responses to sheep E in the C3H/HeJ mice by $(CR2)_2$-IgG1 was equivalent to that in the BALB/c mice (Fig. 5).

We determined the effects of $(CR2)_2$-IgG1 on the response to a soluble T-dependent antigen by immunizing mice with 10 μg of keyhole limpet hemocyanin (KLH) in the presence of 1 mg of $(CR2)_2$-IgG1 or IgG1. The amounts of KLH-specific IgG1, IgG2a, IgG2b, and IgG3 in serum were almost completely suppressed for at least 20 days by $(CR2)_2$-IgG1, whereas the IgM response was not significantly diminished (Fig. 6). These results contrast with the enhancing effect of specific IgG for soluble antigens (*23*). It is thus unlikely that the Fc region of the chimera participates in suppression.

These experiments show that a soluble form of a B lymphocyte receptor can cause immunosuppression in vivo, and they identify CR2 as the complement receptor that mediates the capacity of C3 to enhance the immune response. This finding indicates that the signal-transducing function of the CD19-CR2 complex potentiates the response of B cells to antigen in vivo, confirms studies suggesting a role for CR2 in B cell activation in vitro (7), and clarifies the observation that a monoclonal antibody to murine CR1 that was cross-reactive with CR2 inhibited antibody responses in mice (*24*). An application of the inhibitory activity of $(CR2)_2$-IgG1 may be to prevent primary antibody responses to immunogenic agents used for immunosuppression and cancer therapy, such as xenogeneic monoclonal antibodies.

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25. The Eae I–Xho I fragment encoding SCR-1 and -2 of CR2 [J. J. Weis *et al.*, *ibid.* **167**, 1047 (1988)] was inserted into the 5′ Pst I site of pSNR021 (*19*) with oligonucleotide linkers. J558L myeloma cells were transfected by electroporation at 960 μF and 350V with pSNR021 and pSNRCR2 that had been linearized with Pvu I and selected in medium containing G418 (Gibco) (1 mg/ml). Recombinant proteins were purified from culture supernatants of appropriate clones by affinity chromatography on 5-iodo-NP-Sepharose (*21*), reduced with dithiothreitol, separated on a SDS–polyacrylamide gel (5 to 15% gel), and stained with Coomassie blue.
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Neutralization of Divergent HIV-1 Isolates by Conformation-Dependent Human Antibodies to Gp120

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The spectrum of human immunodeficiency virus type 1 (HIV-1) isolates neutralized by antibodies from HIV-1–infected humans is broader than the spectrum of isolates neutralized by sera from animals immunized with purified gp120 subunits. This broader neutralization was due, in part, to the presence of antibodies to conserved gp120 conformational epitopes. Purified conformation-dependent gp120-specific human antibodies neutralized a wider range of virus isolates than human antibodies directed to linear determinants in gp120 and were also responsible for the majority of the gp120-specific CD4-blocking activity of HIV-1–infected human sera. A gp120 subunit vaccine that effectively presents these conformation-dependent neutralization epitopes should protect against a broader range of HIV-1 variants than a vaccine that presents exclusively linear determinants.

AN HIV-1 VACCINE MUST ELICIT AN immune response that protects against infection by the numerous genetic variants that characterize this virus (*1*). The envelope glycoprotein gp120 of HIV-1 elicits virus neutralizing antibodies (*2*) and protects chimpanzees from a live virus challenge (*3*). However, the neutralizing antibodies induced by subunit immunization with gp120 from a single isolate typically lack the ability to neutralize other HIV-1 isolates (*4–6*). These neutralizing antibodies primarily recognize linear epitopes in the central area of the third hypervariable region (V3) of gp120 (*5*), the principal neutralizing determinant (PND) (7). However, sera from HIV-1–infected humans can neutralize a broad spectrum of virus isolates (*6, 8*), which cannot be explained on the basis of reactivity to linear epitopes in the V3 region (*9*). The cross-reactive neutralizing activity of human sera may be due, in part, to antibodies directed to conformational epitopes in HIV-1 gp120 that have not been efficiently presented by the gp120 vaccine formulations tested to date in animals. To test this hypothesis, we have purified and characterized such antibodies from HIV-1–infected human sera.

