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In vitro evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity

(passive immunization/viral neutralization/phage display/combinatorial libraries/complementarity-determining regions)

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ABSTRACT **A method is described that allows for the improvement of antibody affinity. This method, termed complementary-determining region (CDR) walking, does not require structural information on either antibody or antigen. Complementary-determining regions are targeted for random mutagenesis followed by selection for fitness, in this case increased binding affinity, by the phage-display approach. The current study targets a human CD4-binding-site anti-gp120 antibody that is potently and broadly neutralizing. Evolution of affinity of this antibody demonstrates in this case that affinity can be increased while reactivity to variants of human immunodeficiency virus type 1 is broadened. The neutralizing ability of this antibody is improved, as assayed with laboratory and primary clinical isolates of human immunodeficiency virus type 1. The ability to produce human antibodies of exceptional affinity and broad neutralizing ability has implications for the therapeutic and prophylactic application of antibodies for human immunodeficiency virus type 1 infection.**

The ability to clone human antibodies in large numbers from seropositive individuals (1, 2) or to create them *de novo* by using synthetic approaches (3–5) promises increased application of this class of molecules in the service of human health. There are a number of considerations in choosing an antibody-combining site; these are primarily affinity and specificity. Current molecular methods should allow for experimenter-controlled evolution of binding sites to satisfy demands in both areas. The generation of molecules with exceptional affinities should both increase biological potency and decrease the cost of antibodies as therapeutics.

Currently, there is an increased urgency for the development of molecules for the prophylaxis and therapy of human immunodeficiency virus type 1 (HIV-1) infection. Passive immunotherapy has been successfully used against a number of viruses (6) and indeed has been used to protect chimpanzees against HIV-1 infection (7, 8) and to protect cynomolgus monkeys against simian immunodeficiency virus and HIV type 2 infection (9). One of the major problems in using antibodies as anti-HIV-1 reagents is sequence variation in the envelope proteins of the virus. Because the virus requires the binding of the surface glycoprotein gp120 to the CD4 molecule on the target cell for infectivity (10, 11), the CD4-binding site on gp120 has become a popular target for antiviral antibodies (12–16). However, antibodies to this region are not generally particularly potent in terms of virus neutralization. Furthermore, such antibodies tend to be even less potent against primary isolates of virus than the more commonly used laboratory-adapted strains (17). Using combinatorial libraries, we have isolated human anti-CD4-binding site antibodies with quite exceptional neutralizing ability (18). Nevertheless, we wished to improve the likelihood that these antibodies could succeed in prophylactic and therapeutic application.

In the present report we develop a strategy for evolution of antibody affinity. The method is applied to an HIV-1 neutralizing human antibody directed against the CD4-binding site of gp120. For this antibody, which already shows exceptional neutralizing potency, we show the possibility of increasing affinity, potency, and broadening strain reactivity.

MATERIALS AND METHODS

Reagents, Strains, and Vectors. Oligonucleotides were from Operon Technologies (Alameda, CA). *Escherichia coli*, phage, and the phagemid vector pComb3 are as described (19). The recombinant glycoproteins (rgps) 120 IIIB(LAI) and 120 MN were purchased from American Bio-Technologies (Cambridge, MA) and AgMed (Cambridge, MA), respectively. Reagents for surface plasmon resonance experiments were obtained from Pharmacia.

Library Construction and Selection. *Experiment A.* A *Hin*dIII restriction site was introduced preceding the heavy-chain complementarity-determining region (CDR) I by standard methods into clone HIV-4 (18) in the pComb3 vector (19). Clone HIV-4 (Fab b4) and HIV-12 (Fab b12) are identical. This Fab was selected by panning against rgp120 IIIB(LAI). The GenBank accession no. of HIV-4 sequence is L03147. A CDRI library was constructed by PCR of the above construct with primers (*i*) 5′-GAA-GGT-TTC-TTG-TCA-AGC-TTC-TGG-ATA-CAG-ATT-CAG-TNN-SNN-SNN-SNN-SNN-STG-GGT-GCG-CCA-GGC-CCC-C and (*ii*) primer R3B(20), where N is A,C,G, or T, and S is G or C.

