Antigenic conservation and immunogenicity of the HIV coreceptor binding site

Julie M. Decker,[1,2,3] Frederic Bibollet-Ruche,[1,2,3] Xiping Wei,[1,2,3] Shuyi Wang,[1,2,3] David N. Levy,[1,2,3] Wenquan Wang,[2,4] Eric Delaporte,[5] Martine Peeters,[5] Cynthia A. Derdeyn,[6,7] Susan Allen,[8] Eric Hunter,[6,7] Michael S. Saag,[2] James A. Hoxie,[9] Beatrice H. Hahn,[2,3] Peter D. Kwong,[10] James E. Robinson,[11] and George M. Shaw[1,2,3]

[1]Howard Hughes Medical Institute, [2]Department of Medicine, [3]Department of Microbiology, and [4]Section of Biostatistics, University of Alabama at Birmingham, Birmingham, AL 35294
[5]Institut de Recherche pour le Developpement, University of Montpellier, Montpellier Cedex 5, France
[6]Department of Pathology, [7]Department of Laboratory Medicine, and [8]International Health, Emory University, Atlanta, GA 30329
[9]Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104
[10]Vaccine Research Center, National Institutes of Health, Bethesda, MD 20892
[11]Department of Pediatrics, Tulane University Health Sciences Center, New Orleans, LA 70112

Immunogenic, broadly reactive epitopes of the HIV-1 envelope glycoprotein could serve as important targets of the adaptive humoral immune response in natural infection and, potentially, as components of an acquired immune deficiency syndrome vaccine. However, variability in exposed epitopes and a combination of highly effective envelope-cloaking strategies have made the identification of such epitopes problematic. Here, we show that the chemokine coreceptor binding site of HIV-1 from clade A, B, C, D, F, G, and H and circulating recombinant form (CRF)01, CRF02, and CRF11, elicits high titers of CD4-induced (CD4i) antibody during natural human infection and that these antibodies bind and neutralize viruses as divergent as HIV-2 in the presence of soluble CD4 (sCD4). 178 out of 189 (94%) HIV-1–infected patients had CD4i antibodies that neutralized sCD4-pretreated HIV-2 in titers (50% inhibitory concentration) as high as 1:143,000. CD4i monoclonal antibodies elicited by HIV-1 infection also neutralized HIV-2 pretreated with sCD4, and polyclonal antibodies from HIV-1–infected humans competed specifically with such monoclonal antibodies for binding. In vivo, variants of HIV-1 with spontaneously exposed coreceptor binding surfaces were detected in human plasma; these viruses were neutralized directly by CD4i antibodies. Despite remarkable evolutionary diversity among primate lentiviruses, functional constraints on receptor binding create opportunities for broad humoral immune recognition, which in turn serves to constrain the viral quasispecies.

CORRESPONDENCE
George M. Shaw:
gshaw@uab.edu

Abbreviations used: CD4i, CD4-induced; CRF, circulating recombinant form; IC_{50}, 50% inhibitory concentration; MPER, membrane-proximal external region; Nab, neutralizing antibody; sCD4, soluble CD4.

The antibody response to HIV-1 infection is typically vigorous and sustained, but its effectiveness in virus containment in vivo is uncertain. We and others have shown in acutely infected individuals the rapid development of HIV-1 strain-specific neutralizing antibodies (Nabs) and the equally rapid emergence of virus escape mutations (1–4). Such strain-specific antibody responses are common, and they clearly drive virus selection in vivo (3, 4). More broadly reactive Nabs develop over longer periods (5–7). HIV-1 has evolved a variety of defense mechanisms to avoid antibody recognition, including epitope variation, oligomeric exclusion, conformational masking, glycan cloaking, and steric interference at the virus–cell interface (8–14), and together, they contribute to virus persistence in the face of an evolving antibody repertoire (3, 4). But the precise nature of this evolving antibody response in vivo is incompletely understood. Analysis of HIV-1–specific monoclonal antibodies has revealed variable loop, CD4 binding site, chemokine coreceptor binding site, surface glycan, and membrane proximal gp41 domains as neutralization targets (for reviews see references 13, 14), but the prevalence, titers, and breadth of polyclonal antibody responses to

J.M. Decker and F. Bibollet-Ruche contributed equally to this work.
The online version of this article contains supplemental material.

these epitopes in humans are generally unknown. This is in part a consequence of technical difficulty in identifying epitope-specific neutralizing antibody responses within a larger context of polyclonal neutralizing and nonneutralizing antibody reactivities (15–17).