We purified gp120-specific, conformation-dependent antibodies from the immunoglobulin (Ig) fraction of pooled human sera positive for HIV antibody (Ig^{HIV}) by affinity chromatography using two versions of recombinant gp120 from the HIV-SF2 virus isolate [ARV-2 (*10*)] sequentially: env $2\text{-}3_{SF2}$, a nonglycosylated, denatured version produced in yeast (*11, 12*); and

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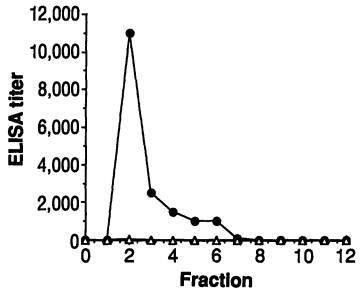

Fig. 1. Purification of antibodies that react with $rgp120_{SF2}$ and not env $2\text{-}3_{SF2}$ from human Ig^{HIV}. The immunoglobulin fraction (50 mg/ml, prepared by ammonium sulfate precipitation [*31*]) of plasma from ten HIV-1 antibody-positivie blood donors (Interstate Blood Bank, Memphis, Tennessee) with high titers of neutralizing antibodies to a broad panel of virus isolates including HIV-SF2, HIV-MN, HIV-BRU, and HIV-ZR6, was chromatographed on an affinity column consisting of purified env $2\text{-}3_{SF2}$ (*32*) coupled to Sepharose 4B (Sigma). The bound env $2\text{-}3_{SF2}$–specific antibodies were eluted as described (*12, 13*). The unbound material from the env $2\text{-}3_{SF2}$–affinity column was adsorbed twice more with fresh env $2\text{-}3_{SF2}$ resin. The final Ig^{HIV} preparation depleted of antibodies to env $2\text{-}3_{SF2}$ was then chromatographed on an affinity column of purified $rgp120_{SF2}$-coupled Sepharose (*32*). The bound $rgp120_{SF2}$-specific antibodies were eluted with 0.1 M glycine (pH 2.5). Fraction (1 ml each) were titered in env $2\text{-}3_{SF2}$ (triangles) and $rgp120_{SF2}$ (circles) antibody ELISAs (*27*).

$rgp120_{SF2}$, a native glycosylated, denatured version produced in mammalian cells (*13*). The env $2\text{-}3_{SF2}$ cannot bind to CD4, the HIV receptor, whereas $rgp120_{SF2}$ has full CD4-binding activity, which depends on gp120 conformation (*14*). The characteristics of env $2\text{-}3_{SF2}$ dictate that only linear epitopes are displayed, whereas $rgp120_{SF2}$ presents both linear and conformational determinants.

We removed antibodies directed to linear determinants in gp120 in the first step in the purification by subjecting Ig^{HIV} to three successive cycles of incubation with env $2\text{-}3_{SF2}$–coupled Sepharose. The env $2\text{-}3_{SF2}$ enzyme-linked immunosorbent assay (ELISA) confirmed that this procedure was effective (Table 1). The env $2\text{-}3_{SF2}$ titer after adsorption was indistinguishable from the background titer observed with nonimmune human Ig (*15*). However, there were still antibodies in the unbound fraction that recognized native $rgp120_{SF2}$ in radioimmunoprecipitation (RIP) assays and gave a signal above background in the $rgp120_{SF2}$ ELISA (Table 1).

The unbound fraction, depleted of antibodies to env $2\text{-}3_{SF2}$, was next chromatographed on an $rgp120_{SF2}$ column. A distinct peak of $rgp120_{SF2}$-specific antibodies, which did not react with env $2\text{-}3_{SF2}$, was recovered from this column (Fig. 1). This affinity column removed most of the remaining $rgp120_{SF2}$-specific antibodies (Table 1). RIP of $rgp120_{SF2}$ was not detected by the unbound fraction from this column, and the $rgp120_{SF2}$ ELISA titer of this fraction was reduced to background, that is, equivalent to the $rgp120_{SF2}$ ELISA of similar concentrations of nonimmune human Ig (*15*).

