Analysis of the Cross-Reactive Anti-gp120 Antibody Population in Human Immunodeficiency Virus-Infected Asymptomatic Individuals

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This study was undertaken to analyze the specificity and neutralizing properties of cross-reactive anti-gp120 antibodies (Abs) in the sera of two human immunodeficiency virus (HIV)-infected asymptomatic individuals. Two panels of murine monoclonal anti-idiotypic Abs (anti-id MAbs) were established against cross-reactive polyclonal anti-gp120 Abs purified from HIV⁺ sera by sequential affinity chromatography using gp120_{SF2}- and gp120_{IIIB}-Sepharose columns. These panels of anti-id MAbs were then used to affinity purify idiotype-positive (Id⁺) anti-gp120 Abs from HIV⁺ sera. The recovery of each of these Id⁺ Abs by purification indicated that several idiotypically distinct cross-reactive anti-gp120 Abs are present in sera over a wide range of concentrations. Immunological and biological studies showed that although all of the Id⁺ Abs were reactive against gp120_{SF2} and gp120_{IIIB}, they exhibited unique epitope specificities and distinct neutralizing activities. Most of the Id⁺ Abs were directed against epitopes in the CD4 attachment site (CD4 site epitopes) of gp120 and exhibited a spectrum of broadly neutralizing activities. On the other hand, a minor population of Id⁺ Abs showed specificity for the V3 region of gp120 and exhibited limited cross-neutralizing activities. Together, these studies indicate that the CD4 site epitope-specific Abs are heterogeneous with respect to their clonality, neutralizing activity, and concentration in sera. This heterogeneity suggests that anti-gp120 Abs to the CD4 attachment site are developed in response to multiple overlapping epitopes present on the original virus isolate and/or epitopes on mutated variants which emerged over time.

Envelope glycoprotein gp120 of human immunodeficiency virus type 1 (HIV-1) contains several epitopes capable of inducing neutralizing antibodies (Abs) in infected individuals. The third hypervariable region (V3) of gp120 is one such region and is known as the major neutralizing epitope (5, 14, 28, 32). Most Abs to this region which have been elicited by either vaccination or natural infection exhibit strain- or type-specific neutralizing activity (5, 14, 18, 28, 32). The hypervariability of the envelope region outside, as well as within, the V3 region suggests that HIV mutants that escape neutralization by V3-specific Abs emerge over time (19).

A second significant neutralizing target of gp120 resides in its CD4 attachment site (CD4 site epitopes). The epitopes in this site are conformationally dependent (3, 7, 30) and composed of several discontinuous regions (11, 22, 31). Recently, we demonstrated that human Abs specific for the CD4 site epitopes, isolated from the sera of HIV-infected individuals, possess broadly neutralizing activity (10). Several examples of human monoclonal Abs (MAbs) specific for this region have also been shown to have broadly neutralizing activity (7, 24, 33). Moreover, functional analysis of Abs with different specificities for this region suggest a diversity in the neutralizing epitopes in this region (4, 6).

Despite extensive studies on different neutralizing targets of gp120, no comprehensive analysis of cross-reactive anti-gp120 Abs elicited by natural infection has been reported.

Characterization of different cross-reactive anti-gp120 Abs in sera of HIV-infected individuals should yield insights into the humoral response against gp120, as well as assist in the design of a broadly effective vaccine.

In this study, polyclonal anti-gp120 Abs cross-reactive to both gp120_{SF2} and gp120_{IIIB} were isolated from the sera of two individuals infected with an MN-like strain of HIV. Two panels of anti-idiotypic (anti-id) MAbs specific for the two preparations of Abs were established and used to purify clonally distinct populations of anti-gp120 Abs bearing unique idiotypes (Id⁺ Abs). Immunochemical and biological analyses of these Id⁺ Abs demonstrated that most of the cross-reactive anti-gp120 Abs in the sera of two HIV-infected asymptomatic individuals are directed to epitopes within the CD4 attachment site whereas a minor population of these Abs is directed to the V3 region.