In the present study, we sought to identify immunogenic, broadly cross-reactive epitopes on the HIV-1 envelope glycoprotein that might serve as targets of the adaptive humoral immune response in naturally infected humans. We hypothesized that conserved requirements for coreceptor binding among diverse lineages of human or simian immunodeficiency viruses might be reflected in conserved antigenicity at the corresponding envelope surface. As a strategy, we took advantage of the wide evolutionary distance that exists between HIV-1 and HIV-2 lineages to probe for conserved neutralization epitopes. The envelope glycoproteins of HIV-1 and HIV-2 are only ~40% homologous in amino acid sequences (18). As a consequence, they generally exhibit weak antigenic cross-reactivity, and sera from HIV-1–infected individuals cross-neutralize HIV-2 poorly, if at all (19–21). Nonetheless, HIV-1 and HIV-2 each require chemokine coreceptor binding for cell entry, with primary non–T cell line–adapted viruses of both types generally using CCR5 (22, 23). Binding of CD4 to HIV-1 gp120 induces conformational changes in the outer and inner envelope domains, the bridging sheet, and the positioning of variable loops V1/V2 and V3 (24–30). These changes lead to exposure of the envelope coreceptor binding site, comprised of the bridging sheet, adjacent surfaces, and possibly the tip of V3. Antibodies that bind to HIV-1 gp120 preferentially (or only) after CD4 engagement are referred to as CD4-induced (CD4i). Typically, these antibodies bind to surfaces that include or are proximal to the bridging sheet where they compete with coreceptor binding and broadly (but not potently) neutralize different HIV-1 strains (28–33). Cross-reactivity between HIV-1–induced CD4i antibodies and HIV-2 has not been reported. Here, we explore the antigenic cross-reactivity and inherent immunogenicity of the coreceptor binding surfaces of HIV-1 and HIV-2 and assess whether HIV-2, in complex with soluble CD4 (sCD4), might be useful as a specific probe for HIV-1–elicited, CD4i-neutralizing antibodies in humans infected by HIV-1 or immunized with candidate HIV-1 vaccines.

RESULTS

Plasma from HIV-1–infected patients neutralizes sCD4-induced HIV-2

Table I shows the extent and kinetics of the Nab response to autologous HIV-1 virus in a patient (133M) after subtype C HIV-1 infection. Nab titers against the earliest detectable virus reached 1:2,500 (50% inhibitory concentration [IC_{50}]) by 11 mo of infection and then subsided. Such a response is typical of patients with newly acquired HIV-1 infection, and it is generally followed rapidly by virus mutation and escape from neutralization (3, 4). To look for more broadly reactive Nabs in this subject, we applied these same plasma specimens to the HIV-2 strain 7312A, a primary CD4-dependent R5 virus (22, 23, 34). As expected, plasma from this HIV-1–infected patient (133M) exhibited no detectable neutralizing activity against HIV-2_{7312A}, a finding consistent with prior studies showing little neutralization cross-reactivity between these highly divergent viral lineages (19, 20). However, when HIV-2_{7312A} was pretreated for 1 h with 9nM sCD4 (equal to the IC_{50} for this virus), the virus became remarkably susceptible to neutralization by 133M plasma, with titers of Nab reaching 1:12,500 by 26 mo after infection (Table I). Similar results were obtained in six additional subjects with primary subtype C HIV-1 infection, whose Nab titers to sCD4-pretreated HIV-2_{7312A} ranged from 1:53 to 1:3,361 and peaked between 8 and 24 mo after acute infection. To determine if the CD4-dependent Nab activity that we observed in plasma from subtype C patients was limited to this virus clade, we studied additional patients chronically infected with HIV-1 subtypes A, B, C, or D. Fig. 1 A depicts the neutralization profile of plasma from four such patients against HIV-2_{7312A} in the absence or presence of sCD4. In each case, there was a dramatic sCD4-dependent shift of 100–10,000-fold in the susceptibility of HIV-2 to neutralization. IC_{50} titers of CD4i Nab titers in these four individuals ranged from 1:750 to 1:20,000. 15 uninfected normal donors had no detectable Nabs to HIV-2_{7312A} with or without sCD4.

