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Immunology

Type-specific neutralization of the human immunodeficiency virus with antibodies to *env*-encoded synthetic peptides

(acquired immunodeficiency syndrome/vaccine/retrovirus)

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ABSTRACT **A synthetic peptide (SP-10-III$_B$) with an amino acid sequence [Cys-Thr-Arg-Pro-Asn-Asn-Asn-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-(Tyr); amino acids 303–321] from the human immunodeficiency virus (HIV) isolate human T-cell lymphotropic virus type III (HTLV-III) HTLV-III$_B$ envelope glycoprotein gp120 was coupled to tetanus toxoid and used to raise goat antibodies to HIV gp120. Goat anti-SP-10-III$_B$ serum bound to the surface of HTLV-III$_B$-infected CEM T cells but not to the surface of HTLV-III$_{RF}$-infected or uninfected CEM T cells. Anti-SP-10-III$_B$ antibodies also selectively bound to gp120 from lysates of HTLV-III$_B$ cells in immunoblot assays. Twenty-one percent of sera (28 of 175) from patients seropositive for HIV contained antibodies that reacted with SP-10-III$_B$ in RIA. Human anti-SP-10-III$_B$ antibodies affinity purified from acquired immunodeficiency syndrome (AIDS) patient serum bound to HTLV-III$_B$-infected cells and immunoprecipitated gp120. Goat antibodies to SP-10-III$_B$ neutralized HTLV-III$_B$ (80% neutralization titer of 1/600), inhibited HTLV-III$_B$-induced syncytium formation, but did not neutralize HIV isolates HTLV-III$_{RF}$ or HTLV-III$_{MN}$ or inhibit syncytium formation with these isolates. Also, goat antiserum to an homologous synthetic peptide [SP-10-III$_{RF(A)}$, (Cys)-Arg-Lys-Ser-Ile-Thr-Lys-Gly-Pro-Gly-Arg-Val-Ile-Tyr] from gp120 of HIV isolate HTLV-III$_{RF}$ inhibited syncytium formation by HTLV-III$_{RF}$, but did not inhibit syncytium formation by HTLV-III$_B$ or by HTLV-III$_{MN}$. Thus, the amino acid sequences of SP-10-III$_B$ and SP-10-III$_{RF(A)}$ define homologous regions of gp120 that are important in type-specific virus neutralization. The identification of these type-specific neutralizing epitopes should facilitate the design of a polyvalent, synthetic vaccine for AIDS.**

The human immunodeficiency virus (HIV) is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (1–4). AIDS is now a world-wide epidemic for which there is no vaccine or cure. Work toward the development of a vaccine for AIDS has centered on the external envelope glycoprotein of HIV, gp120. The gp120 molecule of HIV and recombinant molecules containing amino acid sequences of gp120 have antigenic sites that evoke virus-neutralizing antibody responses in mammals (5–10). Among some HIV isolates, there is substantial genomic heterogeneity localized in hypervariable regions of the *env* gene encoding gp120 (11–15). Differences in *env* genes among HIV isolates can be associated with antigenic (16) or structural changes in gp120 and also can account for the fact that antibodies raised in animals to gp120 frequently neutralize only that isolate (or type) of HIV from which the gp120 is derived (9, 10, 17).

It has been our approach to use synthetic peptides containing hydrophilic amino acids from both conserved and variable regions of HIV gp120 to identify antigenic sites of gp120 using AIDS patient sera and to evaluate the functional importance of these gp120 epitopes with antipeptide antibodies raised in goats. In this study we describe the use of synthetic peptides containing homologous amino acid sequences of gp120 from HIV isolates human T-cell lymphotropic virus type III (HTLV-III) HTLV-III$_B$ and HTLV-III$_{RF}$ (11) to raise high titers of type-specific neutralizing antibodies to these isolates.