The PCR product was gel-purified, digested with *Hin*dIII and *Spe* I, and gel-purified. The product was ligated with *Hin*dIII- and, *Spe* I-digested HIV-4. Subsequent steps were as described (3, 17) to produce phage displaying antibody Fab fragments on their surface. The library, 2×10^7 clones, was affinity-selected by four rounds of panning against gp120 IIIB(LAI) immobilized on Costar 3690 microtiter wells. One well coated with 1 μg of gp120 IIIB was used for each round of selection. After selection, plasmid DNA was prepared, and individual clones were sequenced.

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Abbreviations: CDR, complementarity-determining region; HCDR1 and HCDR3, heavy chain CDR1 or CDR3, respectively; HIV-1, human immunodeficiency virus type 1; PBMC, peripheral blood mononuclear cell; sCD4, soluble CD4; rgp, recombinant glycoprotein.

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Experiment B. Plasmid DNA isolated after experiment A was used as a template for PCR with oligonucleotide primers (*iii*) CCC-TTT-GCC-CCA-GAC-GTC-CAT-ATA-ATA-ATT-GTC-CTG-GGG-AGA-ATC-ATC-MNN-MNN-MNN-MNN-CCC-CAC-TCT-CGC-ACA and (*iv*) FTX-3 (20) to randomize within heavy chain CDR3 (HCDR3) (M = A or C, which gives K = T or G in the complementary strand; NNK doping strategy). The PCR product was gel-purified, digested with *Aat* II and *Xho* I, and gel-purified. This PCR product was ligated with *Aat* II, *Xho* I-digested HIV-4. Subsequent steps were described above to yield a library of 8×10^6 clones. The library was affinity-selected by six rounds of panning against gp120 IIIB. After selection, the gIII fragment was removed, and soluble Fab was produced. The complete amino acid sequences of the variable regions of selected antibodies were deduced by dideoxynucleotide chain-termination sequencing. Fab was purified to homogeneity by affinity chromatography, as described (19).

Surface Plasmon Resonance. The kinetic constants for the binding of Fab to rgp120 IIIB and MN were determined by surface plasmon resonance-based measurements using the BIAcore instrument from Pharmacia. The sensor chip was activated for immobilization with *N*-hydroxysuccinimide and *N*-ethyl-*N'*-(3-diethylaminopropyl)carbodiimide. The proteins, rgp120 IIIB or MN, were coupled to the surface by injection of 50 μl of a 50 μg/ml sample. Excess activated esters were quenched with 15 μl of ethanolamine (1 M and pH 8.5). Typically 4000 resonance units were immobilized. Binding of Fab fragments to immobilized gp120 was studied by injection of Fab in a range of concentrations (0.5–10 μg/ml) at a flow rate of 5 μl/min. The association was monitored as the increase in resonance units per unit time. Dissociation measurements were acquired after the end of the association phase but with a flow rate of 50 μl/min. The binding surface was regenerated with HCl (1 M NaCl and pH 3) and remained active for 20–40 measurements. The association and dissociation rate constants, k_{on} and k_{off}, were determined from a series of measurements as described (20–22). Equilibrium association and dissociation constants were deduced from the rate constants.

Quantitative Infectivity Assay Based on Syncytium Formation. Quantitative neutralization assays with the MN and LA1 (IIIB) strains were done as described (23). Monolayers of CEM-SS target cells were cultured with virus in the presence or absence of Fab, and the number of syncytium-forming units of input virus was determined 3–5 days later. Equivalent amounts of virus were used in the assays to allow direct comparison of Fab concentrations tested. Data represent the average of at least two runs. Assays were repeatable over a virus-surviving fraction range of 1–0.001 within a 2- to 4-fold difference in the concentration of antibody ($P < 0.001$).

Microplaque Neutralization. The quantitative measurement of the reduction of infectivity of primary clinical isolates of HIV-1 was determined with a microplaque assay, as described (24). MT2 cells were used as indicator cells in this assay. The isolation of HIV-1 from frozen peripheral blood lymphocytes obtained from seropositive donors has been described (25). A number of the primary isolates of HIV-1 used in this study have been described (26); these are VL135, VL434, VL069, VL263, and VL596, previously described as isolates 1, 3, 4, 5, and 7, respectively.

