Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target

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Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target

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Broadly neutralizing antibodies (bNAbs), which develop over time in some HIV-1–infected individuals, define critical epitopes for HIV vaccine design. Using a systematic approach, we have examined neutralization breadth in the sera of about 1800 HIV-1–infected individuals, primarily infected with non–clade B viruses, and have selected donors for monoclonal antibody (mAb) generation. We then used a high-throughput neutralization screen of antibody-containing culture supernatants from about 30,000 activated memory B cells from a clade A–infected African donor to isolate two potent mAbs that target a broadly neutralizing epitope. This epitope is preferentially expressed on trimeric Envelope protein and spans conserved regions of variable loops of the gp120 subunit. The results provide a framework for the design of new vaccine candidates for the elicitation of bNAb responses.

Despite more than two decades of research, a protective vaccine against HIV-1 remains elusive. It is widely accepted that such a vaccine will require the elicitation of both T cell–mediated immunity and a broadly neutralizing antibody (bNAb) response (*1*–*3*). All of the known bNAbs provide protection in the best available primate models (*4*–*9*) and, therefore, are considered to be the types of antibodies that should be elicited by a vaccine. Unfortunately, these antibodies tend to display limited breadth and potency against non–clade B viruses, which make up the majority of infections outside North America and Europe, and they recognize epitopes on the virus that so far have failed to elicit bNAb responses when incorporated into a diverse range of immunogens (*10*–*12*). Therefore, in order to develop a successful vaccine, it is of high priority to identify new bNAbs that bind to epitopes that may be more amenable to immunogen design.

We have screened serum from ~1800 HIV-1–infected donors from Thailand, Australia, the United Kingdom, the United States, and several sub-Saharan African countries for neutralization activity and have identified donors who exhibit broad and potent neutralizing serum activity (*13*, *14*). Monoclonal antibodies (mAbs) are currently being generated from these donors by different approaches. In this study, we used a high-throughput strategy to screen immunoglobulin G (IgG)–containing culture supernatants from ~30,000 activated memory B cells from a clade A–infected donor for binding to monomeric recombinant envelope glycoproteins gp120 (HIV-1 primary isolate JR-CSF) and gp41 (HIV-1 strain HxB2) (trimeric gp120 and gp41 complexes, referred to as Env, mediate viral entry) and neutralization activity against HIV-1 primary isolates JR-CSF and SF162 (table S1) (*15*). The memory B cells were cultured at near clonal density, which enabled us to reconstitute the authentic antibody heavy and light chain pair from each culture well.

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A

Clade	No. of viruses	Median IC_{50} (μg/ml) against viruses neutralized with an IC_{50} <50 μg/ml						
		b12	2G12	2F5	4E10	PG9	PG16	PGC14
A	27	6.98	17.10	5.70	6.20	0.16	0.11	41.59
B	31	0.80	0.82	2.41	5.22	0.43	0.70	21.88
C	27	6.46	2.93	31.51	2.97	0.22	0.25	11.97
D	25	1.47	7.71	3.17	4.60	0.10	0.02	38.57
CRF01_AE	10	21.53	>50	0.26	0.51	0.08	0.03	>50
CRF_AG	10	10.40	0.95	0.64	1.42	0.80	0.03	45.10
G	15	3.07	31.03	1.24	1.44	0.29	1.21	>50
F	15	>50	9.23	1.78	2.30	0.09	0.08	25.71
Total	162	2.82	2.43	2.30	3.24	0.22	0.15	25.99

B

Clade	No. of viruses	Percent viruses neutralized						
		b12	2G12	2F5	4E10	PG9	PG16	PGC14
		With an IC_{50} <50 μg/ml						
A	27	30	37	74	96	85	85	11
B	31	58	71	68	97	74	74	29
C	27	33	11	7	96	78	78	19
D	25	48	24	56	96	76	60	8
CRF01_AE	10	30	0	89	100	100	100	0
CRF_AG	10	30	50	80	100	80	60	10
G	15	13	20	80	100	87	73	7
F	15	0	21	87	100	67	64	13
Total	162	35	32	60	98	79	73	15
		With an IC_{50} <1.0 μg/ml						
A	27	0	4	4	0	70	63	0
B	31	32	39	23	0	45	42	3
C	27	7	0	0	11	56	48	0
D	25	12	8	12	8	48	44	0
CRF01_AE	10	11	0	88	80	70	70	0
CRF_AG	10	10	30	60	30	40	50	0
G	15	0	0	27	0	60	33	0
F	15	0	14	13	28	80	79	0
Total	162	11	12	19	12	57	51	1

Table 1. Neutralization activity of mAbs. (**A**) Neutralization potency. Boxes are color-coded as follows: white, median potency >50 μg/ml; green, median potency between 20 and 50 μg/ml; yellow, median potency between 2 and 20 μg/ml; orange, median potency between 0.2 and 2 μg/ml; red, median potency <0.2 μg/ml. CRF_07BC and CRF_08BC viruses are not included in the clade analysis, but are counted toward the total number of neutralized viruses, because there was only one virus tested from each of these clades. (**B**) Neutralization breadth. Boxes are color-coded as follows: white, no viruses neutralized; green, 1 to 30% of viruses neutralized; yellow, 31 to 60% of viruses neutralized; orange, 61 to 90% of viruses neutralized; red, 91 to 100% of viruses neutralized. CRF_07BC and CRF_08BC viruses are not included in the clade analysis, but are counted toward the total number of neutralized viruses, because there was only one virus tested from each of these clades.