Antibody preparations were tested for HIV-SF2–neutralizing activity (Table 1). Some, but not all, of the HIV-SF2–neutralizing activity of the starting material, Ig^{HIV}, was specific for env $2\text{-}3_{SF2}$, consistent with our other findings (*12*). The putative conformation-dependent $rgp120_{SF2}$-specific antibodies recovered from the $rgp120_{SF2}$ column also had HIV-SF2–neutralizing activity. These purified $rgp120_{SF2}$-specific conformation-dependent antibodies, however, contained only a small proportion of the total HIV-SF2–neutralizing activity in the original Ig^{HIV}. A significant proportion of the HIV-SF2–neutralizing activity remained in the unbound fraction after adsorption of both env $2\text{-}3_{SF2}$– and $rgp120_{SF2}$-specific antibodies. The neutralizing antibodies in the unbound fraction must therefore recognize epitopes other than those displayed by these two recombinant antigens.

To ensure that the neutralizing activity

Table 1. ELISA, RIP, HIV-SF2 neutralizing, and CD4-blocking activity of antibody preparations from Ig^{HIV} chromatographed sequentially on env $2\text{-}3_{SF2}$– and $rgp120_{SF2}$-affinity columns. Aliquots of the pooled Ig^{HIV}, the linear-specific antibodies that bound to the env $2\text{-}3_{SF2}$ resin, the env $2\text{-}3_{SF2}$ column flow-through, the conformation-dependent antibodies that bound to the $rgp120_{SF2}$ resin (fractions 2 through 6 in Fig. 1), and the $rgp120_{SF2}$ column flow-through were dialyzed against phosphate-buffered saline (PBS) before their assay for ELISA (*27*), RIP (28), HIV-SF2–neutralizing activity (*12, 13*), and their CD4-blocking activity. Typically, we recover 40 to 60% of the ELISA-reactive antibodies applied to a particular affinity column in the sum of the bound and unbound fractions. Neutralizing activity was tested in duplicate in two independent experiments, and the results were comparable. Shown are the results from one assay run. The neutralizing titers reported are the reciprocal of the dilution of antibody preparation that inhibited viral replication by 50%. To assess blocking of gp120 binding to CD4, we serially diluted antibody preparations and added $rgp120_{SF2}$ (5 μg/ml) to each dilution. After incubation, soluble, recombinant CD4 (2.5 μg/ml) labeled with fluorescein-*N*-hydroxysuccinimide (Molecular Devices) was added to each reaction. The formation of gp120-CD4 complexes was assessed by HPLC column chromatography of 200 μl of reaction mixture on a Zorbax GF-450 gel filtration column (MAC-MOD Analytical). The titer of CD4-blocking activity is defined as the dilution of antibody preparation that blocked the formation of the gp120-D4 complex by 50%. This assay is described in detail in (*29*).

Antibody preparation	Relative concentration*	ELISA titer in units/ml (normalized)†			$rgp120_{SF2}$ RIP activity	Neutralizing activity in units/ml (normalized)†	CD4-blocking activity‡ in units/ml (normalized)†
		env $2\text{-}3_{SF2}$	$gp120_{SF2}$	$V3_{SF2}$			
Ig^{HIV}	1.0	6,329	2,076	105	+	1,000	60 ± 5.1
		(6,329)	(2,076)	(105)		(1,000)	(60 ± 5.1)
env $2\text{-}3_{SF2}$ bound	12.0	43,580	9,473	682	+	2,500	<5
		(3,631)	(790)	(57)		(208)	(ND)§
env $2\text{-}3_{SF2}$ flow-through	0.75	156‖	397	<10¶	+	250	**
		(208)	(529)	(ND)#		(333)	(32.6 ± 9.0)
$rgp120_{SF2}$ bound	12.5	<10	2,060	<10	+	40	**
		(ND)	(165)	(ND)		(3.2)	(13.0 ± 4.0)
$rgp120_{SF2}$ flow-through	0.5	117‖	136‖	<10	−	120	**
		(234)	(272)	(ND)		(240)	(ND)§