RESULTS

CDR Walking. In experiment A, the entire heavy chain CDR (HCDR1), as defined by Kabat *et al.* (27), was targeted for mutagenesis using the overlap PCR mutagenesis strategy described (3). NNS- or NNK-type doping strategies were used with no assumptions made as to the most fit residue at each position. After four rounds of selection for binding to rgp120 IIIB, the sequencing of 12 clones indicated a preference for asparagine (N) at position 31, an aromatic residue at position 32, serine (S) or threonine (T) primarily at position 33, branched hydrophobic residues at position 34, and hydrophobic and/or aromatic residues at position 35 (Fig. 1). Experiment B introduced diversity into HCDR3 at positions 96–99 of the clones that survived the four rounds of selection of experiment A. After six rounds of selection for binding rgp120 IIIB, a strong consensus was seen in both mutagenized CDRs (Fig. 1). At the time these selection experiments were done, only rgp120 IIIB was commercially available. Only in case 3B8 was the starting HCDR3 nucleotide and amino acid sequence identical to the parent, indicating some contamination in the secondary library. The net results of this two-step sequential CDR walk were minimal changes from the starting clone. The parental residues at positions 31, 32, 34, and 99 were strongly or absolutely maintained. The hydrophobic parental residue Val-33 was predominantly hydrophilic threonine or serine after selection. Position 96 appears flexible to a variety of substitutions as does position 98. Position 97 shows a preference for the increased steric bulk of the Tyr-97 → Trp mutation.

Affinity Measurement. After selections, four clones were chosen for further study. Clones were chosen that were related to one another by small changes in amino acid sequence and which displayed the most dramatic change in amino acid identity at positions 96 and 98. The kinetics of binding of purified Fab to two types of rgp120 from the highly divergent isolates MN and IIIB were chosen (28). Comparison of the protein rgp120 MN to rgp120 IIIB revealed 88 amino acid changes in the aligned sequences from rgp120 IIIB, as well as 11 deletions and 5 insertions of amino acids. Binding kinetics were studied in real time by using surface plasmon resonance. The kinetic and calculated equilibrium constants are tabulated for the binding of Fabs to both rgp120s (Table 1). The parental clone HIV-4 binds rgp120 IIIB with ≈10-fold better affinity than rgp120 MN, a trend that is maintained for the evolved clones. The highest-affinity Fab, 3B3, is improved 8-fold in affinity to rgp120 IIIB and 6-fold in affinity to rgp120 MN. The increases in affinities of the evolved Fabs binding to rgp120 IIIB and rgp120 MN are well-correlated as shown in Fig. 2. Thus, without selective pressure for binding to rgp120 MN, increase in affinity to

Experiment A					Experiment B								
CDR1					CDR1					CDR3				
31	32	33	34	35	31	32	33	34	35	96	97	98	99	
N	F	V	I	H						P	Y	S	W	HIV-4
R	Y	T	V	F	N	F	T	L	M	Q	W	N	W	3B1
N	W	S	V	M	N	Y	T	I	M	P	W	T	W	3B2
G	Y	T	L	M	N	F	T	V	H	E	W	G	W	3B3
N	F	T	L	L	N	Y	T	L	I	P	W	N	W	3B4
H	Y	S	L	M	N	F	I	I	M	L	W	N	W	3B6
N	W	V	V	H	N	F	S	I	M	S	W	R	W	3B7
N	F	S	I	M	N	Y	T	I	Q	P	Y	S	W	3B8
N	F	A	I	H	N	F	T	V	H	P	W	R	W	3B9
N	F	T	M	V										
N	F	T	L	Q										
Y	F	T	M	H										
S	Y	P	L	H										

FIG. 1. CDR walking for the selection of improved variants of HIV-1. In experiment A, HCDR1, is randomized over positions 31–35. After selection for binding to rgp120 IIIB the sequences listed in experiment A were observed. Experiment B introduces additional diversity into HCDR3 positions 96–99 in clones that were selected in experiment A. After additional selective pressure to bind rgp120 IIIB, the sequences listed under experiment B were observed. The sequences of the parental clone HIV-4 are shown for comparison.