MATERIALS AND METHODS

Synthetic Peptides. Peptides were synthesized on an Applied Biosystems (Foster City, CA) 430A peptide synthesizer or on a DuPont 2100 peptide synthesizer [peptides SP-10C-III$_B$, SP-10-III$_{RF(A)}$] using chemical and program cycles supplied by the manufacturers. Hydrophilic amino acid sequences (18) and localized secondary structures (19) of HTLV-III$_B$ gp120 were determined as described. Amino acid sequences of HTLV-III$_B$ (ref. 20, clone BH10) *env*-encoded synthetic peptides used in this study are SP-1, Ala-Cys-Val-Pro-Thr-Asp-Pro-Asn-Pro-Gln-Glu-Val-(Tyr), *env*-encoded amino acids 80–91; SP-10, Cys-Thr-Arg-Pro-Asn-Asn-Asn-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-(Tyr), *env*-encoded amino acids 303–321; SP-10A, (Tyr)-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg-Ala-Lys, *env*-encoded amino acids 328–344; SP-10C, (Cys)-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-(Tyr), *env*-encoded amino acids 310–322; SP-11, (Tyr)-Ser-Arg-Ala-Lys-Trp-Asn-Asn-Thr-Leu-Lys-Gln-Ile-Asp-Ser-Lys-Leu-Arg-Glu-Gln-Phe-Gly-Asn-Asn-(Cys), *env*-encoded amino acids 341–363; SP-14, (Tyr)-Asn-Ser-Thr-Gln-Leu-Phe-Asn-Ser-Thr-Trp-Phe-Asn-Ser-Thr-Trp-Ser-Thr-Lys-Gly-Ser-Asn-Asn-Thr-Glu-Gly-Ser-Asp-Thr-Ile-(Cys), *env*-encoded amino acids 393–421; SP-15, (Tyr)-Leu-Thr-Arg-Asp-Gly-Gly-Asn-Ser-Asn-Asn-Glu-Ser-Glu-Ile-Phe-(Cys), *env*-encoded amino acids 461–475; SP-22, Ala-Pro-Thr-Lys-Ala-Lys-Arg-Arg-Val-Val-Gln-Arg-Glu-Lys-Arg-(Cys), *env*-encoded amino acids 504–518. Peptide SP-10-III$_{RF(A)}$ contains the following amino acid sequence from HIV-isolate HTLV-III$_{RF}$ gp120: (Cys)-Arg-Lys-Ser-Ile-Thr-Lys-Gly-Pro-Gly-Arg-Val-Ile-Tyr (14). Amino acids in parentheses were added to facilitate iodination (Tyr) of the peptides or coupling to carrier protein (Cys). A control peptide from the COOH terminus of HTLV-I gp45 was also used: SP-70A, (Tyr)-Pro-Pro-Phe-Ser-Leu-Ser-Pro-Val-Pro-Thr-Leu-Gly-Ser-Arg-(Cys). After synthesis, peptides were deprotected and cleaved from the supporting resin with

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Abbreviations: HIV, human immunodeficiency virus; HTLV-III, human T-cell lymphotropic virus type III; BSA, bovine serum albumin; RIP, radioimmunoprecipitation; TT, tetanus toxoid; MBS, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester; SP, synthetic peptide; AIDS, acquired immunodeficiency syndrome.

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hydrogen fluoride. After solubilization of cleaved peptides in 15–25% (vol/vol) glacial acetic acid, peptides were lyophilized, reconstituted in phosphate-buffered saline (pH 7.2), desalted on a Sephadex G10 column, and again lyophilized. Peptides were conjugated to either bovine serum albumin (BSA) or tetanus toxoid (TT) (provided by F. McCarthy, Wyeth Laboratories) carrier proteins at a molar ratio of 30:1 (peptide to carrier protein) with *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) according to Greene *et al.* (21). Conjugation of peptides to carrier proteins was monitored by trace iodination of peptide or by estimating the molecular weights of conjugates subjected to $NaDodSO_4$/PAGE as described (22). Sequencing of SP-10-III_B and amino acid analysis of SP-10C-III_B confirmed the amino acid content of these peptides.

Cells. H9 T cells infected with HIV isolates HTLV-III_B, HTLV-III_{MN}, and HTLV-III_{RF} as well as uninfected H9 cells were obtained from R. C. Gallo (National Cancer Institute, National Institutes of Health).