Unexpectedly, 97.7% of B cell culture supernatants that neutralized HIV-$1_{JR\text{-}CSF}$ and 46.5% that neutralized HIV-$1_{SF\text{-}162}$ did not bind to monomeric $gp120_{JR\text{-}CSF}$ or $gp41_{HxB2}$, and only 2% of cultures with neutralization activity could neutralize both viruses (fig. S1). Antibody genes were obtained from five B cell cultures that exhibited differing functional profiles; one bound to gp120 and only neutralized HIV-1_{SF162} (PGC14); two bound to gp120 and weakly neutralized both viruses (PGG14 and PG20); and two potently neutralized HIV-$1_{JR\text{-}CSF}$, failed to neutralize HIV-1_{SF162}, and did not bind to gp120 or gp41 in an enzyme-linked immunosorbent assay (ELISA) (PG9 and PG16) (*15*). Analysis of the antibody variable genes revealed two pairs of somatic variants, one of which contained long, heavy-chain complementarity-determining region 3 (CDRH3) loops (PG9 and PG16) (table S2). Long CDRH3 loops have been previously associated with polyreactivity (the ability to bind to a variety of structurally dissimilar antigens with moderate affinity) (*16*); thus, we tested PG9 and PG16 for reactivity against a panel of antigens and confirmed that the antibodies were not polyreactive (fig. S2) (*15*).

All five antibodies were first tested for neutralization activity against a multiclade 16-pseudovirus panel (table S3) (*15*). Two of the antibodies that bound to gp120 in the initial screen (PGG14 and PG20) did not show substantial neutralization breadth or potency against any of the viruses tested, and the third antibody that bound to gp120 (PGC14) neutralized 4 out of 16 viruses with varying degrees of potency. In contrast, the two antibodies that failed to bind recombinant gp120 or gp41 and did not neutralize HIV-1_{SF162} in the initial screen (PG9 and PG16) neutralized a large proportion of the viruses at concentrations less than 1 μg/ml. The observation that 93.3% of B cell cultures that neutralized HIV-$1_{JR\text{-}CSF}$ did not bind to gp120 or gp41 or neutralize HIV-1_{SF162} suggests that this donor's NAb response against HIV-$1_{JR\text{-}CSF}$ might be mediated by antibodies of the PG9 and PG16 type. We next evaluated PG9, PG16, and PGC14 on a large multiclade pseudovirus panel