*The concentration of each antibody preparation relative to the original Ig^{HIV} is listed. This value for the input and unbound fractions was determined with BCA Protein Assay Reagent (Pierce Chemical) and bovine serum albumin used as a reference. We derived the relative concentration of the bound preparations by dividing the volume of Ig^{HIV} applied to each column by the volume of the final pooled peak. †The titer of activity (units per milliliter) in each preparation (relative to starting Ig^{HIV} listed in column 2) is shown. The numbers in parentheses are normalized to the units of reactivity per milliliter of the original Ig^{HIV}. ‡Mean ± SD of triplicate assays. §No CD4-blocking activity detected at a 1:5 dilution of this antibody preparation. ‖The ELISA titer observed with this antibody preparation was in the range of the assay background with nonimmune Ig (*15*). ¶There was no detectable ELISA signal at a 1:10 dilution, the most concentrated dilution tested. #None detected. **The antibody preparations used in the CD4-blocking assay were from a separate column run from the preparations listed in the first two lines of the column. Because the actual concentration relative to the Ig^{HIV} differed from the preparations we used to generate the data in the remainder of the table, only the normalized titers are listed.

Table 2. Recovery of $rgp120_{SF2}$ ELISA-reactive antibodies and neutralizing activity in various fractions from human $rgp120_{SF2}$-specific conformation-dependent antibodies chromatographed on untreated (native) or denatured $rgp120_{SF2}$-affinity columns or on an env $2\text{-}3_{SF2}$ column (*30*).

Column	Antibody preparation	ELISA (units)*	N†
Untreated	Input	2196	+
	Bound	732	+
	Unbound	<10‡	−
$Drgp120_{SF2}$	Input	2196	+
	Bound	<10	−
	Unbound	1088	+
env $2\text{-}3_{SF2}$	Input	2196	+
	Bound	<10	−
	Unbound	1816	+

*The total units of $rgp120_{SF2}$ ELISA-reactive antibodies applied to each column (input), eluted from each column (bound), and in the fraction that did not bind to each column (unbound) are listed. †All of the fractions from each column (Fig. 2) were screened for HIV-SF2–neutralizing activity (N) at a 1:10 dilution, as were the input and unbound antibody preparations. Those preparations or fractions that neutralized HIV-SF2 are indicated as positive (+) and those that failed as negative (−). ‡No detectable $rgp20_{SF2}$ ELISA-reactive antibodies observed at a 1:10 dilution, the most concentrated dilution tested.

exhibited by the purified antibodies from the $rgp120_{SF2}$ affinity column was not due to the incomplete removal of reactivity to linear V3-region determinants by env $2\text{-}3_{SF2}$, we tested the antibody preparations from both affinity columns in a $V3_{SF2}$ ELISA (Table 1). Adsorption with env $2\text{-}3_{SF2}$ effectively bound antibodies to $V3_{SF2}$, and these antibodies were recovered in the env $2\text{-}3_{SF2}$–specific fraction. There were no $V3_{SF2}$-reactive antibodies in the input Ig for the $rgp120_{SF2}$ column or in the putative gp120-specific conformation-dependent fraction recovered from the $rgp120_{SF2}$ column. Thus, the neutralization by the antibodies eluted from the $rgp120_{SF2}$ column must be due to reactivity with epitopes other than linear $V3_{SF2}$ determinants.

If the human antibodies that recognize $rgp120_{SF2}$ and not env $2\text{-}3_{SF2}$ are specific for conformation-dependent epitopes in gp120, they should not bind to denatured $rgp120_{SF2}$. Antibodies specific for $rgp120_{SF2}$ were rechromatographed on an affinity column of untreated $rgp120_{SF2}$; on a column of $rgp120_{SF2}$ that had been denatured, reduced, and alkylated ($Drgp120_{SF2}$); and on a column of env $2\text{-}3_{SF2}$ (Fig. 2 and Table 2). The $rgp120_{SF2}$-specific antibodies were bound by the untreated $rgp120_{SF2}$ column, and these antibodies eluted as a distinct peak that did not react with env $2\text{-}3_{SF2}$. In contrast, no $rgp120_{SF2}$-specific antibodies were detected in any of the fractions eluted from the $Drgp120_{SF2}$- or env $2\text{-}3_{SF2}$–affinity columns. However, $rgp120_{SF2}$-specific antibodies were present in the unbound fractions from these two affinity columns (Table 2). The fractions from these affinity columns were also screened for HIV-SF2–neutralizing activity (Table 2). The neutralizing activity cochromatographed on all three columns with the $rgp120_{SF2}$ ELISA-reactive antibodies. Thus, the $rgp120_{SF2}$-specific human-neutralizing antibodies that have been purified from the Ig^{HIV} recognize conformational epitopes and not carbohydrate moieties in $rgp120_{SF2}$.

