 normal human sera (E/C). Serum samples with reactivity ≥2.0 were considered positive and were further evaluated in competition RIP assay to determine the percentage of total anti-gp120 antibodies reactive with SP-22.

selected for high reactivity to SP-22. Sera 1 and 2 were obtained from asymptomatic HIV-seropositive hemophiliacs, and serum 3 was obtained from a patient with AIDS. From previous studies, it was determined that all three of these sera contained antibodies that neutralized HIV *in vitro* (A.J.L., T.J.M., and D.P.B., unpublished observations). As shown in Fig. 2*A*, SP-22–BSA completely inhibited the reactivity of serum 1 to purified gp120 in immunoblot assay (lane 2). In contrast, equivalent amounts of BSA treated with the coupling agent MBS (BSA–MBS, lane 1) or synthetic peptide SP-73 (from HTLV-I gp45) coupled to BSA (lane 3) did not inhibit antibody reactivity to gp120. The lack of reactivity of normal human serum to gp120 is shown in lane 4. Also, when sera 2 and 3 were preabsorbed with SP-22–BSA and tested in immunoblot assay against Triton X-100 lysates of HTLV-III$_B$-infected H-9 cells, antibody reactivity to gp120 was selectively removed (data not shown). Preincubation of sera 2 or 3 with equivalent amounts of either BSA–MBS or BSA–SP-73 conjugates did not inhibit antibody binding to gp120 in this assay. In competition RIP assay, SP-22–BSA conjugates also inhibited the binding of antibodies in serum 1 (Fig. 2*B*) to ^{125}I-labeled gp120. However, in contrast to results obtained by immunoblotting in which SP-22 completely inhibited serum 1 reactivity to gp120, SP-22–BSA inhibited serum 1 reactivity to gp120 by only 60% in RIP assay. Other control antigens (SP-73–BSA, BSA–MBS) did not inhibit precipitation of gp120 by serum 1. Similar results were obtained in competition RIP assay with serum 2 (data not shown). Next, purified gp120, Friend leukemia virus gp71, and recombinant proteins encoded by the *env* gene of HTLV-III$_B$ were used to evaluate the specificity of HIV$^+$ patient antibody binding to ^{125}I-labeled SP-22–BSA conjugates in competition RIP assay. When preincubated with 0.1, 1, or 2 μg of either gp120 or recombinant protein *env-9* that contains the COOH-terminal amino acid sequence of gp120 shared by SP-22, serum 1 antibodies were inhibited in a