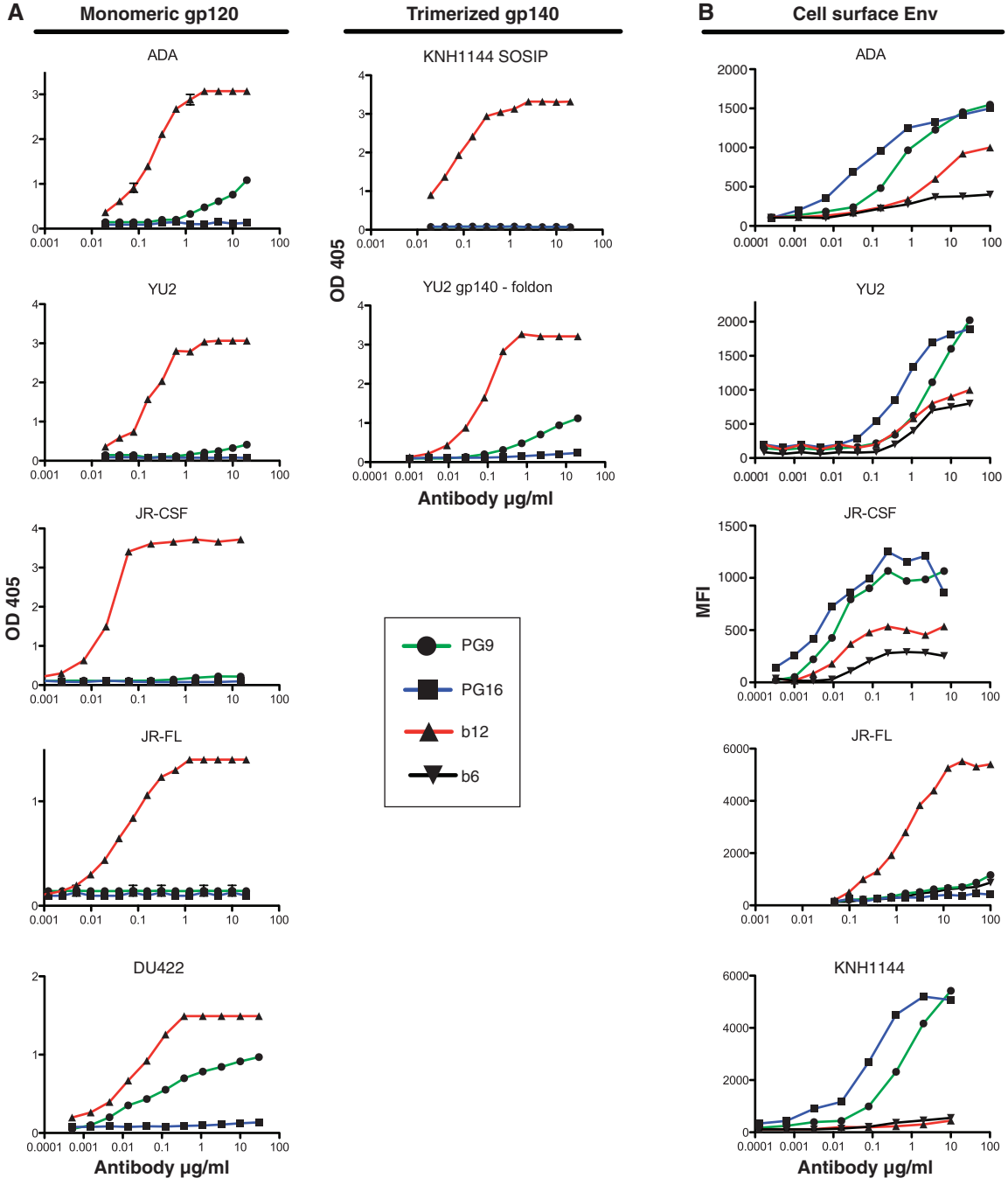

Fig. 1. Antigen-binding properties of PG9 and PG16. (**A**) Binding of PG9 and PG16 to monomeric gp120 and artificially trimerized gp140 constructs as determined by ELISA. Antigens were coated directly onto ELISA wells in the experiments shown, but similar results were also obtained when antigens were captured onto wells with antibodies against noncompetitive epitopes. OD, optical density (absorbance at 450 nm). (**B**) Binding of PG9 and PG16 to Env expressed on the surface of 293T cells as determined by flow cytometry. The bNAb b12 was used as a control for ELISA assays; b12, which binds with similar affinity to both cleaved and uncleaved forms of Env, and the nonneutralizing antibody b6, which only binds to uncleaved Env, are included in the cell surface–binding assays to show the expected percentages of cleaved and uncleaved Env expressed on the cell surface (*19*). Experiments were performed in duplicate, and data are representative of at least three independent experiments. MFI, mean fluorescence intensity.

consisting of 162 viruses, to further assess the neutralization breadth and potency of these three antibodies (Table 1, and tables S4 and S5). The bNAbs b12, 2G12, 2F5, and 4E10, and the donor's serum, were also included in the panel for comparison. Overall, PG9 and PG16 demonstrated a remarkable combination of neutralization breadth and potency. PG9 neutralized 127 out of 162 and PG16 119 out of 162 viruses with a potency that frequently considerably exceeded that noted for the four control bNAbs. The antibody concentration required to inhibit HIV activity by 50% or 90%, respectively (IC_{50} and IC_{90} values), for neutralized viruses across all clades were an order of magnitude lower for PG9 and PG16 than for any of the four existing bNAbs (Table 1A, and tables S4 and S5), and both mAbs showed an overall greater breadth of neutralization than b12, 2G12, and 2F5 (Table 1B, and tables S4 and S5). At low antibody concentrations, PG9 and PG16 also demonstrated greater neutralization breadth than 4E10 (Table 1B). Furthermore, both mAbs potently neutralized one virus (IAVI-C18) that exhibits resistance to all four existing bNAbs (table S4). Inspection of the mAb neutralization curves revealed that, whereas the PG9 neutralization curves usually exhibited sharp slopes, the neutralization curves for PG16 sometimes exhibited gradual slopes or plateaued at less than 100% neutralization (fig. S3 and table S3). Although neutralization curves with similar profiles have been reported previously (*17*, *18*), the mechanism for this is not well understood. Comparison of the neutralization profile of the serum with the neutralization profile of PG9, PG16, and PGC14 revealed that these three antibodies could recapitulate the breadth of the serum neutralization in most cases (table S4). For example, almost all of the viruses that were neutralized by the serum with an IC_{50} > 1:500 were neutralized by PG9 and/or PG16 at <0.05 μg/ml. The one case where this did not occur was against HIV-1_{SF162}, but this virus was potently neutralized by PGC14. Although PG9 and PG16 are somatic variants, they exhibited different degrees of potency against a number of the viruses tested. For instance, PG9 neutralized HIV-$1_{6535.30}$ ~185 times as potently as PG16, and PG16 neutralized HIV-$1_{MGRM-AG-001}$ ~440 times as potently as PG9. In some cases, the two antibodies also differed in neutralization breadth; PG9 neutralized nine viruses that were not sensitive to PG16, and PG16 neutralized two viruses that were not sensitive to PG9. On the basis of these results, it appears that this donor's broad serum neutralization might be mediated by somatic antibody variants that recognize slightly different epitopes and display varying degrees of neutralization breadth and

Table 2. Alanine mutations that decrease PG9 or PG16 neutralization activity. Amino acid numbering is based on the sequence of HIV-1_{HxB2}. Boxes are color coded as follows: white, the amino acid is identical among 0 to 49% of all HIV-1 isolates; light blue, the amino acid is identical among 50 to 90% of isolates; dark blue, the amino acid is identical among 90 to 100% of isolates. Amino acid identity was determined based on a sequence alignment of HIV-1 isolates listed in the HIV sequence database at http://hiv-web.lanl.gov/content/hiv-db/mainpage.html. In descriptions of the gp120 domains, C refers to constant domains and V refers to variable loops. Neutralization activity is reported as fold increase in IC_{50} value relative to WT JR-CSF and was calculated using the equation [(IC_{50} mutant)/(IC_{50} WT)]. Boxes are color coded as follows: green, substitutions that had a negligible effect on neutralization activity; yellow, 4- to 9-fold IC_{50} increase; orange, 10- to 100-fold IC_{50} increase; red, >100-fold IC_{50} increase. Experiments were performed in triplicate, and values represent an average of at least three independent experiments.