MATERIALS AND METHODS

Recombinant proteins. Recombinant gp120_{IIIB}, a glycoprotein of HIV_{IIIB} origin, is secreted from a *Drosophila* cell line and was obtained from Smith-Kline Beecham, King of Prussia, Pa. Recombinant gp120_{SF2}, an envelope glycoprotein of HIV_{SF2} origin, is secreted from Chinese hamster ovary (CHO) cells and was obtained from Chiron, Emeryville, Calif. The quality of recombinant gp120 molecules was assessed by a radioimmunoassay, which revealed good binding of both types of gp120 to the CD4 molecules on SupT1 cells. Recombinant soluble CD4 (sCD4) was prepared by transfection of a truncated CD4 gene into CHO cells. The

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sCD4 secreted into the culture fluid of CHO cells (representing 95% of the amino-terminal extracellular portion of the molecule) was purified on an anti-CD4 Ab-conjugated Sepharose column.

Isolation of anti-gp120 Abs. Cross-reactive anti-gp120 Abs were isolated from the sera of HIV-infected individuals by affinity chromatography. Serum, inactivated by detergent (1% Nonidet P-40) and heat (1 h at 56°C), was first passed over a gp120_{SF2}-Sepharose 4B column. After the column was washed extensively with phosphate-buffered saline (PBS), the column-bound Ab was eluted with citrate buffer at pH 2.8 and dialyzed against PBS. The anti-gp120_{SF2} Abs that cross-reacted with gp120_{IIIB} were then isolated by affinity chromatography by using a gp120_{IIIB}-Sepharose column and conditions similar to those described above.

Development of anti-id MABs. Anti-id MABs specific for cross-reactive anti-gp120 Abs were developed by methods previously described by us (4). BALB/c mice were immunized with cross-reactive affinity-purified anti-gp120 Abs isolated from two HIV⁺ sera. All immunizations consisted of two subcutaneous injections of 50 µg of Ab in syntex Adjuvant Formulation (SAF) containing 25 µg of *N*-acetyl-muramyl-L-alanyl-D-isoglutamine (Syntex, Palo Alto, Calif.) at 2-week intervals and were followed by a single intraperitoneal injection of 50 µg of Ab in incomplete Freund adjuvant 3 days prior to fusion. Splenocytes were fused with the cell line SP-2/0, and the resulting hybrids were selected in hypoxanthine-aminopterin-thymidine medium. The culture fluids were tested by enzyme-linked immunosorbent assay (ELISA), and the positive clones were subcloned at least twice. Anti-id MABs were purified from the ascites by using protein A-Sepharose columns.

ELISA. Screening of hybridomas secreting anti-id MABs was performed by previously described methods (4). Immulon microtiter plates (Dynatech Laboratory, Chantilly, Va.) were coated at 300 ng per well with the following human immunoglobulin (Ig) preparations in PBS: the cross-reactive anti-gp120 Abs used for immunizations, protein G-purified Ig preparations from the same serum depleted of anti-gp120 Abs and myeloma proteins representing all isotype combinations of the human heavy and light chains (Binding Site, San Diego, Calif.). Culture fluids from hybridomas were added to Ig-coated wells and incubated for 3 h. The specific binding activity of MABs in culture fluid to human Ig preparations was detected by goat anti-mouse Ig Abs coupled to alkaline phosphatase and a phosphatase substrate (Sigma, St. Louis, Mo.). To test the binding activities of HIV⁺ serum and the Id⁺ Abs to V3 region-derived peptides, microtiter plates were coated overnight with 0.25 µg of each peptide per well in PBS. Different concentrations of Id⁺ Abs or dilutions of serum were added to the wells and incubated for 2 h. Ab-binding activity was detected by goat anti-human Ig (heavy- and light-chain) Abs coupled to alkaline phosphatase and a phosphatase substrate. The sequences of V3 region peptides from HIV-1 strains used for binding assays were as follows: strain SF2, TRPNNTRKSIYIGPGRAFH TTGRIIGDIRKAHC; strain MN, YNKRKRIHIGPGRAFYT TTKNIIG; strain IIIB, NNTRKSIRIQRGPGRAFVTIGK IG; strain RF, NNTRKSITKGPGRVIYATGQIIIG; strain SC, NNTTSHIHIGPGRAFYTGDIIIG; strain WMJ-2, NN VRRSLSIGPGRAFRTREGN.