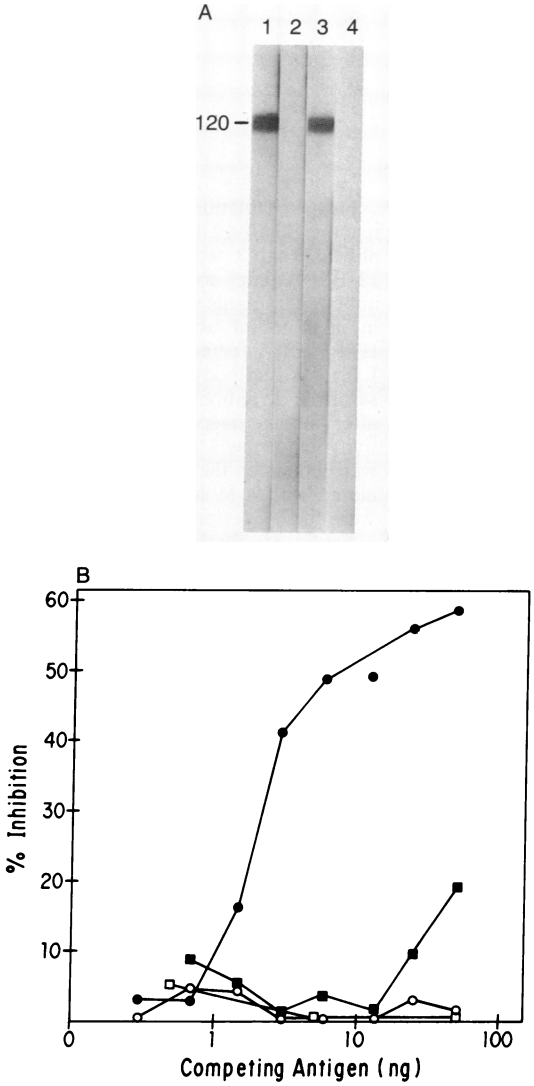

FIG. 2. Inhibition of HIV$^+$ patient antibody reactivity to gp120 by synthetic peptide SP-22. HIV$^+$ patient antibodies (serum 1) or a normal human serum control were preincubated with peptide–BSA conjugates and reactivity was tested either to purified gp120 in immunoblot assay (*A*) or to ^{125}I-labeled gp120 in competition RIP assay (*B*). (*A*) Inhibition of serum 1 antibody binding to purified gp120 by SP-22–BSA (lane 2), whereas equivalent amounts of BSA treated with coupling agent MBS (lane 1) or peptide conjugate SP-73–BSA (lane 3) containing an amino acid sequence from HTLV-I gp45 did not inhibit antibody binding to gp120. Lack of reactivity of normal human serum to purified gp120 is shown in lane 4. (*B*) Serum 1 was preincubated with peptides conjugated to BSA and then tested for the ability to precipitate ^{125}I-labeled HIV gp120. Antibodies in serum 1 were inhibited from precipitating gp120 by SP-22–BSA (●) derived from the COOH terminus of gp120 but not by BSA treated with coupling agent MBS (□) or by HTLV-I peptide SP-73–BSA (○). Partial inhibition was seen with peptide SP-1–BSA from the NH_2 terminus of HIV gp120 (■).

dose-dependent manner from binding to SP-22–BSA conjugates by 60–90% (data not shown). Incubation of serum 1 with equivalent amounts of Friend leukemia virus gp71 or recombinant protein pE3 encoded by an NH_2-terminal region of gp120 (lacking the COOH-terminal amino acid sequence of SP-22) did not inhibit antibody binding to SP-22–BSA.

Affinity Purification of Anti-SP-22 Human Antibodies. To evaluate the ability of anti-SP-22 antibodies to inhibit either HIV infection or HIV-induced syncytium formation *in vitro*, serum 3 was passed over an SP-22–BSA–Sepharose affinity column and anti-SP-22 antibodies were recovered by elution

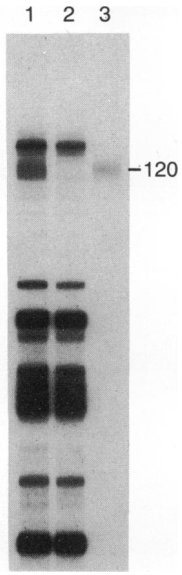

FIG. 3. Isolation of anti-gp120 antibodies reactive to SP-22 by affinity chromatography. Serum 3 was passed over an SP-22–BSA affinity column and the bound and unbound fractions were collected and tested in immunoblot assays against lysates of HTLV-III_B-infected H-9 cells as described. Lanes: 1, antibody reactivity of untreated serum 3; 2, antibody reactivity of serum 3 after passage over affinity column; 3, reactivity of serum 3 antibodies to gp120 that were bound to the SP-22–BSA column and eluted with 4 M $MgCl_2$. Bound antibodies were further tested for the ability to inhibit syncytium formation and to neutralize HIV *in vitro* (Table 1).

with 4 M $MgCl_2$. Both the unbound and the bound serum fractions were then tested for reactivity to SP-22 and gp120 in immunoblot and RIP assays. The SP-22 affinity column removed most or all of the immunoblot reactivity to gp120 from the HIV^+ patient serum 3 (Fig. 3, lane 2). After elution with 4 M $MgCl_2$, these anti-gp120 antibodies were recovered from the column (lane 3). The reactivity of untreated serum 3 is shown in lane 1. When tested in RIP assay (Table 1), serum antibodies that bound to the SP-22 affinity column and were eluted with 4 M $MgCl_2$ also bound ^{125}I-labeled SP-22–BSA, whereas the unbound fraction contained essentially no antibodies reactive with SP-22. In addition, ≈70% of the total precipitating anti-gp120 antibodies were bound to the SP-22 column as determined in RIP assays (Table 1). As a negative control, serum 3 was also passed over an SP-70A–Sepharose affinity column containing a synthetic peptide derived from the COOH terminus of HTLV-I gp45. As expected, essentially all of the antibodies that reacted with SP-22 and >90% of precipitating antibodies to gp120 were contained in the unbound fraction. When tested in syncytium inhibition and neutralization assays *in vitro*, affinity-purified anti-SP-22 patient serum antibodies failed to inhibit HIV-induced syncytium formation (data not shown) and virus infectivity (Table 1) as measured by reverse transcriptase production and by levels of HIV p24 in culture supernatants. In contrast, the unbound serum fraction that passed through the SP-22 column retained the ability to inhibit syncytium formation and neutralized HIV *in vitro* as determined by reverse transcriptase production and by the HIV p24 competition RIA.