Table I. Neutralization of HIV-1 and HIV-2 by sequential plasma specimens from an HIV-1 seroconverter

Patient 133M	HIV-1 133M virus[a]	HIV-2 7312A virus	HIV-2 7312A virus + sCD4
mo			
2	22[b]	0	154
6	250	0	63
8	333	0	105
11	2,500	0	833
14	1,667	0	2,000
18	1,429	0	5,556
20	1,136	0	7,143
23	1,053	0	11,111
26	556	0	12,500

[a]The HIV-1 gp160 *env* gene from patient 133M was PCR amplified and cloned from uncultured month-2 PBMCs and used to prepare pseudotyped virus.
[b]Reciprocal IC_{50} titer of neutralizing antibodies as determined in JC53BL-13 cells (reference 3).

HIV-1 CD4i monoclonal antibodies neutralize sCD4-induced HIV-2

If the broadly cross-reactive neutralizing antibody activity that we observed in HIV-1–infected patient plasma is due to classical CD4i antibodies, then prototypic CD4i monoclonal antibodies derived from HIV-1–infected patients, which have been extensively characterized against HIV-1 envelope glycoproteins (28–33), might be expected to cross-neutralize HIV-2 in a CD4-dependent fashion. Fig. 1 B demonstrates this to be the case. Without sCD4, the CD4i monoclonals 17b, 21c, and