Antisera. Antisera to peptide conjugates were raised in goats by subcutaneous immunization in two sites at day 0 with 28 mg of conjugate suspended in 1 cc of complete Freund's adjuvant followed by booster immunizations approximately every 2 weeks for 2–3 months in incomplete Freund's adjuvant. Serum was collected before immunization and 2 weeks after boosting.

Immunoassays. Binding of antibodies from HIV-seropositive subjects or from immunized animals to *env*-encoded synthetic peptides in RIA was determined as previously described (22). Immunoblotting (23) and radioimmunoprecipitation (RIP, ref. 22) assays were done as previously described.

Neutralization and Syncytium Formation Assays. HIV neutralization assays were done as described (9) by incubating 25–100 infectious units of HTLV-III_B, HTLV-III_{MN}, or HTLV-III_{RF} with heat-inactivated (56°C, 30 min) serum samples for 30 min at 37°C before incubation of virus with H9 cells (10^5 cells per well). After infected H9 cells were grown in culture for 10 days, supernatants were tested for reverse transcriptase activity. Inhibition of syncytium formation assays was done in microtiter plates (Costar A/2 cluster 96 wells) by mixing 7.5×10^4 uninfected CEM cells with 5×10^3 CEM cells infected with HTLV-III_B or HTLV-III_{RF} with or without anti-gp120 antibodies as described (10). After incubation (24 hr, 37°C in 5% CO_2/95% air), the number of giant cells in duplicate wells was determined by microscopic examination (×40 magnification).

RESULTS

HIV Patient Antibody Reactivity to Synthetic Peptides from HTLV-III_B gp120. Sera from AIDS patients (n = 12) were tested in RIA for antibody reactivity to synthetic peptides SP-1, -10, -10A, -11, -14, -15, and -22 containing hydrophilic amino acid sequences from HTLV-III_B gp120 (Fig. 1*A*). Six of 12 sera (50%) reacted with peptide SP-10 by greater than three times the mean control cpm value obtained with normal human serum controls (n = 4). By the same criterion, 7 of 12 sera had positive reactivity to peptide SP-22 from the COOH terminus of gp120 (22), used here as a positive control. To determine the reactivity of large numbers of AIDS patient sera with SP-10, a more extensive screen of 175 sera from HIV-seropositive individuals was undertaken. We found that 21% of seropositive subjects had positive reactivity to SP-10-III_B in RIA (data not shown).

To evaluate further the reactivity of AIDS patient antibodies to synthetic peptide SP-10-III_B, an affinity column containing BSA–peptide conjugate SP-10-III_B was used to isolate peptide-specific anti-gp120 antibodies from 2-ml aliquots of an AIDS patient serum with antibody reactivity to SP-10. Affinity columns containing equal amounts of synthetic peptides SP-10A, -11, -14, -15, and -22 coupled to BSA were used as controls. Antibodies eluted from affinity columns with 4 M $MgCl_2$ and dialyzed were tested for reactivity to HTLV-III_B *env*-encoded synthetic peptides in RIA and ^{125}I-labeled gp120-III_B in RIP assay. Antibodies recovered from SP-10-III_B, SP-10A-III_B, and SP-22-III_B affinity columns reacted specifically with the corresponding peptide in RIA but not to other *env*-encoded synthetic peptides (data not shown). When tested in RIP assay, antibodies recovered from SP-10-III_B, SP-10A-III_B, and SP-22-III_B affinity resins all immunoprecip-