To determine whether SP-22 nonreactive anti-gp120 antibodies would neutralize HIV, serum 3 antibodies that did not bind to the SP-22 affinity column were additionally passed over a gp120 affinity column and the bound antibodies were eluted with 4 M $MgCl_2$. When tested in HIV neutralization assays, both the bound and unbound fractions from the gp120 affinity column contained neutralizing antibodies (Table 1), in agreement with results of a previous study (12). When the antibodies that bound to the gp120 affinity column were eluted and tested for reactivity to gp120 in immunoblot and RIP assays, the bound antibody fraction precipitated gp120 in RIP assays (Table 1) but did not react with gp120 in immunoblot assays (not shown).

To determine whether the epitope or epitopes on gp120 identified by the anti-SP-22 affinity-purified human antibodies were conserved on other strains of HIV, immunoblot assays were performed using lysates of H-9 cells infected with HTLV-III_{MN} and Haitian HIV isolate HTLV-III_{RF}. Anti-SP-22 antibodies from serum 1 as well as untreated HIV^+ serum 1 reacted with gp120 from both HTLV-III_{MN} and HTLV-III_{RF} (not shown).

DISCUSSION

Results presented here support the conclusion that the COOH terminus of gp120 contains an immunodominant antigen that is recognized by 45% of all individuals seropositive for HIV. This COOH-terminal sequence of gp120 is absolutely conserved in isolates HTLV-III_{BH10}, HTLV-III_{MN}, AIDS-associated retrovirus type 2, and LAV (14), while a single conservative amino acid change [lysine to arginine, envelope amino acid 507 (15)] is found in Haitian isolate HTLV-III_{RF}. Because this region is so highly conserved, the immunogenic COOH-terminal epitope was also likely to be conserved among differing strains of HIV. In this study, results of immunoblot assays with affinity-purified anti-SP-22 antibodies and cell lysates containing HTLV-III_{MN} and HTLV-III_{RF} viral proteins confirmed that the COOH-terminal epitope on gp120 was conserved in divergent HIV isolates. With sera from some HIV^+ patients, up to

Table 1. Effect of affinity-purified anti-gp120 human antibodies on HTLV-III_B replication *in vitro*

Affinity column	HIV^+ patient serum fraction	Reactivity to SP-22	Reactivity to gp120	Neutralization of HTLV-III_B as measured by: Reverse transcriptase production, cpm	Neutralization of HTLV-III_B as measured by: Production of viral P24, ng/ml
SP-22 (BSA)	Bound	15,031	88,904	225,692	20
	Unbound	104	33,259	2,697	<0.3
SP-70A (BSA)	Bound	44	1,110	206,775	20
	Unbound	5,963	12,312	9,504	<0.3
gp120*	Bound	ND	44,730	4,000	1.2
	Unbound	ND	1,467	2,228	<0.3

Synthetic peptide–BSA conjugates or purified HIV gp120 were coupled to CNBr-activated Sepharose and used as affinity columns for purification of antibodies from HIV^+ patient serum. Peptide SP-22 is derived from the COOH terminus of HIV gp120; peptide SP-70A contains sequences from the COOH terminus of HTLV-I gp45 (see *Materials and Methods*). Data shown are representative of two experiments performed with SP-22–BSA and SP-70–BSA affinity columns. HIV^+ patient serum was passed over affinity columns and the bound and unbound fractions were collected as described. Data are expressed as Δcpm obtained in RIP assays using sera passed over affinity columns and ^{125}I-labeled SP-22–BSA or ^{125}I-labeled gp120. Serum fractions were tested at a dilution of 1:32 for their ability to neutralize infection of HTLV-III_B and are described in *Materials and Methods*. Virus infection was monitored on the basis of both reverse transcriptase activity and viral p24 core antigen (competition RIA) released into the supernatant of challenged H9 cells. The untreated serum completely blocked infection at a dilution of up to 1:100. ND, not done.