Mutation	Gp120 domain	Fold IC_{50} increase relative to wild-type	
		PG9	PG16
V127A	C1 (V1/V2 stem)	30	57
N134A	V1	5	23
N156A	C1 (V1/V2 stem)	280	1500
S158A	C1 (V1/V2 stem)	>2000	>2000
F159A	C1 (V1/V2 stem)	>2000	>2500
N160K	V2	>2000	>2500
T162A	V2	>2000	>2500
D167A	V2	5	30
Y173A	V2	1400	1000
F176A	V2	>5000	>7000
V181A	V2	200	250
P299A	V3 (base)	200	1400
K305A	V3 (stem)	50	2800
I307A	V3 (tip)	10	3000
I309A	V3 (tip)	9	150
F317A	V3 (tip)	3	1400
Y318A	V3 (tip)	2	1000
N392A	V4	7	23
I420A	C4	9	11
I423A	C4	40	14
I424A	C4	10	9

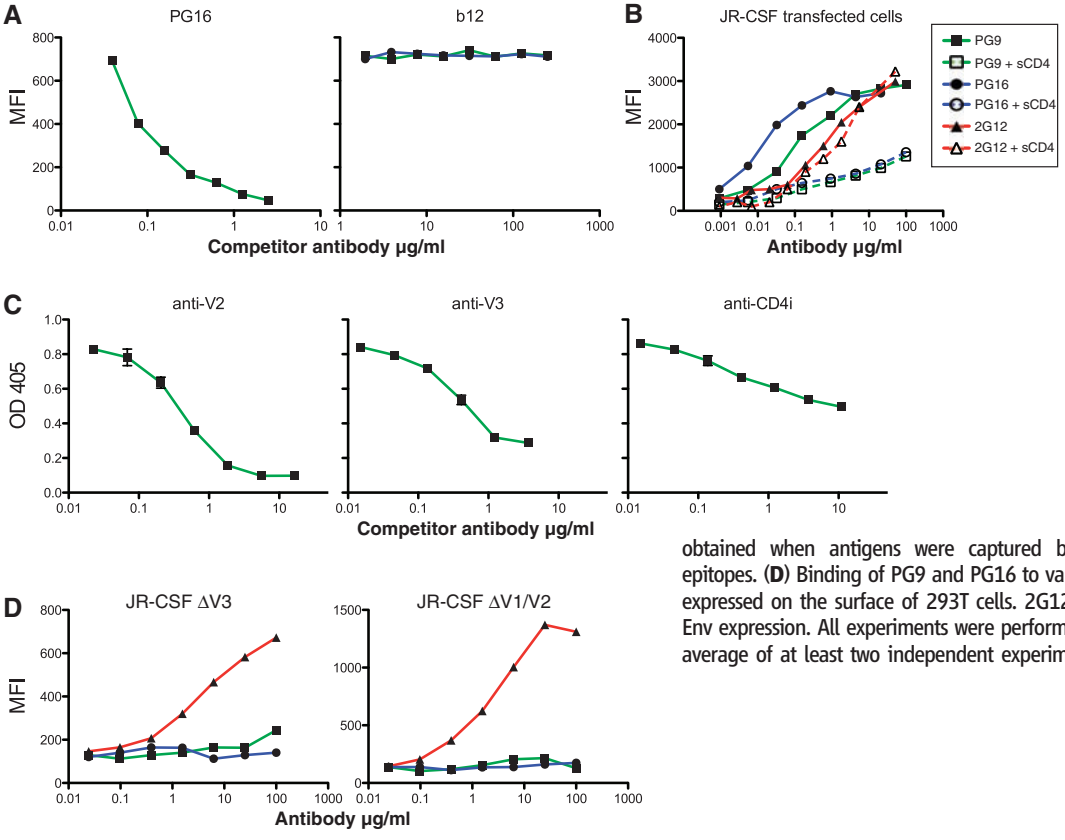

Fig. 2. Mapping the PG9 and PG16 epitopes. (**A**) Competition of PG9 and PG16 with each other and with the CD4–binding site (CD4bs) antibody b12 for cell surface Env binding. The competitor antibody is indicated at the top of each graph. MFI, mean fluorescence intensity. (**B**) Effect of soluble CD4 (sCD4) on the binding of PG9 and PG16 to cell surface Env. 2G12 is included to control for CD4-induced shedding of gp120. (**C**) Competition of PG9 with antibodies against the V2 loop (10/76b), V3 loop (F425/b4e8), and the CD4i site (X5) for gp120 binding. Antigens were coated directly onto ELISA wells in the experiments shown, but similar results were also obtained when antigens were captured by antibodies against noncompetitive epitopes. (**D**) Binding of PG9 and PG16 to variable loop–deleted HIV-1_{JR-CSF} variants expressed on the surface of 293T cells. 2G12 is included to control for cell surface Env expression. All experiments were performed in duplicate, and data represent an average of at least two independent experiments.

potency against any given virus. Selection for these types of antibodies may reflect the immune system's response to an ever-evolving viral envelope target.