Table 1. Binding and neutralization data for evolved Fab reacting with laboratory isolates of HIV-1

Fab	gp120 type	k_{on}, $M^{-1}s^{-1}$	k_{off}, s^{-1}	K_a, M^{-1}	K_d, M	IC_{50} μg/ml	IC_{50} M
HIV-4	IIIB	7.6×10^4	4.8×10^{-4}	1.6×10^8	6.3×10^{-9}	3.9×10^{-2}	7.7×10^{-10}
HIV-4	MN	3.4×10^4	1.5×10^{-3}	2.3×10^7	4.4×10^{-8}	3.0×10^{-1}	5.9×10^{-9}
3B1	IIIB	8.5×10^4	1.1×10^{-4}	7.7×10^8	1.3×10^{-9}	2.2×10^{-2}	4.4×10^{-10}
3B1	MN	1.4×10^5	1.8×10^{-3}	7.8×10^7	1.3×10^{-8}	9.2×10^{-3}	1.9×10^{-10}
3B3	IIIB	8.4×10^4	6.5×10^{-5}	1.3×10^9	7.7×10^{-10}	4.7×10^{-2}	9.4×10^{-10}
3B3	MN	1.6×10^5	1.2×10^{-3}	1.3×10^8	7.5×10^{-9}	5.5×10^{-3}	1.1×10^{-10}
3B4	IIIB	7.7×10^4	3.6×10^{-4}	2.1×10^8	4.8×10^{-9}	5.0×10^{-2}	9.9×10^{-10}
3B4	MN	8.6×10^4	4.1×10^{-3}	2.1×10^7	4.8×10^{-8}	2.0×10^{-2}	3.9×10^{-10}
3B9	IIIB	4.5×10^4	1.8×10^{-4}	2.5×10^8	5.0×10^{-9}	6.6×10^{-2}	1.3×10^{-9}
3B9	MN	8.1×10^4	1.1×10^{-3}	7.4×10^7	1.4×10^{-8}	7.8×10^{-3}	1.6×10^{-10}

The ability of parental and evolved Fabs to bind rgp120 IIIB and MN was determined by surface plasmon resonance (20–22). The equilibrium association and dissociation constants were calculated from the experimentally determined kinetic constants where $K_a = k_{on}/k_{off}$ and $K_d = k_{off}/k_{on}$. The interpolated IC_{50} values of Fab-neutralizing MN and IIIB viral stocks, as determined with the quantitative infectivity assay based on syncytium formation (23), are given in μg/ml and in molar units.

rgp120 IIIB is accompanied by increased affinity to rgp120 MN. Though a series of single-point mutations is not available in the clones examined to assign changes in affinity directly, comparison of Fab 3B3 with Fab 3B9 and Fab 3B1 with Fab 3B4, which each differ at two positions, suggests change of Pro-96 to glutamine or glutamate as the most productive change. Further examination of acidity changes within the evolved Fabs suggests affinity increases are correlated with decreased pI values of Fabs. The antigens rgp120 IIIB and MN have basic calculated pI values of 9.5 and 9.3, respectively. pI considerations may also contribute to the anomalous behavior of Fab 3B9, which is the only Fab that is increased in pI, as compared with the parent.

Neutralization Studies. Quantitative neutralization assays with the laboratory-adapted strains MN and LAI (IIIB) were done to determine potency of the Fabs (23). As shown in Fig. 3*A*, the evolved Fabs are clearly improved in their abilities to neutralize infectivity of the MN viral stock. The binding affinity of Fabs to rgp120 MN is well-correlated with ability to neutralize the MN stock (Fig. 4). The highest-affinity Fab, 3B3, is improved 54-fold with respect to neutralization of the MN isolate in this assay (Table 1). A different MN viral stock was used in these studies than had been used in the initial characterization of Fab HIV-4 (18). This result is reflected in a difference in activity, as compared with this previous report. Studies with the LAI (IIIB) viral stock show a clustering of Fabs with similar potencies (Fig. 3*B*). With this viral stock a range of reactivity of only 3-fold is noted with the most potent Fab, 3B1, showing a modest 2-fold increase in potency. With both viral stocks, Fabs demonstrate exceptional potency in the 10^{-10} M range (Table 1). To further