Isolation of Id⁺ Abs. Id⁺ Abs from HIV⁺ sera were isolated by methods described earlier (4). Briefly, 80 to 160 ml of the serum used for cross-reactive anti-gp120 Ab preparations was applied to an anti-id Ab-Sepharose 4B

TABLE 1. Binding of sera to peptides derived from the V3 regions of various gp120

Serum and dilution	Binding ^a to peptide from:					
	MN	SF2	IIIB	RF	SC	WMJ-2
I						
1/100	>3.0	2.08	0.34	0.38	1.38	1.33
1/200	2.73	1.36	0.14	0.14	0.80	0.75
1/400	1.51	0.80	0.06	0.06	0.37	0.33
1/800	0.57	0.56	0.06	0.03	0.15	0.16
II						
1/100	>3.0	1.30	0.36	0.29	1.70	1.20
1/200	2.74	0.76	0.16	0.13	1.05	0.65
1/400	2.01	0.45	0.06	0.07	0.56	0.29
1/800	1.11	0.30	0.03	0.04	0.28	0.12

^a Specific binding of sera I and II to V3 region peptides derived from gp120_{MN}, gp120_{SF2}, gp120_{IIIB}, gp120_{RF}, gp120_{SC}, and gp120_{WMJ-2} was determined by ELISA. Microtiter plates were coated with V3 peptides at 250 ng per well. Binding activity is expressed as optical density at 405 nm.

column. After extensive washing, the bound Id⁺ Abs were eluted with citrate buffer, pH 2.8, and dialyzed against PBS.

Radioimmunoassay. Recombinant gp120_{SF2} and gp120_{IIIB} were iodinated by Bolton-Hunter reagent (NEN, Wilmington, Del.). To analyze epitope specificities, microtiter plates were coated for 18 h with 400 ng of Id⁺ Abs in PBS and blocked for 1 h with 1% bovine serum albumin in PBS. After blocking, ¹²⁵I-labeled gp120_{SF2/IIIB} (100,000 cpm/50 µl per well), preincubated for 1 h with or without sCD4 (at 2.5 µg/50 µl per well), was added to microtiter plates. Following 3 h of incubation, the plates were washed and the bound radioactivity was measured. For the competitive-inhibition assay, plates were coated with 200 ng of Id⁺ Abs in PBS. Radiolabeled gp120 (50,000 cpm/50 µl per well) and various concentrations of Id⁺ Abs were preincubated for 1 h, and the mixtures were added to the wells. At 3 h later, the plates were washed and the bound radioactivity per well was measured. Percent inhibition was calculated as [1 - (cpm with inhibitor/cpm without inhibitor)] × 100.

Neutralization assay. A quantitative neutralization assay with HIV-1 laboratory strains was performed as previously described (16, 35). Monolayers of CEM-SS target cells were cultured with different HIV strains in the presence or absence of different neutralizing Abs, and 3 to 5 days later, numbers of syncytium-forming units were determined. An equivalent amount of virus was used in the assays to allow direct comparison of the various Ab concentrations tested. The assays were repeatable over a virus-surviving fraction range of 1 to 0.001 within a range of two- to fourfold differences in Ab concentration ($P < 0.001$).

RESULTS

Characterization of HIV⁺ sera. Sera from two HIV⁺ asymptomatic individuals (sera I and II) were selected for analysis of cross-reactive neutralizing anti-gp120 Ab populations. Prior to analysis, the virus strains responsible for the seroconversion of these individuals were determined. Since the V3 region of gp120 is specific for each HIV isolate, analysis of the humoral response to this region indicated the strain of HIV that had infected these individuals. Therefore, the sera were tested for the ability to bind to V3 region-derived peptides. As indicated in Table 1, both sera showed strong binding activity to the MN V3 peptide and weak

TABLE 2. Specificity of anti-id MAbs

Serum and anti-id MAb	Binding ^a to:	
	Anti-gp120 Ab ^b	Normal Ig ^c
I		
AB	1.903	0.087
AC	0.162	0.059
AD	0.740	0.070
AL	0.411	0.031
AO	1.307	0.042
AP	0.401	0.099
BZ	0.465	0.033
BJ	0.162	0.049
II		
CP	0.291	0.057
CX	0.234	0.070
DA	0.205	0.037
DI	0.413	0.037
FE	0.475	0.062
FD	0.578	0.096

^a Specific binding of anti-id MAbs to anti-gp120 from serum I or II was tested by ELISA. Microtiter plates were coated with 300 ng of anti-gp120 or normal Ig per well. Binding activity is expressed as optical density at 405 nm.