The inability of PG9 and PG16 to bind monomeric $gp120_{JR\text{-}CSF}$ or $gp41_{HxB2}$ in the initial screen while potently neutralizing HIV-$1_{JR\text{-}CSF}$ suggested that the epitope targeted by these antibodies might be preferentially expressed on trimeric HIV Env. This possibility was investigated by comparing the ability of PG9 and PG16 to bind monomeric gp120 from several different strains, artificially trimerized gp140 constructs, and trimeric Env expressed on the surface of transfected cells (*15*). Although both antibodies bound with high affinity to cell surface Env, PG16 did not bind to any of the soluble gp120 or gp140 constructs, and PG9 bound only weakly to monomeric gp120 and trimerized gp140 from certain strains (Fig. 1). We hypothesized that PG9 and PG16 do not exhibit exclusive specificity for cleaved HIV-1 trimers because it has been previously shown that a substantial fraction of cell surface Env is composed of uncleaved gp160 molecules (*19*). Indeed, this was confirmed by the fact that both antibodies bound with high affinity to cleavage-defective HIV-1_{YU2} trimers expressed on the surface of transfected cells (fig. S4) (*15*). These results suggest that there are significant structural differences between soluble, recombinant gp140 and uncleaved gp160 expressed on the surface of transfected cells.

We next sought to define the PG9 and PG16 epitopes. We suspected that they would recognize the same or overlapping epitopes because the two antibodies are somatic variants. Indeed, both antibodies competed for binding to HIV-$1_{JR\text{-}CSF}$–transfected cells (Fig. 2A) (*15*). Soluble CD4, a soluble version of the receptor for Env, diminished binding of both PG9 and PG16 to cell surface Env, although neither antibody competed with the CD4–binding site antibody b12 for trimer binding (Fig. 2, A and B). This result suggests that conformational changes induced by CD4 binding cause a loss of the epitope targeted by the antibodies. Competition ELISAs revealed that PG9 competed for gp120 binding with antibodies against the V2 and V3 variable loops of gp120, and to a lesser extent, with CD4-induced (CD4i) antibodies (which recognize an epitope on gp120 dependent on CD4 binding) (Fig. 2C and fig. S5) (*15*). Neither PG9 nor PG16 bound to HIV-$1_{JR\text{-}CSF}$ variants with deleted V1 and V2, or V3, variable loops expressed on the surface of transfected cells, which further suggested that the variable loops are critical components of the epitopes (Fig. 2D). To dissect the fine specificity of PG9 and PG16, we performed alanine scanning using a large panel of HIV-$1_{JR\text{-}CSF}$ Env alanine mutants that have been described previously (*20*–*23*), as well as several new alanine mutants. We generated pseudoviruses incorporating single Env alanine mutations, and PG9 and PG16 were tested for neutralization activity against each mutant pseudovirus (*15*). Mutations that resulted in viral escape from PG9 and PG16 neutralization were considered important for formation of the PG9 and PG16 epitopes (Table 2 and table S6). On the basis of these data, and consistent with the competition experiments, residues that form the epitopes recognized by PG9 and PG16 are primarily located in conserved regions of the V2 and V3 loops of gp120 (Table 2 and fig. S6). Certain co-receptor binding site mutations also had an effect on PG9 and PG16 neutralization, albeit to a lesser extent (table S6). PG9 and PG16 were largely dependent on the same residues, although PG16 was more sensitive to V3 loop substitutions than PG9. Although neither antibody bound to wild-type HIV-$1_{JR\text{-}FL}$–transfected cells, a glutamic acid to lysine (E to K) mutation at position 168 in the V2 loop of HIV-$1_{JR\text{-}FL}$ generated high-affinity PG9 and PG16 recognition (fig. S7). Asparagines N156 and N160, sites of V2 N-glycosylation, were critical in forming the PG9 and PG16 epitopes because substitutions at these positions resulted in escape from antibody neutralization (Table 2 and fig. S8). HIV-1_{SF162} contains a rare N to K polymorphism at position 160 (which is nonpermissive for N-glycosylation), and mutation of this residue to an N renders this isolate sensitive to PG9 and PG16 (fig. S9). Deglycosylation of monomeric gp120 abolished binding of PG9 (fig. S8), which confirmed that glycans are important, directly or indirectly, in forming the epitope (*15*).

The preferential binding of PG9 and PG16 to native trimers could either be because their epitopes span more than one gp120 subunit or because the antibodies recognize a single subunit in a conformation that is stabilized on the trimer. To address this question, we studied the binding profiles of PG9 and PG16 to mixed HIV-1_{YU2} trimers, in which two gp120 subunits contained point mutations to abolish binding of the two antibodies (*15*). A third substitution that abrogates binding of 2G12, which binds with high affinity to both monomeric gp120 and trimeric Env, was also introduced into the same construct as an internal control. Cell surface–binding analysis revealed that all three antibodies bound to the mixed trimers with similar apparent affinity compared with wild-type trimers, and all binding was saturated at a similar lower level (fig. S10). This result suggests that the preference of PG9 and PG16 for trimeric Env is due to gp120 subunit presentation in the context of the trimeric spike rather than gp120 cross-linking.