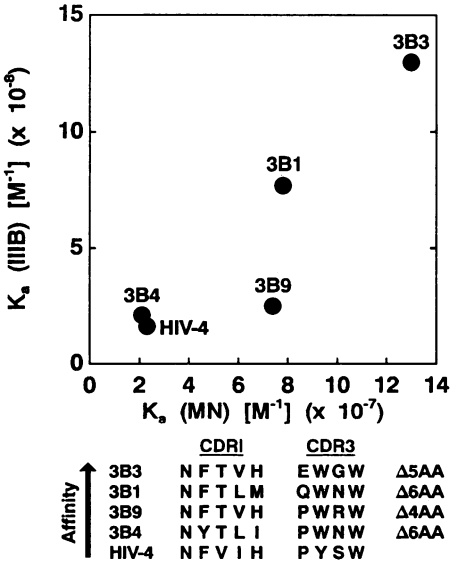

FIG. 2. The affinity increases of evolving Fabs for binding the divergent envelope proteins rgp120 IIIB and rgp120 MN are well-correlated. Affinities were determined by using the surface plasmon resonance technique (20–22). The sequences of evolved clones are ranked as compared with the parent, and changes in the amino acid (AA) sequence from the parent are shown as ΔAA. See also Table 1.

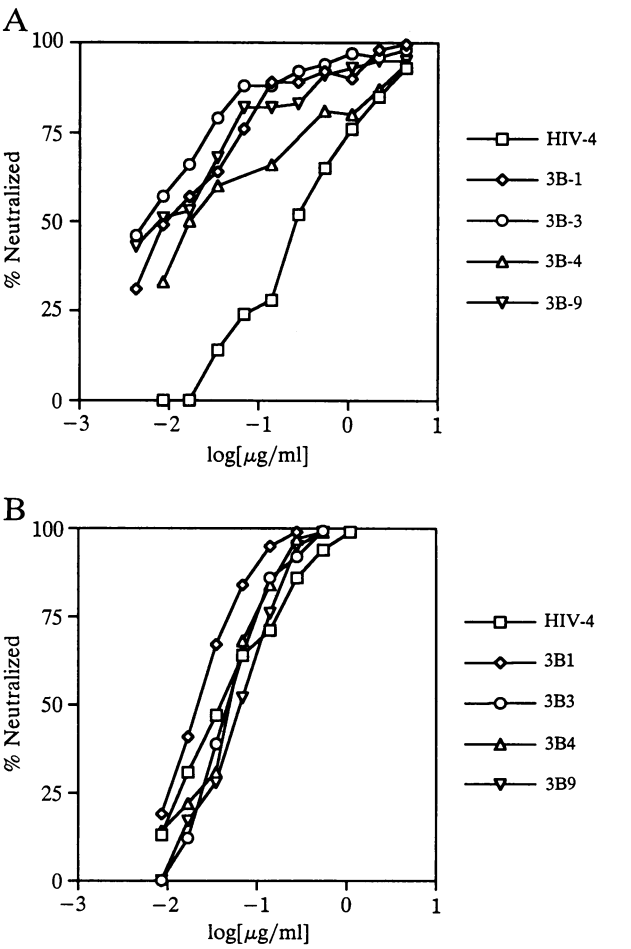

FIG. 3. Evolved Fabs are potent in neutralization assays with laboratory isolates of HIV-I. (*A*) Parental and improved Fabs are compared in a quantitative infectivity assay based on syncytium formation (23) with MN viral stock. (*B*) Comparison with LAI (IIIB) viral stock. Results indicate the average of at least two assays.

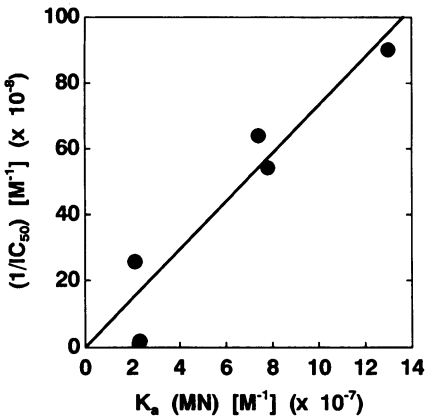

FIG. 4. Affinity increases are well correlated with increased potency in the quantitative infectivity assay with the MN viral stock. IC_{50} values for the reduction of infectivity were interpolated from Fig. 3A. The equilibrium association constant for binding rgp120 MN was determined as in Table 1.