We investigated whether these gp120-specific conformation-dependent antibodies could neutralize other isolates that differ from HIV-SF2 in the amino acid sequence of gp120, including isolates that differed significantly in their V3-region amino acid sequences. The neutralizing activity of these purified gp120-specific conformation-dependent antibodies was compared to purified env $2\text{-}3_{SF2}$–specific antibodies (Fig. 3). The conformation-dependent gp120-specific antibodies effectively cross-neutralized HIV-MN, HIV-BRU, and HIV-ZR6. In contrast, the env $2\text{-}3_{SF2}$–specific antibodies cross-neutralized only the HIV-MN isolate. The low titer of neutralization of HIV-BRU and HIV-ZR6 by the conformation-dependent antibodies is consistent with the inherently lower sensitivities of these two isolates to serum neutralization (right panel, Fig. 3) than the sensitivities of HIV-SF2 and HIV-MN. The differential sensitivity to serum neutralization of individual HIV-1 isolates has been well documented (*6, 16*).

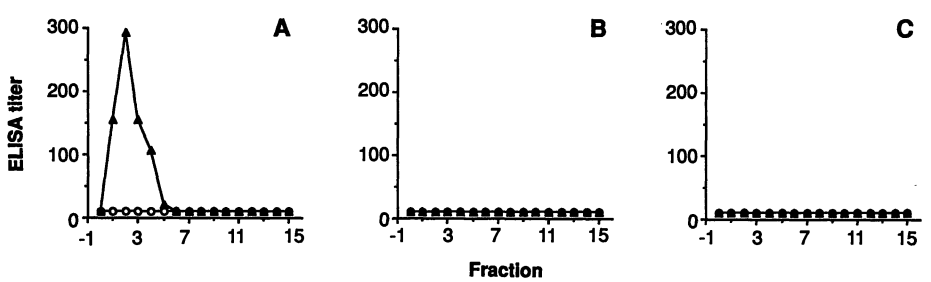

Fig. 2. Reduction and alkylation of $rgp120_{SF2}$ eliminates the binding of putative gp120-specific conformation-dependent ELISA-reactive antibodies purified from human Ig^{HIV}. Pooled rgp120-specific antibodies prepared as described as Fig. 1 were diluted in PBS containing 1% fetal bovine serum (as a source of carrier protein), and we adsorbed aliquots to affinity columns of untreated $rgp120_{SF2}$ (**A**), $Drgp120_{SF2}$ (**B**), or env $2\text{-}3_{SF2}$ (**C**). We washed the columns and then treated them with 0.1 M glycine (pH 2.5) to elute any bound antibodies. Fractions (1 ml each) were collected and assayed for $rgp120_{SF2}$ and env $2\text{-}3_{SF2}$ antibodies by ELISA (*27*). Shown are the titers of antibodies of fractions collected from each column reactive with env $2\text{-}3_{SF2}$ (circles) and $rgp120_{SF2}$ (triangles).

The domains of gp120 that participate in binding to CD4 are likely targets for the conformation-dependent gp120-specific neutralizing antibodies that we have purified from Ig^{HIV} because CD4 binding is known to require the correct conformation of gp120 and to involve discontinuous regions (*14, 17*). Moreover, a human HIV-1–neutralizing monoclonal antibody with a fairly broad spectrum of virus-neutralizing activity that also blocks CD4 binding has been shown to be directed to a conformational determinant in gp120 (*18*). When the antibody preparations from the sequential fractionation of Ig^{HIV} on env $2\text{-}3_{SF2}$ and $rgp120_{SF2}$ affinity columns were tested for their ability to block the binding of gp120 to CD4, this activity was detected only in the conformation-dependent fraction and not in the fraction specific for linear determinants (Table 1).