Others have shown that NAbs that bind to epitopes encompassing parts of the V2 or both the V2 and V3 domains can exhibit potency comparable to that of PG9 and PG16, although these antibodies have thus far displayed strong strain-specificity (*17*, *24*). The epitopes recognized by these antibodies have been shown to differ from that of the clade B consensus sequence only by single–amino acid substitutions, which suggested the existence of a relatively conserved structure within the V2 domain (*17*, *18*). Here, we have confirmed that this region serves as a potent neutralization target and demonstrated that antibodies that recognize conserved parts of V2 and V3 can have broad reactivity.

Given that this is the first attempt at direct functional screening of such a large number of antibodies, it seems likely that the approach will generate further bNAbs, in particular those that bind poorly to recombinant forms of Env. The neutralization breadth exhibited by PG9 and PG16, particularly against non–clade B isolates, suggests that vaccine-induced antibodies of similar specificity might provide protection against a diverse range of the most prevalent HIV-1 isolates circulating worldwide. Furthermore, the exceptional neutralization potency exhibited by these antibodies in vitro suggests that antibodies of this specificity might provide protection at relatively modest serum concentrations achievable by vaccination. Immunogens designed to focus the immune response on conserved regions of variable loops in the context of the trimeric spike will determine whether these types of antibodies can be readily elicited by a vaccine.

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Comprehensive Mapping of Long-Range Interactions Reveals Folding Principles of the Human Genome

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We describe Hi-C, a method that probes the three-dimensional architecture of whole genomes by coupling proximity-based ligation with massively parallel sequencing. We constructed spatial proximity maps of the human genome with Hi-C at a resolution of 1 megabase. These maps confirm the presence of chromosome territories and the spatial proximity of small, gene-rich chromosomes. We identified an additional level of genome organization that is characterized by the spatial segregation of open and closed chromatin to form two genome-wide compartments. At the megabase scale, the chromatin conformation is consistent with a fractal globule, a knot-free, polymer conformation that enables maximally dense packing while preserving the ability to easily fold and unfold any genomic locus. The fractal globule is distinct from the more commonly used globular equilibrium model. Our results demonstrate the power of Hi-C to map the dynamic conformations of whole genomes.

The three-dimensional (3D) conformation of chromosomes is involved in compartmentalizing the nucleus and bringing widely separated functional elements into close spatial proximity (*1*–*5*). Understanding how chromosomes fold can provide insight into the complex relationships between chromatin structure, gene activity, and the functional state of the cell. Yet beyond the scale of nucleosomes, little is known about chromatin organization.

Long-range interactions between specific pairs of loci can be evaluated with chromosome conformation capture (3C), using spatially constrained ligation followed by locus-specific polymerase chain reaction (PCR) (*6*). Adaptations of 3C have extended the process with the use of inverse PCR (4C) (*7*, *8*) or multiplexed ligation-mediated amplification (5C) (*9*). Still, these techniques require choosing a set of target loci and do not allow unbiased genomewide analysis.

Here, we report a method called Hi-C that adapts the above approach to enable purification of ligation products followed by massively parallel sequencing. Hi-C allows unbiased identification of chromatin interactions across an entire genome.We briefly summarize the process: cells are crosslinked with formaldehyde; DNA is digested with a restriction enzyme that leaves a 5′ overhang; the 5′ overhang is filled, including a biotinylated residue; and the resulting blunt-end fragments are ligated under dilute conditions that favor ligation events between the cross-linked DNA fragments. The resulting DNA sample contains ligation products consisting of fragments that were originally in close spatial proximity in the nucleus, marked with biotin at the junction. A Hi-C library is created by shearing the DNA and selecting the biotin-containing fragments with streptavidin beads. The library is then analyzed by using massively parallel DNA sequencing, producing a catalog of interacting fragments (Fig. 1A) (*10*).

We created a Hi-C library from a karyotypically normal human lymphoblastoid cell line (GM06990) and sequenced it on two lanes of an Illumina Genome Analyzer (Illumina, San Diego, CA), generating 8.4 million read pairs that could be uniquely aligned to the human genome reference sequence; of these, 6.7 million corresponded to long-range contacts between segments >20 kb apart.

We constructed a genome-wide contact matrix M by dividing the genome into 1-Mb regions ("loci") and defining the matrix entry m_{ij} to be the number of ligation products between locus i and locus j (*10*). This matrix reflects an ensemble average of the interactions present in the original sample of cells; it can be visually represented as a heatmap, with intensity indicating contact frequency (Fig. 1B).

We tested whether Hi-C results were reproducible by repeating the experiment with the same restriction enzyme (HindIII) and with a different one (NcoI). We observed that contact matrices for these new libraries (Fig. 1, C and D) were extremely similar to the original contact matrix [Pearson's $r = 0.990$ (HindIII) and $r = 0.814$ (NcoI); P was negligible ($<10^{-300}$) in both cases]. We therefore combined the three data sets in subsequent analyses.