^b Anti-gp120 Abs were isolated from either serum I or serum II by affinity purification on gp120_{SF2}- and gp120_{IIIB}-conjugated Sepharose columns.

^c Normal Igs were prepared from anti-gp120 Ab-depleted serum I or serum II by affinity purification using protein G.

binding to SC V3 and WMJ-2 V3 peptides. This suggests that the donors of the HIV⁺ sera were infected with MN or an MN-like strain of HIV-1. When tested for neutralizing activity against laboratory strains MN, IIIB, and RF, both sera neutralized all three strains effectively. The neutralizing activity against MN was only slightly higher than that of the other two strains, indicating that in addition to MN V3-specific neutralizing Abs, the sera contained a substantial amount of broadly neutralizing Abs (data not shown).

Isolation of different populations of cross-reactive anti-gp120 Abs by anti-id MAbs. To fractionate different cross-reactive anti-gp120 Ab populations in sera, two panels of anti-id MAbs were established. Total cross-reactive anti-gp120 Abs from HIV⁺ sera I and II were independently prepared by sequential affinity purification on two different gp120 (IIIB and SF2)-conjugated Sepharose columns. The purified Abs were used to immunize mice for development of anti-id MAbs. The anti-id MAbs that showed specific binding to the total cross-reactive anti-gp120 Abs but not to the anti-gp120-depleted normal Ig fraction of the sera were selected for further analysis (Table 2). By this criterion, 34 anti-id MAbs to serum I anti-gp120 Abs and 19 anti-id MAbs to serum II anti-gp120 Abs were selected for further analysis.

To determine whether the anti-id MAbs cross-recognize the same population of anti-gp120 Abs, the Id⁺ Ab corresponding to each anti-id MAb was isolated from the sera by using anti-id MAb-Sepharose columns. The Id⁺ Ab isolated by each anti-id MAb was then tested for binding activity to the other anti-id MAbs in the panel. In this assay, 8 of 34 anti-id MAbs specific for serum I Abs and 6 of 19 anti-id MAbs specific for serum II Abs recognized unique idiotopes in the cross-reactive anti-gp120 Ab populations (data not shown). The rest of the anti-id MAbs recognized the same Id⁺ Ab specific for the anti-id MAbs listed in Table 2. In addition, on the basis of their recovery from sera by affinity purification, the concentrations of the individual Id⁺ Abs in

TABLE 3. Composition of Id⁺ Abs in HIV⁺ sera

Serum and Id ⁺ Ab	Concn ^a (μg/ml)
I	
AB ⁺	50
AC ⁺	23
AD ⁺	25
AL ⁺	2.7
AO ⁺	4
AP ⁺	12
BJ ⁺	5.8
BZ ⁺	8.8
II	
CP ⁺	36
CX ⁺	21
DA ⁺	6.7
DI ⁺	8.3
FD ⁺	17
FE ⁺	5.1

^a Total recovery of different Id⁺ Abs from serum I or II by affinity chromatography was used as an index to estimate the concentration of each Id⁺ Ab. The serum was repeatedly passed through each anti-id MAb column until each serum sample was depleted of the Id⁺ Ab.

the sera were estimated (Table 3). The data indicate that the sera contained different populations of cross-reactive anti-gp120 Abs over a wide range of concentrations.

Characterization of different cross-reactive anti-gp120 Abs.

The immunological properties of various Id⁺ Abs that constitute the cross-reactive anti-gp120 Ab were analyzed. (i) Their direct binding to recombinant gp120s from two different HIV strains was determined. All of the Id⁺ Abs from serum I, except AD⁺ Ab and AP⁺ Ab, bound very effectively to gp120_{IIIB} and gp120_{SF2} (Table 4). Only weak reactivity was detected in the binding of AD⁺ and AP⁺ Abs to gp120_{IIIB} (Table 4). Most of the Id⁺ Ab from serum II also exhibited strong binding activity to both recombinant gp120s. However, FD⁺ Ab bound to gp120_{IIIB} very weakly (Table 5). (ii) The ability of sCD4 to inhibit the binding of Id⁺ Abs to gp120 was determined. sCD4 inhibited the binding of