The data presented here and in other reports (*9, 12, 13*) suggest that at least three categories of neutralizing antibodies are induced in HIV-1–infected humans. The first category, antibodies to linear determinants in gp120, is dominated by antibodies directed to the V3 region (*5, 19*). However, in order to detect or purify such antibodies, it is necessary to use a peptide or recombinant antigen that contains a V3-region PND homologous with that of the isolate or isolates that have infected each individual. Because the central V3-region sequences of both HIV-SF2 and HIV-MN are highly homologous with the consensus, central V3 sequence of HIV-1 field isolates prevalent in the United States (*7*), it was not surprising that env $2\text{-}3_{SF2}$ bound neutralizing antibodies from Ig^{HIV} and that these antibodies effectively cross-neutralized HIV-MN. Purified env $2\text{-}3_{SF2}$–specific antibodies did not neutralize HIV-BRU. Thus, this Ig preparation must be deficient in neutralizing antibodies specific for the V3-core amino acids GPGRAF (*20*) conserved in HIV-BRU, HIV-MN, and HIV-SF2. The second category, antibodies to gp120 conformational epitopes, neutralizes a broader spectrum of HIV-1 isolates than the first category. We have demonstrated that this category of antibodies exists in pooled high-titer human Ig^{HIV}. It would be of interest to determine how prevalent such antibodies are among HIV-1–infected individuals by analysis of individual serum specimens. Our results demonstrate that the mechanism of neutralization by at least a portion of this antibody

fraction is likely to be through blocking of gp120 binding to CD4. We cannot rule out the possibility that there are other conformational epitopes that are targets of human neutralizing antibodies, in addition to those involved in CD4 binding, such as the conformational V3 epitopes described (*21*). However, it is unlikely that these antibodies could account for the broadly reactive cross-neutralization observed with conformation-dependent gp120- specific antibodies purified from humans, unless one assumes a highly conserved architecture of the V3 region in the face of considerable amino acid divergence (for example, the V3 sequences of HIV-SF2 and HIV-ZR6). The last category, antibodies that do not recognize linear or conformational epitopes in gp120 subunits, could be directed to oligomers of gp120 (*22*), epitopes in gp41 (*23*), or the viral core protein p17 (*24*). Alternatively, certain neutralization epitopes in gp120 might not be available when the protein is coupled to the solid support because they are buried or lost as a result of subtle changes in the conformation of gp120 that do not eliminate its ability to bind CD4.

These findings are important in the design of a successful gp120-based subunit vaccine against HIV-1. The presentation of both linear and conformational epitopes of gp120 could induce protective humoral immunity to a broader spectrum of virus isolates than a strategy that relies on the induction of antibodies to linear determinants alone. Furthermore, sequence variation in the V3 region, which has been shown to result in neutralization escape mutants (*25*), might be offset by the use of a gp120 vaccine that induces antibodies to both linear and conformational epitopes. We have observed that sera from primates immunized with multiple doses of $rgp120_{SF2}$, but not env $2\text{-}3_{SF2}$ formulated in high-potency adjuvants, neutralize a broad range of HIV-1 isolates (*26*). It will be important to determine whether these neutralizing antibodies recognize gp120-specific conformational epitopes and block the binding of gp120 to CD4, as do the human antibodies that we have characterized here.