We first tested whether our data are consistent with known features of genome organization (*1*): specifically, chromosome territories (the tendency of distant loci on the same chromosome to be near one another in space) and patterns in subnuclear positioning (the tendency of certain chromosome pairs to be near one another).

We calculated the average intrachromosomal contact probability, $I_n(s)$, for pairs of loci separated by a genomic distance s (distance in base pairs along the nucleotide sequence) on chromosome n. $I_n(s)$ decreases monotonically on every chromosome, suggesting polymer-like behavior in which the 3D distance between loci increases with increasing genomic distance; these findings are in agreement with 3C and fluorescence in situ hybridization (FISH) (*6*, *11*). Even at distances greater than 200 Mb, $I_n(s)$ is always much greater than the average contact probability between different chromosomes (Fig. 2A). This implies the existence of chromosome territories.

Interchromosomal contact probabilities between pairs of chromosomes (Fig. 2B) show that small, gene-rich chromosomes (chromosomes 16, 17, 19, 20, 21, and 22) preferentially interact with each other. This is consistent with FISH studies showing that these chromosomes frequently colocalize in the center of the nucleus

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ERRATUM

Post date 19 February 2010

Reports: "Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target" by L. M. Walker *et al.* (9 October 2009, p. 289). Sequence information for the variable domains of the neutralizing antibodies was not provided at the time of publication; GenBank accession numbers are GU272043 to GU272052. Patents have been filed related to the work on the method of isolation of the broadly HIV-neutralizing human antibodies, the neutralization profile for multiple clades and strains of HIV viruses that demonstrates the breadth and potency of activity, and the epitope on V2 and V3 loops that PG9 and PG16 recognize (U.S. provisional patent application numbers USSN 61/161,010; USSN 61/165,829; and USSN 61/224,739, respectively).

LETTERS

edited by Jennifer Sills

Sustainable Foresting: Easier Said Than Done

IT IS COMMONLY HELD THAT PLANTING FORESTS HELPS TO MITIGATE CLIMATE CHANGE, because forests sequester carbon dioxide into long-lived biomass and soils (*1*). However, our personal experience shows that managed forests are unlikely to increase the land carbon sink unless foresters are paid a fair price for the ecosystem services they provide.

Six years ago, we bought 200 ha of mountain spruce forest and 35 ha of deciduous forest from the German government, under the condition that we would use the forest to grow and sell wood in a sustainable manner and that we would employ local labor. The spruce forest was a monoculture; the deciduous forest contained a rich flora of about 15 tree species.

The first obstacle we ran into was deer. To convert the spruce forest from a monoculture to a mixed forest, we supported the early successional rowan trees, but the deer ate the rowan tree bark. The resulting wood rot caused the trees to break. At that point, we had to choose between returning to a less environmentally friendly spruce monoculture or upsetting the public by clearing, replanting, and fencing in our mixed forest to keep out the deer. Fencing is unpopular with the German public, who are entitled to use private forest for recreation.

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Clearing requires permission by German forest law; permits are granted after wind or pest damage We opted for clearcutting in a few cases, but found that the more environmentally friendly approach of doing so prevented recovery of the forest. Leaving slash on site was well received by nature conservationists because of the habitats provided by the dead wood, but the spruce slash stimulated nitrification, and tall thickets of nettle prevented tree regeneration. When slash was removed and sold to a power station, the cost of collecting the slash was as high as the income received. Our action also upset the nature conservationists. However, the forest did regenerate.

An attempt to return to a more natural vegetation at a mountain site also met with a mixed response from conservationists. When wind damage forced us to clearcut an old spruce stand, we planted a mix of beech, sycamore, and fir. Landscape conservationists (who want to preserve the original spruce) complained that we had changed the appearance of the mountain, whereas nature conservationists applauded the fact that the new habitat supports rare insects, bats, and birds. Yet, sycamore and fir are susceptible to deer browsing and must be fenced for about 50 years. Given the carbon cost of fencing and the soil carbon loss after clearing, the climate mitigation potential of this clearcut will likely be negative over the next 60 years (*1*).

We also found that selling our wood was not as easy as we had envisioned. A 150-year beech could not pass through the saw mill and was not perfect for veneers; it went as cheap firewood. The price for spruce peaks at a breast-height diameter of 20 to 25 cm (typical of a 60-year-old tree) because modern construction beams are glued compound woods, not solid cuts from big trees. The forest carbon pool will fall in response to this demand for small trees. Such trees can be logged selectively with modern harvesting machines, but the machines require a 4-m-wide skidder trail every 20 m; the resulting 20% loss of forested land diminishes the capacity of the forest to absorb carbon.

Furthermore, maintaining biodiversity turns out to be a commercially risky management strategy. Industry demands uniform pieces of wood of the same species, mainly beech or spruce. The only way to maintain our 15-species mix is to sell to the niche market for high-quality stems of rare woods.

These observations show that in Germany, and likely elsewhere, there is little incentive to manage land in a climate-friendly manner. Only when ecosystem services such as mitigating climate, biodiversity, and recreation create income will they be able to compete with the market for wood as timber, pulp, or an energy source.