TABLE 4. Effect of sCD4 on binding of gp120 to Id⁺ Abs isolated from serum I

Id ⁺ Ab ^a and controls	Binding of ¹²⁵ I-labeled gp120 ^b to Id ⁺ Abs			
	gp120 _{IIIB}		gp120 _{SF2}	
	- sCD4	+ sCD4	- sCD4	+ sCD4
AB ⁺	17,225	5,452	11,482	3,955
AC ⁺	14,198	4,454	9,226	2,723
AD ⁺	558	644	17,053	15,016
AL ⁺	13,183	5,578	9,945	4,165
AO ⁺	15,631	6,613	11,557	4,206
AP ⁺	589	854	2,941	3,060
BJ ⁺	6,016	1,244	4,452	945
BZ ⁺	14,006	3,810	10,464	3,185
F105 ^c	6,206	1,695	13,749	2,619
Normal Ig	133	121	114	122

^a Id⁺ Abs were isolated by using anti-id MAbs developed to the cross-reactive anti-gp120 Abs of serum I or II.

^b Binding of ¹²⁵I-labeled gp120 (SF2 and IIIB) to Id⁺ Abs is expressed as counts per minute bound to microtiter wells coated with 0.4 μg of the respective Id⁺ Ab per well in the absence (- sCD4) or the presence (+ sCD4) of 10 μg of sCD4 per ml.

^c Human neutralizing MAb specific for the CD4 site epitope (24).

TABLE 5. Effect of sCD4 on binding of gp120 to Id⁺ Abs isolated from serum II

Id ⁺ Ab ^a and control	Binding of ¹²⁵ I-labeled gp120 ^b to Id ⁺ Abs			
	gp120 _{IIIB}		gp120 _{SF2}	
	- sCD4	+ sCD4	- sCD4	+ sCD4
CP ⁺	10,765	4,224	4,250	1,773
CX ⁺	2,099	1,863	8,299	6,986
DA ⁺	6,592	2,434	4,566	1,856
DI ⁺	5,497	2,220	5,749	3,217
FE ⁺	8,972	3,224	3,300	1,037
FD ⁺	425	528	4,648	2,676
Normal Ig	150	111	123	133

^a See Table 4, footnote a.^b See Table 4, footnote b.

AB⁺, AC⁺, AL⁺, AO⁺, BJ⁺, and BZ⁺ Abs of serum I and CP⁺, DA⁺, DI⁺, and FE⁺ Abs of serum II to both gp120s. These inhibition data indicate that the Id⁺ Abs recognize epitopes within or around the CD4 attachment site of gp120. In the case of FD⁺ Ab, binding to gp120_{SF2}, but not that to gp120_{IIIB}, was weakly inhibited by sCD4 (Table 5). (iii) Binding of Id⁺ Abs to synthetic V3 region-derived peptides was determined. As listed in Table 6, AP⁺ and AD⁺ Abs of serum I and FD⁺ Ab of serum II bound to the V3 peptide, indicating that these are specific for the V3 region of gp120. However, the finding that binding of FD⁺ Ab to gp120_{SF2}

TABLE 6. Binding of Id⁺ Abs to a V3 region-derived peptide

Id ⁺ Ab and control	Conc (μg/ml)	Binding activity ^a
AB ⁺	2	0.034
	0.5	0.026
AC ⁺	2	0.036
	0.5	0.025
AP ⁺	2	0.845
	0.5	0.278
AO ⁺	2	0.041
	0.5	0.028
AD ⁺	2	1.502
	0.5	0.386
AL ⁺	2	0.042
	0.5	0.028
BJ ⁺	2	0.034
	0.5	0.028
BZ ⁺	2	0.032
	0.5	0.027
CP ⁺	2	0.034
	0.5	0.025
CX ⁺	2	0.040
	0.5	0.025
DA ⁺	2	0.032
	0.5	0.025
DI ⁺	2	0.034
	0.5	0.025
FE ⁺	2	0.029
	0.5	0.023
FD ⁺	2	0.572
	0.5	0.135
Normal IgG	2	0.039
	0.5	0.028

^a The specificity of Id⁺ Abs for the V3 region-derived peptide from gp120_{SF2} was determined by ELISA. Microtiter plates were coated with 500 ng of the V3 peptide per well. Binding activity is expressed as optical density at 405 nm.