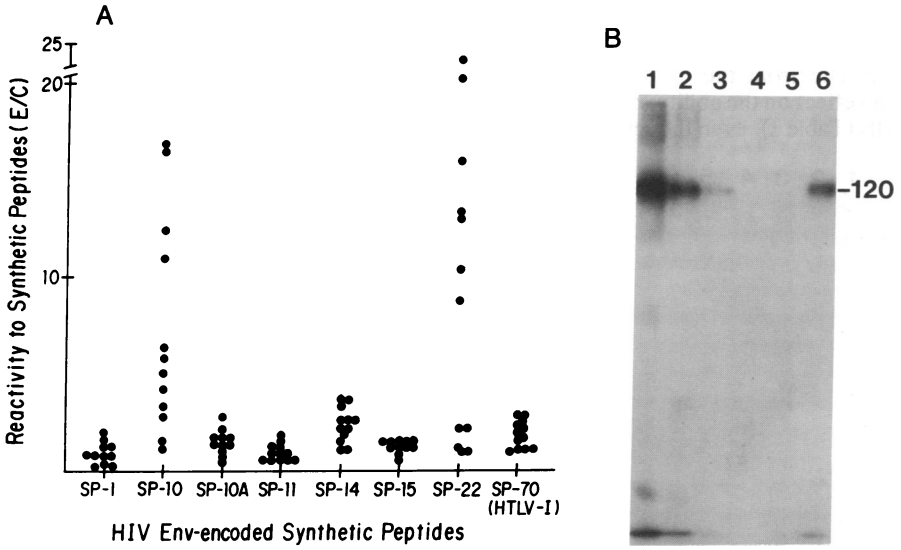

FIG. 1. Reactivity of AIDS patient antibodies to HTLV-III_B *env*-encoded synthetic peptides. (*A*) Sera from 12 AIDS patients were tested in RIA for reactivity to synthetic peptides SP-1, -10, -10A, -11, -14, -15, and -22 containing amino acid sequences derived from HTLV-III_B gp120. Synthetic peptide 70A from HTLV-I gp46 envelope molecule was used as a negative control. Data are expressed as a ratio of experimental to control cpm values (E/C). Ratios >3.0 were considered positive. Four normal human sera were used to establish control cpm values. (*B*) Affinity columns containing synthetic peptides SP-1, -10, -10A, -11, -14, -15, and -22 were used to isolate antipeptide antibodies from an AIDS patient serum. Affinity purified antibodies were then tested for reactivity to gp120 in RIP assay. Antibodies recovered from columns containing SP-10 (lane 1), SP-10A (lane 2), and SP-22 (lane 6) precipitated ^{125}I-labeled gp120 in RIP assays, whereas eluates from columns containing SP-11, -14, or -15 did not.

itated ^{125}I-labeled gp120-III$_B$ (Fig. 1*B*), whereas eluates from affinity columns containing SP-11, -14, and -15 did not. In addition, affinity-purified human anti-SP-10-III$_B$ antibodies bound to the surface of H9 cells infected with HTLV-III$_B$ in indirect immunofluorescence assay using flow cytofluorometry (data not shown). Because of data indicating that AIDS patient antibodies bound to SP-10, we next evaluated the ability of SP-10 to evoke a neutralizing antibody response in animals.

Goat Antisera to SP-10-III$_B$ Bound to gp120-III$_B$. HTLV-III$_B$ *env*-encoded synthetic peptide SP-10-III$_B$ conjugated to TT was used to raise antisera to gp120-III$_B$ in two goats. Antisera and preimmune serum controls were then evaluated in three assays: RIA for antipeptide reactivity, immunoblotting assays for reactivity to gp120, and indirect immunofluorescence using flow cytofluorometry for antibody binding to the surface of HIV-infected cells. Goat antiserum raised to SP-10-III$_B$ reacted specifically with SP-10-III$_B$ conjugated to BSA in RIA but not to the gp120 COOH-terminal peptide SP-22–BSA used as a negative control (data not shown). Conversely, goat antiserum to SP-22-III$_B$–TT reacted specifically with SP-22-III$_B$–BSA but not to SP-10-III$_B$–BSA (data not shown). When tested in immunoblot assay against lysates of HTLV-III$_B$-infected cells, goat anti-SP-10-III$_B$ antiserum reacted with gp120-III$_B$ (Fig. 2). When tested in indirect immunofluorescence analysis against uninfected CEM cells and against CEM cells infected with either HTLV-III$_B$ or HTLV-III$_{RF}$, goat anti-SP-10-III$_B$ antiserum reacted only with the surface of HTLV-III$_B$-infected cells (Fig. 3).