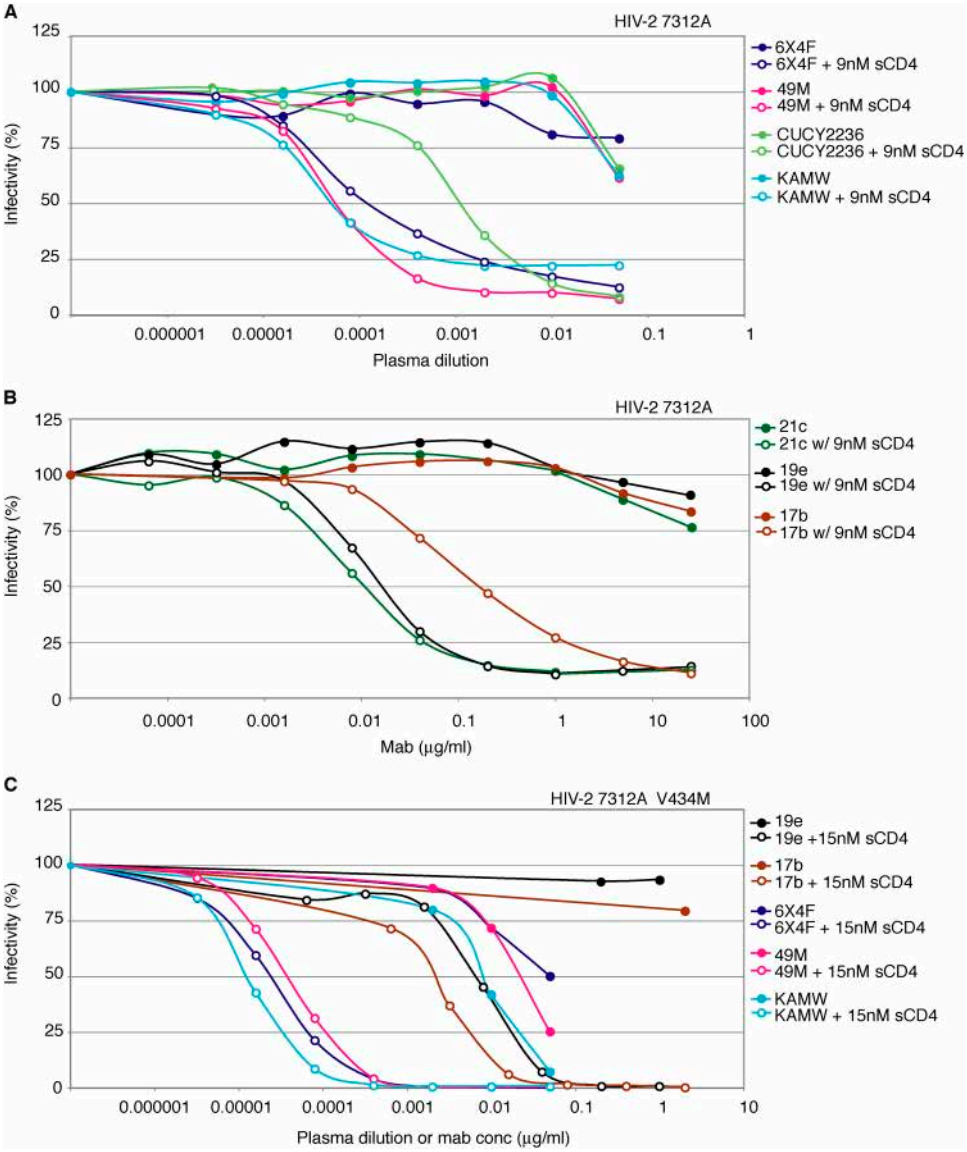

Figure 1. CD4-dependent neutralization of HIV-2 by HIV-1 antibodies. Neutralization of HIV-2_{7312A} (A and B) and HIV-$2_{7312A/V434M}$ (C) infectivity in JC53BL-13 cells (reference 3) was mediated by plasma from patients with HIV-1 clade A (6X4F), B (CUCY2236), C (49M), or D (KAMW) infection or by the HIV-1 CD4i monoclonal antibodies 21c, 19e, or 17b. sCD4 concentrations correspond to the IC_{50} values specific for each virus.

19e failed to neutralize HIV-2_{7312A}. In the presence of sCD4, a dramatic shift in the neutralization curves was observed with all three antibodies neutralizing HIV-2_{7312A} potently (Fig. 1 B). It is notable that for both the CD4i polyclonal (Fig. 1 A) and monoclonal (Fig. 1 B) antibodies, the extent of neutralization reached only ~90%, and in the case of the clade D plasma KAMW, 80%. This is due in part to a time- and concentration-dependent interaction between sCD4 and the gp120 envelope glycoprotein because higher sCD4 concentrations and more prolonged preincubation times (30–120 min) increased the extent of HIV-2_{7312A} neutralization by both monoclonal and polyclonal CD4i antibodies (unpublished data). Steric accessibility or affinity of CD4i antibodies to their cognate epitopes may also influence the extent of virus neutralization because a single mutation (V434M) in the bridging sheet of HIV-2_{7312A}, making this amino acid the same as in HIV-1 (see Site-directed mutagenesis of the HIV-2 bridging sheet alters HIV-1 CD4i antibody recognition), resulted in a marked shift of the neutralization curves of 17b and 19e and of three HIV-1 patient plasmas (shown in Fig. 1 C) to the left and downward, resulting in 100% neutralization of infectious virus.

Multiple primary HIV-2 strains are susceptible to HIV-1 CD4i antibody neutralization

Neutralization of HIV-2 by HIV-1–elicited CD4i antibodies is not restricted to HIV-2_{7312A} and derivative strains. HIV-

Table II. Neutralization titers of HIV-1 monoclonal antibodies and patient plasma against different HIV-2 viruses

Moab	Epitope	7312A	UC-1	7312A V434M	7312A H419R	7312A Q422L
E51	CD4i	−/−[a]	−/13.0	−/4.0	−/22.0	−/−
17b	CD4i	−/0.16	−/9.4	8.0/0.002	15.0/0.002	−/−
48d	CD4i	−/−	−/−	−/−	−/−	−/−
31H	CD4i	−/3.71	−/1.58	−/0.62	−/1.42	−/−
23e	CD4i	−/−	−/−	−/−	−/−	−/−
21c	CD4i	−/0.011	−/0.005	−/0.94	−/0.014	−/0.03
X5	CD4i	−/−	−/−	−/2.5	−/−	−/−
412d	CD4i	−/−	−/−	−/−	−/−	−/−
19e	CD4i	−/0.017	−/0.009	−/0.006	−/0.005	−/0.01
ED47	CD4i	−/−	−/−	−/−	−/4.7	−/−
ED49	CD4i	−/5.4	−/12.0	−/2.4	−/3.3	−/3.0
b12	CD4bs	−/−	−/−	ND	ND	ND
F105	CD4bs	−/−	−/−	ND	ND	ND
F91	CD4bs	−/−	−/−	ND	ND	ND
15e	CD4bs	−/−	−/−	ND	ND	ND
2F5	gp41	−/−	−/−	ND	ND	ND
447-52D	V3	−/−	−/−	ND	ND	ND
19b	V3	−/−	−/−	ND	ND	ND
C011	V3	−/−	−/−	ND	ND	ND
2580	V3	−/−	−/−	ND	ND	ND
2442	V3	−/−	−/−	ND	ND	ND
2G12	glycan	−/−	−/−	ND	ND	ND
A32	gp120	−/−	−/−	ND	ND	ND
C11	gp120	−/−	−/−	ND	ND	ND
2.6C	HIV-2/gp120	−/−	−/−	ND	ND	ND
1.7A	HIV-2/gp120	0.016/0.011	0.005/0.007	0.017/0.009	0.023/0.017	0.009/0.009
Patient ID	**HIV-1 subtype**					
6X4F	A	−/10,000	370/76,923	20/41,667	4,065/96,937	ND
21X0F	A	−/6,667	500/13,699	63/17,241	222/47,619	ND
37X4F	A	−/3,846	−/1,333	59/68,027	435/65,240	ND
BAMA0037	B	36/4,167	83/3,448	40/16,667	48/4,167	ND
SMST1012	B	67/7,692	370/9,090	48/13,514	192/4,348	ND
KIMA9001	B	31/1,136	36/1,563	37/6,250	21/1,612	ND
200M	C	−/2,941	91/5,000	31/4,348	77/7,692	ND
49M	C	−/17,241	385/17,241	45/27,027	333/65,189	ND
42F	C	−/5,000	263/6,251	−/52,632	−/18,181	ND
KAMW	D	−/18,868	53/18,519	143/83,333	27/26,316	ND
sCD4		9 nM	3 nM	15 nM	28 nM	6 nM