FIG. 1. Inhibition of binding of AB⁺ (A) and CP⁺ (B) Abs to gp120_{SF2} by other id⁺ Abs. Microtiter plates were coated with 200 ng of either AB⁺ or CP⁺ Ab in PBS per well and blocked with 1% bovine serum albumin-PBS. ¹²⁵I-labeled gp120_{SF2} (50,000 cpm) preincubated with different id⁺ Abs was then added to the microtiter wells. After 3 h of incubation, the plates were washed and the bound radioactivity was counted. Percent inhibition was calculated as described in Materials and Methods. The results represent means of two experiments.

was weakly influenced by sCD4 suggests that the V3 epitope recognized by this Id⁺ Ab is located close to the CD4 attachment site of gp120_{SF2}. Although CX⁺ Ab showed binding to both gp120s, it neither bound to any of the V3 peptides nor showed specificity for the epitopes in the CD4 attachment site. Thus, we were unable to assign an epitope specificity to this Ab.

To understand the topography of the CD4 site epitopes recognized by Id⁺ Abs, the ability of each Id⁺ Ab to compete with other Id⁺ Abs for binding to radiolabeled gp120 was determined. The binding of AB⁺ Ab to gp120_{SF2} was inhibited by all CD4 site epitope-specific Id⁺ Abs of serum I but not by the V3 region-specific AD⁺ Ab (Fig. 1A). BJ⁺ Ab competed with AB⁺ Ab only partially. This suggests that either BJ⁺ Ab recognizes an epitope located distantly from that recognized by AB⁺ Ab or BJ⁺ Ab binds to gp120_{SF2} with a lower affinity than does AB⁺ Ab. The finding that the direct binding activity of BJ⁺ Ab to gp120_{SF2} is lower than that of AB⁺ Ab to gp120_{SF2} favors the latter explanation. The binding of CP⁺ Ab to gp120_{SF2} was inhibited by all Id⁺ Abs specific to CD4 site epitopes from serum II but not by FD⁺ Ab and CX⁺ Ab (Fig. 1B). In addition, when other Id⁺ Abs were tested as described above, similar inhibitions were observed (data not shown). Overall, the

FIG. 2. Neutralizing activities of id^+ Abs. The neutralizing activities of id^+ Abs from sera I (A) and II (B) were tested against three different HIV-1 isolates (RF, IIIB, and MN). Neutralizing activity is expressed as percent inhibition of syncytium formation at different id^+ Ab concentrations. Percent inhibition was calculated as $[(1 - V_n)/V_o] \times 100$, where V_n is the number of syncytium-forming units of virus in the presence of id^+ Abs and V_o is the number of syncytium-forming units in the control well without id^+ Abs. The results represent means of two experiments.

inhibition data indicate that the epitopes for the Id^+ Abs are in close proximity to one another.

Neutralizing activity of Id^+ Abs. The ability of each Id^+ Ab to neutralize three different strains of HIV-1 was determined (Fig. 2A and B). The Id^+ Abs specific for the CD4 site epitopes, as well as those to the V3 region, exhibited a broad spectrum of neutralizing activities against multiple strains of HIV-1. Among the CD4 site epitope-specific Id^+ Abs of serum I, AC^+ , AL^+ , AB^+ , and BZ^+ Abs neutralized all three strains. AO^+ Ab neutralized MN efficiently and IIIB weakly but did not neutralize the RF strain, whereas BJ^+ Ab neutralized only MN. Of the CD4 site epitope-specific Id^+ Abs from serum II, CP^+ and DI^+ Abs neutralized all three strains, whereas DA^+ and FE^+ Abs neutralized only the MN and IIIB strains. All three V3-specific Id^+ Abs of serum I

(AD^+ and AP^+ Abs) and serum II (FD^+ Ab) effectively neutralized MN but did not neutralize the IIIB strain. Both AP^+ and FD^+ Abs also neutralized the RF strain. The CX^+ Ab, with unknown specificity, failed to neutralize any of the strains tested. In general, CD4 site epitope-specific Id^+ Abs exhibited a spectrum of broadly neutralizing activities, while V3-specific Id^+ Abs neutralized a single strain efficiently with limited cross-neutralizing activity (Fig. 2A and B).