Goat Antisera to Peptides SP-10-III$_B$ and SP-10-III$_{RF(A)}$ Inhibit Syncytium Formation and Neutralize HIV Isolates HTLV-III$_B$ and HTLV-III$_{RF}$ in a Type-Specific Manner. Antisera to SP-10-III$_B$–TT raised in two goats inhibited syncytium formation by HTLV-III$_B$ and also neutralized HTLV-III$_B$ infection of H9 cells in culture (Table 1). In contrast, anti-SP-10-III$_B$ antisera did not neutralize HTLV-III$_{RF}$ or HTLV-III$_{MN}$ or inhibit syncytium formation by these isolates. Conversely, goat antiserum to peptide SP-10-III$_{RF(A)}$ containing an homologous amino acid sequence from HIV isolate HTLV-III$_{RF}$, gp120 inhibited syncytium formation by HTLV-III$_{RF}$, but had no effect on syncytium formation induced by HTLV-III$_B$ or HTLV-III$_{MN}$. Control antisera raised to synthetic peptide SP-22-III$_B$–TT conjugate containing a conserved amino acid sequence from the COOH terminus of HTLV-III$_B$ gp120 had no effect on the ability of the three HIV isolates to infect H9 cells (Table 1), even though goat anti-SP-22 antiserum reacted with HTLV-III$_B$ gp120 in immunoblot assay (data not shown). Also, antiserum to the TT carrier treated with cross-linking agent MBS (TT–MBS) did not inhibit HIV-induced syncytium formation (Table 1). Fig. 4 shows a titration of goat no. 70 anti-SP-10-III$_B$ antiserum in neutralization assays with HTLV-III$_B$. The antibody titer for 80% neutralization of HTLV-III$_B$ with antiserum obtained after three immunizations (second bleed) was 1:600.

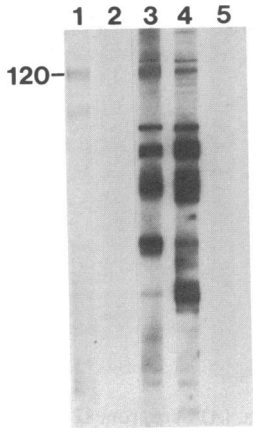

FIG. 2. Reactivity of anti-SP-10-III$_B$ antiserum to gp120 in immunoblot assay. Antiserum raised in a goat to SP-10-III$_B$-TT was tested in immunoblot assay for reactivity to gp120 in lysates of H9 cells infected with HTLV-III$_B$. Lanes: 1, reactivity of anti-SP-10-III$_B$ antiserum to gp120; 2, preimmune goat serum; 3, AIDS patient serum no. 1; 4, AIDS patient serum no. 2; 5, normal human serum.

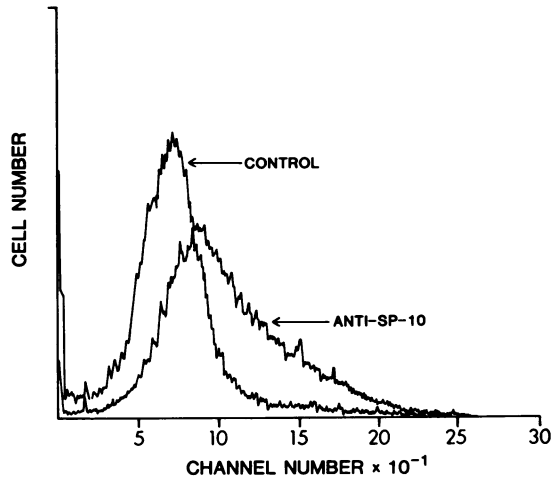

FIG. 3. Goat anti-SP-10-III$_B$ serum binds to HTLV-III$_B$ but not to HTLV-III$_{RF}$-infected H9 T cells. The reactivity of goat anti-SP-10 serum and autologous preimmune control sera was compared on either uninfected CEM cells, CEM cells infected with HIV isolate HTLV-III$_B$, or CEM cells infected with HIV isolate HTLV-III$_{RF}$, using flow cytofluorometry and a Coulter EPICS V cytofluorograph. Goat anti-SP-10 serum (1:200) reacted with 40% of HTLV-III$_B$-infected CEM T cells compared to HTLV-III$_B$-infected CEM cells incubated with control (preimmune) goat serum (1:200). Neither goat anti-SP-10 nor control (preimmune) serum (1:50) reacted with noninfected CEM cells (data not shown). Also, neither control (preimmune) nor anti-SP-10 serum (1:50) bound to CEM cells infected with the HTLV-III$_{RF}$ isolate of HIV (data not shown). As a positive control, antibodies from AIDS patient serum reacted with 45% of CEM cells infected with HTLV-III$_B$ and 42% of CEM cells infected with HTLV-III$_{RF}$.