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15. The Ig fraction from nonimmune (HIV-1–seronegative) pooled human sera, prepared in a manner identical to that described for the Ig^{HIV}, yields a titer of approximately 130 to 150 in both the env $2\text{-}3_{SF2}$ and $rgp120_{SF2}$ ELISAs. However, nonimmune Ig does not give a detectable signal, even at a 1:10 dilution, when tested in the $V3_{SF2}$ ELISA.
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27. The procedure for the ELISA for antibodies to env $2\text{-}3_{SF2}$, $rpg120_{SF2}$, and the SF2-V3 peptide (amino acids 301 to 332 of HIV-SF2 Env; TRPNNNTRKSIYIGPGRAFHTTGRIIGDIRKAHC) was as described (*12, 13*). The ELISA titer is the reciprocal of the dilution that resulted in an absorbance corresponding to 50% (usually an absorbance of 0.6) of the maximum signal in the assay. The results are from the mean of duplicate assays. The standard deviation was less than or equal to 10% of the values reported.
28. To test antibodies for RIP activity, $rgp120_{SF2}$ was biosynthetically labeled by incubation of [^{35}S]Met and [^{35}S]Cys with a CHO cell line that continuously secretes $rgp120_{SF2}$. Amounts of $rgp120_{SF2}$ were measured by an antigen-capture ELISA. Equivalent amounts (100 ng) of rgp120 were complexed with normalized volumes of affinity-fractionated human sera in constant-volume reactions, precipitated with protein A–Sepharose, separated by electrophoresis on SDS gels and autoradiographed. Band intensities were compared to intensities of bands precipitated by 20 μg of purified Ig from HIV$^+$ and HIV$^-$ human sera. Positive signals (+) indicate reactivity equivalent to the HIV$^+$ control; negative (−) indicates no signal, as with the HIV$^-$ human sera.
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30. Affinity columns were prepared as described (*12, 13*). Reduced and alkylated $rgp120_{SF2}$ [G. E. Means and R. E. Feeney, *Chemical Modification of Proteins* (Holden-Day, San Francisco, 1971)] was purified by gel-filtration HPLC on a Du Pont GF-450 column. The purity of the product was checked by SDS-gel electrophoresis and gel-filtration HPLC. The antigens coupled to each column were tested for CD4 binding by incubation of the resin with soluble recombinant CD4. The untreated $rgp120_{SF2}$ Sepharose bound CD4, whereas the env $2\text{-}3_{SF2}$ and $Drgp120_{SF2}$ resins did not.
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32. The env $2\text{-}3_{SF2}$ was purified to greater than 90% purity as described (*12*). The $rgp120_{SF2}$ was purified (>95% purity) as follows (i) clarification and concentration of the cell culture supernatant from CHO cells secreting $rgp120_{SF2}$, (ii) diethylaminoethyl (DEAE) anion-exchange chromatography at pH 8, (iii) fractionation on a high-performance liquid chromatography (HPLC) phenyl hydrophobic-interaction column, (iv) chromatography on an HPLC ether hydrophobic-interaction column, and (v) gel-filtration chromatography. Typical yields were 20 to 25% with a 250-fold purification.

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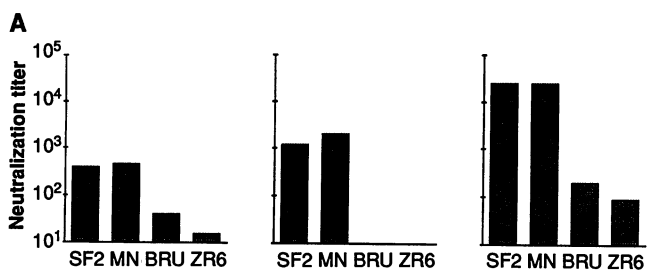

B

V3-region sequences	
SF2	CTRPNNNTRKSIYI GPGRAFHTTGRIIGDIRKAHC
MN	CTRPNYNKRKRIHI GPGRAFYTTKNIIGTIRQAHC
BRU	CTRPNNNTRKSIRIQRGPGRAFVTIGKI GNMRQAHC
ZR6	CTRPYKNTRQSTPI GLGQALYTTRGRTKIIGQAHC

Fig. 3. (**A**) Comparison of the neutralizing activity for divergent HIV-1 isolates of human antibodies to linear- and conformation-dependent epitopes in HIV-SF2 gp120. (**Left panel**) $rgp120_{SF2}$ conformation-dependent antibodies, (**middle**) env $2\text{-}3_{SF2}$–specific linear antibodies, and (**right**) unfractionated human HIV$^+$ serum) tested for neutralization of HIV-SF2, HIV-MN, HIV-BRU, and HIV-ZR6 as described (*12, 13*) (Table 1). The $rgp120_{SF2}$- and env $2\text{-}3_{SF2}$–specific antibodies were prepared by the methods described in Fig. 1. However, the $rgp120_{SF2}$ conformation-dependent antibodies tested were fivefold more concentrated than the antibody preparation used in Table 1. As a control, a single, high-titer human HIV$^+$ antiserum with neutralizing activity to a broad panel of HIV-1 isolates was tested in parallel. (**B**) The sequence of the V3 regions of each of the isolates in (A). Core region indicated in boldface. The single-letter amino acid code is as follows: A, Ala; R, Arg; N, Asn; D, Asp; C, Cys; Q, Gln; E, Glu; G, Gly; H, His; I, Iso; L, Leu; K, Lys; M, Met; F, Phe; P, Pro; S, Ser; T, Thr; W, Trp; Y, Tyr; and V, Val.