DISCUSSION

We analyzed the different populations of cross-reactive Abs in two asymptomatic individuals infected with an MN-like strain of HIV-1. This analysis was achieved by isolation and characterization of various populations of Abs (Id^+ Abs)

TABLE 7. Immunological and biological properties of Id⁺ Abs isolated from sera I and II

Serum and Id ⁺ Ab	Concn (μg/ml) in serum	Binding to:			Inhibition of binding to gp120 by sCD4 ^a	Virus neutralization ^b		
		gp120 _{SF2} ^c	gp120 _{IIIB} ^c	V3 peptide ^d		MN	IIIB	RF
I								
AB ⁺	50	+++	+++	—	+	++	+++	+
AC ⁺	23	+++	+++	—	+	+	+++	++
AD ⁺	25	+++	+	+	—	+++	—	—
AL ⁺	2.7	++	++	—	+	++	+	+
AO ⁺	4	+++	+++	—	+	+++	+	—
AP ⁺	12	++	+	+	—	+++	—	++
BJ ⁺	5.8	++	++	—	+	+	—	—
BZ ⁺	8.8	+++	+++	—	+	++	++	+
II								
CP ⁺	36	++	+++	—	+	++	+++	++
CX ⁺	21	+++	++	—	—	—	—	—
DA ⁺	6.7	+++	++	—	+	+	+	—
DI ⁺	8.3	+++	+++	—	+	+	++	+
FE ⁺	5.1	++	+++	—	+	+	++	—
FD ⁺	17	++	+	+	— ^e	+++	—	+

^a Inhibition of binding is expressed as follows: —, no inhibition; +, inhibition.

^b Quantitative neutralization assays of HIV-1 were performed as described in Materials and Methods, and the results are expressed as follows (IC₅₀, 50% inhibitory concentration): +++, 2 μg/ml > IC₅₀; ++, 2 μg/ml < IC₅₀ < 10 μg/ml; +, 10 μg/ml < IC₅₀ < 50 μg/ml; —, no detectable neutralizing activity below 50 μg/ml.

^c Binding is expressed as follows: —, no binding; +, weak binding; ++, moderate binding; +++, strong binding.

^d Binding is expressed as follows: —, no binding; +, binding.

^e Binding to gp120_{SF2} was weakly inhibited by sCD4 (Table 5).

by using anti-id MAbs. A summary of the data (Table 7) indicates that most of the cross-reactive anti-gp120 Abs are specific to the CD4 site epitopes and heterogeneous with respect to clonality, neutralizing activity, and concentration in serum. A minor population of the cross-reactive anti-gp120 Abs is directed to the V3 region.

Idiotypes are ubiquitous markers of Ig variable regions which are recognized by anti-id Abs (1, 12, 13, 23, 26, 29). Idiotypes can be unique and associated with an Ab of a given specificity (private Id) (1, 12) or shared by Abs with different specificities (cross-reactive Id) (1, 34). On the basis of the linkage between idiotypes and epitope specificity of an Ab, we selected anti-Id MAbs that are specific to the anti-gp120 Abs but not to Abs with unrelated specificity (normal Ig). Such anti-id MAbs were used to isolate and characterize various populations of cross-reactive anti-gp120 Abs elicited by natural infection. The advantages of this approach over other techniques, such as generation of human MAbs, are that (i) targeted Ab populations, such as the cross-reactive anti-gp120 Abs, can be comprehensively analyzed with respect to their immunological and biological properties, and (ii) by determining the concentration of each Ab population (Id⁺ Abs) in serum, the humoral response against individual epitopes accumulated over the course of infection can be estimated. The fact that the various Id⁺ Abs isolated by this anti-id approach exhibit different epitope specificities and neutralizing activities enabled us to effectively analyze the cross-reactive anti-gp120 Ab population in HIV⁺ sera.

In the testing of Id⁺ Ab binding to the V3 peptide (Table 6), three Id⁺ Abs (AP⁺, AD⁺, and FD⁺) were found to be specific to the V3 region. Although these Id⁺ Abs cross-reacted with gp120s from two strains, they showed weak binding activity to gp120_{IIIB} (Tables 4 and 5). This low level of binding explains the inability of these Id⁺ Abs to neutralize the IIIB strain of HIV. Among the V3-specific Id⁺ Abs, AP⁺ and FD⁺, but not AD⁺, neutralized the RF strain.

Furthermore, the fact that these V3-specific Id⁺ Abs were isolated from the sera of individuals who were infected with an MN-like strain of HIV could explain the stronger neutralizing activities observed with these Id⁺ Abs against the MN strain. Collectively, these data indicate that a subpopulation of V3-specific Abs in HIV-infected individuals cross-reacts with multiple strains of HIV, as described by ourselves (10) and others (8, 21). However, the ability of these Abs to neutralize multiple strains of HIV is limited mainly by their lower affinity for a certain strain.