DISCUSSION

In this study we describe the use of *env*-encoded synthetic peptides coupled to TT to raise high titers of isolate-specific, anti-HIV neutralizing antibodies. Goat antisera were raised in two goats to synthetic peptide SP-10-III$_B$, containing at the COOH terminus an amino acid sequence from a hydrophilic, hypervariable region of HTLV-III$_B$ gp120. Sera from both goats neutralized HTLV-III$_B$ and inhibited HTLV-III$_B$-induced syncytium formation but did not inhibit infection of T cells with HIV isolates HTLV-III$_{RF}$ or HTLV-III$_{MN}$. Moreover, when six amino acids (Thr-Arg-Pro-Asn-Asn-Asn) from the NH_2-terminal region of SP-10-III$_B$ were deleted, the resulting synthetic peptide (SP-10C-III$_B$) also induced type-specific antibodies that neutralized only HTLV-III$_B$ as determined by inhibition of syncytial cell formation (Table 1). These six NH_2-terminal amino acids of SP-10-III$_B$ are highly conserved among HIV isolates, whereas the COOH-terminal sequence of SP-10-III$_B$ is highly variable. Thus, deletion of a conserved region at the NH_2 terminus of SP-10-III$_B$ would not be expected to abolish the ability of peptide SP-10C-III$_B$ to evoke a type-specific antibody response to HTLV-III$_B$. Also, the homologous peptide SP-10-III$_{RF(A)}$ containing an amino acid sequence derived from HTLV-III$_{RF}$ gp120 elicited antibodies that blocked syncytium formation by isolate HTLV-III$_{RF}$ but not by HTLV-

Table 1. Effect of anti-SP-10 antisera on infectivity of HIV isolates HTLV-III_B, HTLV-III_{RF}, and HTLV-III_{MN}

Goat	Inoculum*	Immunizations, no.	Time postimmunization, days	Syncytium inhibition using HIV isolates†			Neutralization of HIV isolates‡		
				III_B	III_{RF}	III_{MN}	III_B	III_{RF}	III_{MN}
70	SP-10-III_B-TT	0	0	−	−	−	<10	<10	<10
		2	29	−	−	−	50	<10	<10
		3	72	+ (40)	−	−	600	<10	<10
		4	96	+ (80)	−	−	250	<10	<10
		5	112	+ (80)	−	−	ND	ND	ND
		6	131	+ (160)	−	−	ND	ND	ND
86	SP-10-III_B-TT	0	0	−	−	−	<10	<10	<10
		1	19	−	−	−	<10	<10	<10
		2	23	+ (10)	−	−	100	<10	<10
		3	48	+ (10)	−	−	ND	ND	ND
69	SP-10C-III_B-TT	0	0	−	−	−	<10	ND	ND
		3	43	+ (20)	−	−	40	ND	ND
76	SP-10-$III_{RF(A)}$-TT	0	0	−	−	−	ND	ND	ND
		1	15	−	+ (40)	−	ND	ND	ND
		2	29	−	+ (80)	−	ND	ND	ND
		3	43	−	+ (80)	−	ND	ND	ND
		4	62	−	+ (160)	−	ND	ND	ND
84	SP-22-III_B-TT (Control)	0	0	−	−	−	<10	<10	<10
		2	23	−	−	−	<10	<10	<10
		3	48	−	−	−	<10	<10	ND
		4	63	−	−	−	<10	<10	ND
		5	81	−	−	−	<10	<10	ND
80	TT-MBS (Control)	0	0	−	−	−	<10	<10	ND
		2	21	−	−	−	<10	<10	ND
		3	36	−	−	−	<10	<10	ND
		4	54	−	−	−	<10	<10	ND

*Synthetic peptides were coupled to TT with MBS.
†Values in parentheses are the inverse of serum dilutions that inhibited the number of syncytia (60–80 per well) by >80%.
‡Neutralization was determined by evaluation of reverse transcriptase activity in supernatants of H9 cells cultured for 10 days in the presence of 100 infectious units of HIV isolates. Values are inverse of antiserum dilutions that inhibited reverse transcriptase activity by >80%. ND, not done.