*The unbound fraction of HIV^+ patient serum from an SP-22 affinity column was also passed over a gp120 affinity column and both bound and unbound serum fractions were collected.

100% of the total antibody response to gp120 in immunoblot assay and as much as 79% of antibody reactivity in RIP assay were mapped to the COOH terminus of gp120 by using synthetic peptide SP-22 in competitive binding studies. Blocking of antibody reactivity to gp120 in RIP or immunoblotting assays was directly attributable to an antigen or antigens on peptide SP-22, since control peptides from HTLV-I gp45, as well as BSA alone, did not inhibit antibody reactivity. Furthermore, patient antibody binding to SP-22 could be inhibited by purified gp120 or recombinant protein env-9 containing the COOH-terminal region of gp120, supporting the conclusion that similar antigens were present on gp120, SP-22, and recombinant protein env-9.

When peptide SP-22 was used as an immunoadsorbent, it was possible to adsorb from HIV^+ patient sera all of the detectable anti-gp120 antibodies that reacted in immunoblot assays. Thus, the COOH-terminal region of gp120 contained the major antigen or antigens identified with these sera in immunoblot assays. However, these same patient sera also contained additional anti-gp120 antibodies that did not bind to SP-22 but that did react with gp120 in RIP assay (Table 1). When purified on a gp120 affinity column, these SP-22 nonreactive antibodies neutralized HIV *in vitro*, reacted with ^{125}I-labeled gp120 in RIP assay, but did not react with gp120 in immunoblot assays. These data suggested that neutralizing antibodies were directed against epitopes that were not at the COOH terminus of gp120 and that were denatured by immunoblotting. However, in regard to the latter observation, it is also possible that the RIP assay was more sensitive in detecting antibody reactivity to gp120 than the immunoblot assay; thus, affinity-purified neutralizing antibodies to gp120 might not be as readily identified in immunoblot assay as in RIP assay. Although neutralizing anti-gp120 antibodies did not react with gp120 in immunoblot assays, further studies are needed to determine directly whether neutralizing antibodies react with epitopes on HIV gp120 that are denatured in immunoblot assays.

Although the COOH-terminal antigen of gp120 was highly immunogenic, there was no correlation between the clinical status of HIV^+ patients and antibody reactivity to SP-22 in RIP assays (Fig. 1). These data suggested that antibodies to the COOH terminus of gp120 might not be protective or beneficial to the host. This hypothesis was supported by studies presented here in which anti-SP-22 antibodies were purified over an SP-22 affinity column and tested for the ability to inhibit HIV-induced syncytium formation and to neutralize HIV *in vitro*. Although all of the antibodies to SP-22 and 73% of the antibodies to gp120 detectable in RIP assay were bound by the SP-22 affinity column, the unbound serum fraction retained its ability to neutralize HIV *in vitro*. Moreover, affinity-purified anti-SP-22 antibodies recovered from the affinity column bound to gp120 in immunoblot and RIP assays but failed to neutralize HIV *in vitro* (Table 1). Also, we have found that rabbit and mouse polyclonal antibodies raised to SP-22 as well as goat antisera to recombinant protein env-9 (containing the SP-22 amino acid sequence) do not inhibit virus infection in the same *in vitro* assays (not shown). Since the anti-SP-22 antibodies in our present study did not inhibit virus infection *in vitro*, they may also do little to protect the host from HIV infection *in vivo*. Further studies in animal models are necessary to determine whether anti-SP-22 antibodies are beneficial to the host. If these antibodies are not protective, it may be necessary to delete this immunodominant region from any HIV envelope component being considered as a vaccine so that the humoral immune response to HIV can be redirected to other antigenic sites on gp120 that induce protective immunity.

Retroviral-encoded synthetic peptides have proven useful in mapping the human antibody response to retroviral antigens (24–27). Techniques similar to those described here and in the cited studies may prove useful in mapping the reactivity of neutralizing antibodies to epitopes on human retroviral proteins and ultimately in the design of synthetic vaccines for raising neutralizing antibodies to HIV.

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