Our earlier study showed that CD4 site epitope-specific Id⁺ Abs isolated from a pool of HIV⁺ sera exhibited a spectrum of neutralizing activities against multiple strains of HIV-1 (4). We attributed these differences in neutralizing activity to the existence of antigenically diverse epitopes around the CD4 attachment site of gp120. However, it should be pointed out that in that study the Id⁺ Abs were isolated from a pool of HIV⁺ sera from four different individuals. Since these individuals might have been infected with different strains of HIV, the diversity in the neutralizing activity of Id⁺ Abs may be the result of a pooled response to multiple strains of HIV. In contrast, in this study, we analyzed the CD4 site epitope-specific Id⁺ Abs in individual HIV⁺ sera. The Id⁺ Abs specific for the CD4 site epitopes of an individual showed a variety of neutralizing activities, indicating that multiple partially overlapping epitopes in the CD4 attachment site originated from a single or limited HIV strain within the individual. The broad range of recovery of Id⁺ Abs by affinity purification showed that Id⁺ Abs are present in a wide range of concentrations in sera (Table 3). We believe that this is due to the presence of multiple overlapping epitopes around the CD4 attachment site that induce distinct immune responses which accumulated over time.

Although it has been documented that an HIV-infected individual has multiple isolates derived from a single strain

by a series of mutation events (20, 25, 27), it is not clear whether the various CD4 site epitopes reside in only the original infectious isolate or whether they are present in some or all of the mutationally derived isolates of that individual. In our studies, to define CD4 site epitope-specific Abs, an inhibition assay with sCD4 was employed. This assay does not distinguish the exact location of the epitopes recognized by each Ab. Hence, a cross-inhibition assay was performed which indicated that all of the CD4 site epitopes recognized by different Id⁺ Abs coexist in the same gp120 molecule. As suggested by others (6) and us (4), some of the CD4 site epitopes could completely overlap the overall structure of a CD4 attachment site, whereas others could partially overlap it. If this is the case, all CD4 site epitopes are not necessarily located within the conserved structure of the CD4 attachment site. Thus, the virus may be able to undergo mutation in the CD4 site epitopes which do not influence the overall structure of the virus attachment and thereby escape neutralization. Indeed, a recent study indicated that a CD4 site epitope-specific human MAb prepared during the early stage of infection could not neutralize the mutated virus isolated at a later stage of infection of the same individual (5a). In this regard, it will be very important to find out whether any of the CD4 site epitope-specific Id⁺ Abs can neutralize various viral mutants. This will provide crucial information on whether truly conserved neutralizing targets exist at the CD4 attachment site.

Epitope analysis of different neutralizing Id⁺ Abs showed that six of eight Id⁺ Abs from serum I and four of six Id⁺ Abs from serum II are specific for the epitopes around the CD4 attachment site. The data on the recovery of Ab purification showed that CD4 site epitope-specific Id⁺ Abs constitute a major portion of the cross-reactive neutralizing Abs of sera I and II. These data, in conjunction with the neutralization data, suggest that most of the cross-reactive neutralizing anti-gp120 Abs in HIV-infected asymptomatic individuals are directed to the CD4 site epitopes. Since the Ab response to the CD4 attachment site is weaker than that for the V3 site early in infection (7, 17), the dominance of the CD4 site epitope-specific Ab response in the cross-reactive Ab population seen in our study could be attributed to the accumulation of immune responses to this relatively conserved site. The response to the CD4 site epitopes would dominate over that to the rapidly mutating V3 region, and thus over time most of the broadly neutralizing activity would be directed to the CD4 site epitopes of gp120.

Despite the diversity in the different epitopes and lower immunogenicity, the CD4 attachment site still represents an attractive target to circumvent antigenic variation. The ability of HIV to mutate in this region is potentially restricted by the requirements for attachment to target cells. Hence, it is conceivable that Abs to this region have inherently broad neutralizing activity. Moreover, because the attachment region is conformational and consists of noncontiguous residues (11, 22, 31), the broadly neutralizing epitopes of the CD4 attachment site cannot be represented by linear peptides (2, 15) for peptide-based vaccines. In light of these constraints, anti-id Ab-based vaccines could offer an alternative approach to elicit and/or boost CD4 attachment site-specific broadly neutralizing Abs (9).

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