delineate functional changes that accompany increased affinity, neutralization studies with primary clinical isolates of HIV-1 were done. Primary clinical isolates of HIV-1 were grown in peripheral blood mononuclear cells (PBMCs) (25). As controls in this microplaque assay (24), IIIB and MN stocks, as well as isolate VL069, were propagated in H9 cells. Additionally, assays with all stocks were done with a pooled plasma from 13 HIV-1-seropositive individuals. In all cases MT2 cells were used as indicator cells. The results are shown in Table 2. In these assays, the highest-affinity Fab, 3B3, is now able to neutralize an additional four isolates, as compared with the parent Fab, HIV-4. Fifty-percent neutralization of isolates VL135 and VL530 by Fab 3B3 at 38.9 and 29.5 μg/ml, respectively, is significant because the parent, Fab HIV-4, showed insignificant levels of neutralization, ≈10%, at 50 μg/ml. Neutralization of these isolates with pooled positive plasma shows that these isolates are relatively resistant to neutralization, as compared with the laboratory isolates grown in H9 cells. Neutralization of MN and IIIB stocks by antibody 3B3 is improved ≈5-fold in this assay. A host-cell effect is noted with isolate VL069. Propagation of this isolate in H9 cells results in a sensitization to neutralization. This effect has been noted previously and is discussed in detail elsewhere (29). In these assays the intrinsic error of the interpolated titers averages ±30%.

Table 2. Neutralization of primary clinical isolates of HIV-1 with natural and evolved Fab

Virus	Host cell	Amount for 50% neutralization, μg/ml HIV-4	3B3	Titer + PHP
VL135	PBMC	>50	38.9	1:33
VL263	PBMC	17.0	6.6	<1:10
VL596	PBMC	33.1	17.0	1:10
VL069	PBMC	>50	>50	<1:10
VL434	PBMC	>50	10.5	1:10
VL114	PBMC	>50	5.2	<1:10
VL172	PBMC	>50	>50	1:10
VL530	PBMC	>50	29.5	<1:10
VL750	PBMC	>50	>50	1:10
IIIB	H9	0.36	0.068	1:767
MN	H9	0.18	0.044	1:24,000
VL069	H9	3.6	3.5	1:1,200

The potency of the HIV-4 parental clone and Fab 3B3 to neutralize primary clinical isolates was measured in a microplaque assay (24). Virus was either propagated in PBMC or H9 cells. The neutralizing ability of pooled human plasma from 13 HIV-I-seropositive patients (+PHP) is shown for comparison as the titer of the serum dilution. Furthermore, the laboratory-adapted stocks IIIB and MN were also tested in this variant assay for comparison. A host-cell effect is shown for isolate VL069 grown in H9 cells (29).

DISCUSSION

The present study shows the feasibility of improving antibody affinity and function where specific structural information on both antibody and antigen is not available and the antibody already possesses high affinity. The current approach termed "CDR walking" is a variant of our synthetic-antibody approach for the generation of additional specificities *in vitro* (3–5). Practically, there is one important difference. CDR walking involves a limited introduction of diversity into the CDR regions of a defined antibody, as contrasted with the synthetic approach for the generation of new specificities where structural diversity is stressed over library completeness. Diversity in the present case is limited to 6 or fewer amino acid residues with an NNK- or NNS-doping strategy so as to ensure near-complete representation of all possible amino acid combinations. Selection from the library with the phage-display technique then allows for the refinement of the contact between antigen and antibody, which may result from unpredictable sequence changes in the region of interest. Repeated introduction of diversity into CDRs followed by stringent selections should allow for the refinement of human antibodies to levels of affinity far beyond those generated by the immune response. Two strategies are evident for the application of this approach, either sequential or parallel optimization of CDRs. Parallel optimizations makes the assumption that the optimized loops will exhibit additivity in free energy changes when the individually optimized loops are combined (30, 31). In many cases, additivity will likely be observed. Sequential optimization takes into account that additivity may not always be observed and that optimal binding may result from the interdependence of loops. Such interdependence could result from coordinated structural changes on binding antigen and is supported by recent evidence that suggests induced-fit mechanisms may best describe antibody–antigen recognition (32, 33). The two-step sequential walk reported here demonstrates the potential of this strategy. Both sequential and parallel approaches are being examined at present. In this initial study HCDR1 and HCDR3 were chosen for optimization. These CDRs were targeted because rearrangements of these CDRs have been observed on binding of another antibody to antigen (33). Residues 96–99 of HCDR3 were targeted because chain-shuffling experiments indicated this region is a hotspot during the natural maturation of this antibody (34). After the randomization and selection protocol that sampled mutations of 9 amino acid positions, higher-affinity Fabs resulted that had mostly modest changes from the parent (Fig. 1). The most radical change involves the Pro-96 → Glu mutation found in the highest-affinity clone 3B3, which is 8-fold improved in binding rgp120 IIIB. Interestingly, this mutation is also observed in the *in vivo* response, as revealed by chain shuffling. The *in vivo* antibodies that share this mutation are, however, of lower affinity than HIV-4 and have accumulated many additional somatic mutations throughout their sequence (34).