III_B or HTLV-III_{MN}. The extracellular envelope domains of isolates HTLV-III_B and HTLV-III_{RF} differ in 21.4% of amino acids (14). Despite this divergence, the SP-10 region of gp120 from both HIV isolates appears to be an important target for virus neutralization. These data suggest that synthetic peptides SP-10-III_B, SP-10-$III_{RF(A)}$, and homologous peptides from other HIV isolates might be combined in a synthetic vaccine capable of inducing a polyvalent, neutralizing-antibody response to divergent HIV isolates. Data presented here are unique in that, while there have been a

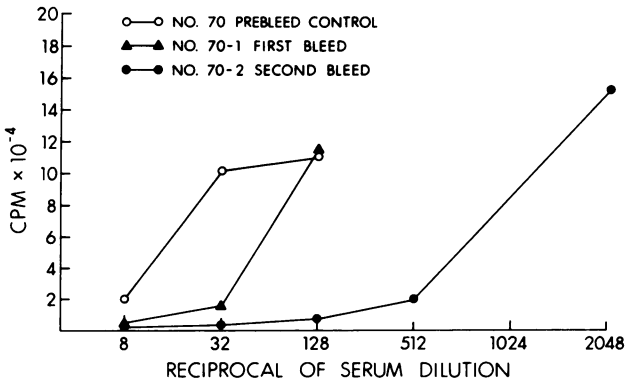

FIG. 4. Neutralization of HTLV-III_B with goat antisera to synthetic peptide SP-10 containing an amino acid sequence from HTLV-III_B gp120. Shown are cpm values obtained in reverse transcriptase assay when HTLV-III_B and goat sera were preincubated and then cultured for 10 days with H9 T cells. ○, Preimmune serum; ▲, anti-SP-10 antiserum, first boost; ●, anti-SP-10 antiserum, second boost.

number of studies on the use of HIV *env*-encoded synthetic peptides to raise neutralizing anti-gp120 antibodies (24–26), type-specific neutralization of HIV with antisera against the variable sequences of the SP-10 region has not been shown. Moreover, there are no reports of antisynthetic peptide antisera that can block HIV-induced syncytium formation.

Syncytium induction by HIV is mediated by the interaction of the CD4 molecule with gp120 on the surface of cells (27–33). Because antibodies directed to the SP-10 region of gp120 inhibited syncytium induction by HIV, the SP-10 region of gp120 must either contain or be closely associated with sites of gp120 that interact with the CD4 molecule. Recently, two groups (33, 34) have mapped sites of gp120–CD4 interaction to regions of the COOH-terminal half of gp120, none of which include the SP-10 region. These results indicate that the SP-10 region does not interact directly with CD4; rather, antibody binding to the SP-10 region may either sterically inhibit gp120–CD4 interaction or interfere with an event occurring after binding. In this regard, we have observed that the SP-10 peptide itself and goat antibodies to SP-10 do not inhibit antigen-induced proliferative responses of normal peripheral blood T cells (B.F.H. and T.J.P., unpublished work), and thus do not interfere with normal CD4 function—an important prerequisite for the use of SP-10 as a vaccine component for AIDS.

In this study we have used SP-10-like peptides coupled to the carrier molecule TT. However, other carrier molecules may be more appropriate for use in anti-HIV vaccines. For instance, Cease *et al.* (35) have identified two major helper T-cell epitopes of gp120 (T1 and T2) using a computer program to predict amino acid sequences found in amphipathic α-helices. Because it is likely that maximal protective

responses to HIV will require primed preexisting T-cell as well as B-cell responses, it will be important to covalently link SP-10-like peptides to peptides containing appropriate helper T-cell epitopes and to evaluate these conjugates for their ability to induce anti-HIV neutralizing antibodies and, as well, to induce HIV-specific T-cell responses in primates.

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Note Added in Proof. Since submission of this manuscript, Rusche *et al.* (36) have also identified a region overlapping the SP-10 region of HIV gp120 that is important in type-specific neutralization of HIV.

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