[a]Values preceding the slash marks denote the IC_{50} in μg/ml for monoclonal antibodies and in reciprocal dilutions for patient plasma specimens, each in the absence of sCD4. Values following the slash marks denote IC_{50} values in the presence of sCD4. sCD4 concentrations were adjusted to correspond to the IC_{50} specific for each virus as indicated in the bottom row. Dashes denote absent neutralization defined as IC_{50} >25 μg/ml for monoclonal antibodies or <1:20 for human plasma. Neutralization assays were performed in JC53BL-13 cells (reference 3).

2_{UC-1} and HIV-$2_{ST/SXB1}$, two other well-characterized HIV-2 R5-tropic viruses (22, 35), along with five additional primary HIV-2 patient isolates also demonstrated striking neutralization susceptibility to HIV-1–elicited CD4i monoclonal antibodies and to HIV-1–infected patient plasma in patterns that were similar (but not identical) to HIV-2_{7312A}. Results for HIV-2_{7312A} and HIV-2_{UC-1} are compared in Table II. Each virus was susceptible to 21c and 19e and to a lesser extent 17b, 31H, and ED49. HIV-2_{UC-1} was more susceptible to E51 and 31H, but much less susceptible to 17b, compared with HIV-2_{7312A}. Both viruses were completely resistant to neutralization by 13 different HIV-1–elicited non-CD4i hu-