The key issue in producing antibodies to HIV-1 for therapeutic or prophylactic purposes is that they should be highly potent (of high affinity and neutralizing ability) and be cross-reactive with a wide range of isolates. These are usually two opposing characteristics. We have chosen HIV-4, as it recognizes a conformational epitope on gp120 that overlaps the CD4-binding site of gp120 and is broadly and potently active (19). If HIV-4 is truly recognizing the conserved features (shape) of the CD4-binding region, it should be

possible to increase its affinity to gp120 for many or all viral strains, as to date all HIV-1 isolates use CD4 as their primary receptor. This phenomenon is, indeed, observed as shown in Fig. 2. Binding has been increased to MN and IIIB, two highly divergent isolates (28). Selective pressure could have been applied to favor cross-reactivity by selecting with a mixture of divergent gp120s; however, this did not prove to be necessary in the present investigation. The present strategy was dictated because only rgp120 IIIB was commercially available when the selection experiments were done. Potency as judged by quantitative neutralization assays with MN and LAI (IIIB) stocks is improved as well (Fig. 3). With the MN isolate affinity is well-correlated with neutralizing ability (Fig. 4). Neutralization of MN and LAI stocks with soluble CD4 (sCD4) revealed IC_{50} values of 0.6 nM and 0.8 nM, respectively (35). As shown in Table 1, the parental clone and Fab 3B1 have IC_{50} values of 0.8 nM and 0.4 nM, respectively, for the LAI stock. For MN, the parental clone and Fab 3B3 have IC_{50} values of 6 nM and 0.1 nM, respectively. The ability of these evolved monovalent Fabs to neutralize with potencies equivalent to sCD4 is distinctive. The lack of correlation of rgp120 IIIB affinity with neutralizing ability may reflect the sensitivity of the assay conditions in this range. In a recent multicenter study of human and mouse anti-HIV-1 antibodies, no bivalent antibody has demonstrated such potency (36).

Can a single CD4-site antibody fulfill the promises once made by sCD4 as a therapeutic agent? Primary clinical isolates often require 1000-fold more sCD4 for neutralization than laboratory isolates (37–39). This fact may be the primary contributor to the failure of sCD4. As shown in Table 2, the highest-affinity Fab also demonstrates improved ability to neutralize primary clinical isolates. Four isolates not neutralized by the parent are now neutralized by Fab 3B3. For isolate VL114 a titration with bivalent CD4 IgG, predicted to have high activity, yielded an IC_{50} of 10 μg/ml, as compared with 5.2 μg/ml for Fab 3B3. As most of the CDR residues have yet to be optimized, it should be possible to further evolve this Fab to affinities 100 or 1000 times those reported here. These preliminary results suggest that broadly reactive antibodies of exceptional affinity can be prepared. Such antibodies will likely be of use at least to prevent vertical transmission of virus and in cases of accidental exposure. Ideally a mixture of such antibodies directed against several epitopes would be used. It remains to be demonstrated whether antibodies alone can be effective in cases where lymph nodes and thymus are seeded with the virus; however, they will likely be valuable components in combination therapies (40) and perhaps as key targeting agents in future therapies.

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