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1. A. Thuille, E. D. Schulze, *Glob. Change Biol.* **12**, 325 (2006).
2. We thank J. Gash for editorial help and discussion.

Responsible Researchers Required

THE NEWS OF THE WEEK STORY "A DARK TALE behind two retractions" (R. F. Service, 18 December 2009, p. 1610) omitted important issues regarding the training of young scientists. Students and postdocs involved in this debacle may have learned excellent experimental protocols and techniques, but did any of them have training in responsible conduct of research? And if so, how did that training influence their actions? When co-workers present research results, we trust them to be open, honest, and forthright. The high stakes of losing two papers in top-tier journals for all involved, including a pretenure faculty member whose tenure decision may have been affected by the retractions, remind us that we should pursue science for the sake of discovery and self-actualization rather than less savory goals such as power, financial gain, and prestige.

Disappointingly, the News story did not discuss subsequent action that could preclude this type of situation from arising again. Challenging results that cannot be reproduced requires substantial courage. At what point do we draw the line between results that "have

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not yet been reproduced" and those that "cannot be reproduced"? Without guidance about responsible conduct of research, students lack the tools and experience to question protocols or recognize potentially risky situations. Both the National Institutes of Health (*1*) and the National Science Foundation (*2*) have written new guidelines for required training in responsible conduct of research. These changes suggest that the scientific community now acknowledges that training young scientists means more than teaching them how to design and perform experiments and interpret data. We hope that high-profile laboratories, such as Peter Schultz's, will lead by example and will endorse the concept that real-time, face-to-face, responsible-conduct training by qualified professionals is not only desirable but necessary to help avert horror stories like this in the future.

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1. NIH, "Update on the Requirement for Instruction in the Responsible Conduct of Research" (http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-019.html).
2. NSF, Responsible Conduct of Research (http://edocket.access.gpo.gov/2009/E9-19930.htm).

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

Oil and Water Do Mix

IN THE 20 NOVEMBER 2009 SECTION OF This Week in *Science* (p. 1040), the piece "Methane's path to captivity" invoked a widespread scientific misconception: the idea that oil and water repel each other. In fact, water molecules and hydrophobic substances—including oil—attract one another, although only weakly. The false belief in repulsion traces to the original use of the misleading descriptive term "hydrophobic." In this case, the summary refers to a Report by M. R. Walsh *et al.* (*1*), but nowhere does this Report assert that hydrophobic substances repel or are repelled by water. A valid exposition of the interaction of water with hydrophobic substances can be found in an article in *Nature* by D. Chandler (*2*). Chandler states that "the term hydrophobic (water-fearing) is commonly used to describe substances that, like oil, do not mix with water. Although it may

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look as if water repels oil, in reality the separation of oil and water in ambient conditions is not due to repulsion…but to particularly favorable hydrogen bonding between water molecules." Also widely in current use is the term "superhydrophobic," referring to surfaces that often are described, erroneously, as repelling water. A paper by L. Zhai *et al*. (*3*) deals with this topic. The authors explain, "Water repellant in the context of our work simply means that water droplets placed on the surface will roll off freely at a small angle. This is how we and many others define the superhydrophobic state (contact angle greater than 150° coupled with a small rolling angle)." Not surprisingly, the forces involved—cohesion, adhesion, and gravity—are all attractive.

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2. D. Chandler, *Nature* **417**, 491 (2002).
3. L. Zhai *et al*., *Nano Lett*. **6**, 1213 (2006).

CORRECTIONS AND CLARIFICATIONS

News Focus: "The little wasp that could," by E. Pennisi (15 January, p. 260). The caption, "Eaten Alive," should read: Larvae of *Euplectrus walteri* (inset) ingest blood from a caterpillar. They develop attached to the outside of the caterpillar's body.

Perspectives: "Explaining Bird Migration" by O. Gilg and N. G. Yoccoz (15 January, p. 276). The second affiliation for Olivier Gilg should have been as follows: Lab Biogeosciences, UMR CNRS 5561, Eq Eco Evo, Université de Bourgogne, 21000 Dijon, France.

This week in *Science*: "Targeting DNA gyrase" (4 December 2009, p. 1317). In the fifth line, "DNA gyrase GTPase activity" should have read "DNA gyrase ATPase activity."

Reports: "Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target" by L. M. Walker *et al*. (9 October 2009, p. 289). Sequence information for the variable domains of the neutralizing antibodies was not provided at the time of publication; GenBank accession numbers are GU272043 to GU272052. Patents have been filed related to the work on the method of isolation of the broadly HIV-neutralizing human antibodies, the neutralization profile for multiple clades and strains of HIV viruses that demonstrates the breadth and potency of activity, and the epitope on V2 and V3 loops that PG9 and PG16 recognize (U.S. provisional patent application numbers USSN 61/161,010; USSN 61/165,829; and USSN 61/224,739, respectively).

Reports: "Activation of the cellular DNA damage response in the absence of DNA lesions" by E. Soutoglou and T. Misteli (13 June 2008, p. 1507). The horizontal panels in rows 1 and 6 of Fig. 1A were inadvertently duplicated during figure production. The correct row 6 panel is reproduced here. The conclusion that tethering of LacR-Chk1 does not lead to accumulation of γ-H2AX at the tethering site is not affected and is supported by the quantitation shown in the original Fig. 1B.