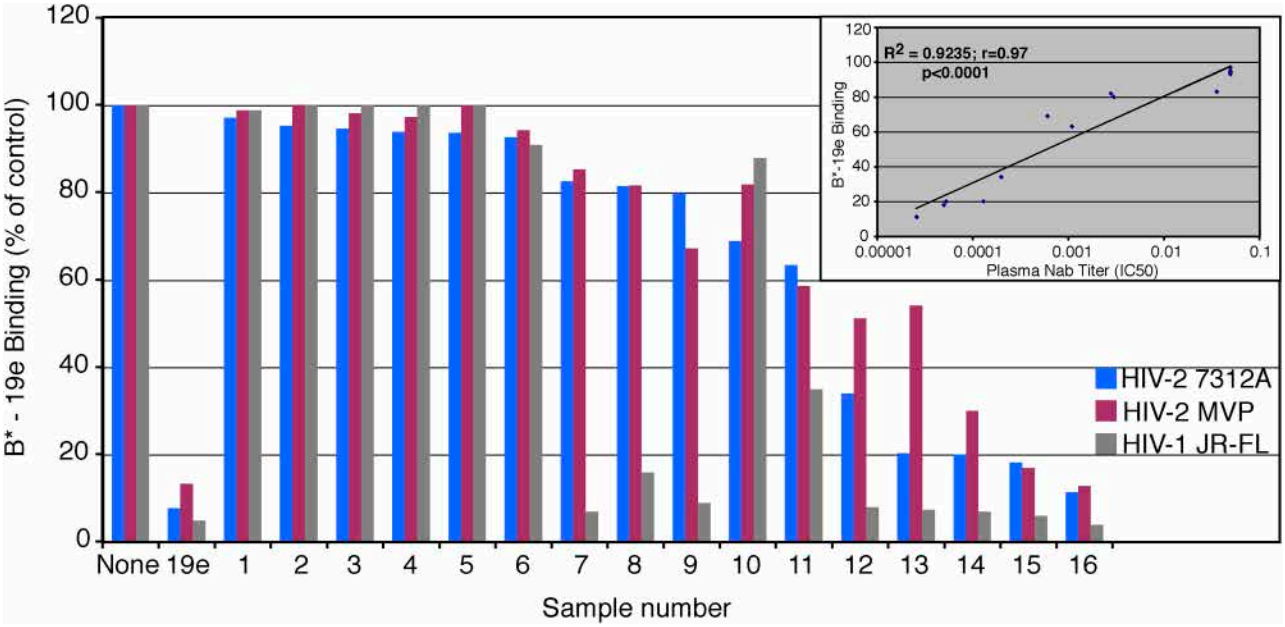

Figure 2. Blocking of biotinylated 19e binding to HIV-1 and HIV-2 gp120–sCD4 complexes by human plasma samples from either normal uninfected donors (sample nos. 1–5) or HIV-1–infected subjects (sample nos. 6–16). Unlabeled 19e effectively competed with biotinylated (B*) 19e for binding to all gp120-sCD4 complexes and served as a positive control.

man monoclonal antibodies, including those targeting the CD4 binding site (CD4bs), V3 loop, surface glycans, and gp41. HIV-2_{UC-1} was also compared with HIV-2_{7312A} in its susceptibility to neutralization by a subset of 10 HIV-1 clade A, B, C, and D patient plasmas (Table II, bottom). CD4-dependent Nab titers against HIV-2_{UC-1} were at least twofold higher than for HIV-2_{7312A} in two patients (6X4F and 21X0F), threefold lower in one patient (37X4F), and not substantially different in seven others. For each HIV-1 antibody-positive plasma specimen tested, there was a one-to-three log CD4-dependent shift in the HIV-2_{UC-1} neutralization curve (Table II, bottom).

HIV-1 CD4i antibody binding to HIV-2 glycoprotein correlates with neutralization

CD4i antibodies in HIV-1 plasma that neutralize HIV-2 infection might also be expected to compete directly with HIV-1 CD4i monoclonal antibodies for binding to HIV-2 gp120–sCD4 complexes. Fig. 2 shows the results of an assay using 16 human plasma samples (11 HIV-1 positive; 5 normal uninfected controls) to compete with biotin-conjugated 19e for binding to HIV-2_{7312A}, HIV-$2_{MVP15132}$, or HIV-1_{JR-FL} gp120–sCD4 complexes. A mock-treated sample did not inhibit biotin-labeled 19e binding, which was normalized to 100%. Unlabeled 19e competed efficiently with biotin-labeled 19e binding to each of the three HIV glycoproteins. The five normal control specimens (sample nos. 1–5) showed no significant competition for biotinylated 19e binding to any of the three HIV envelope glycoproteins. The 11 HIV-1–positive patient specimens, however, competed variably with 19e for binding to both HIV-1 and HIV-2 glycoproteins. Sample nos. 13–16 showed the strongest competition against 19e for HIV-2_{7312A} binding, and these samples also exhibited the highest neutralization titers against HIV-2_{7312A} (reciprocal mean IC_{50} = 0.00007 ± 0.00005). Sample nos. 6–9 showed the least competition with 19e for binding HIV-2_{7312A}, and these had the lowest Nab titers against this virus (IC_{50} = 0.023 ± 0.024). Other samples were intermediate in binding and neutralization activity. There was a highly significant correlation between the titers of Nab measured against HIV-2_{7312A} and the efficiency with which these plasma specimens competed with 19e for HIV-2_{7312A} binding (R^2 = 0.94; r = 0.97; $P < 0.0001$). With the exception of sample no. 10, the HIV-1–positive patient plasma specimens competed for 19e binding to the HIV-1_{JR-FL} glycoprotein more efficiently than to either of the two HIV-2 glycoproteins.

To further examine the correlation between antibody binding and neutralization, we tested a large number of biotin-labeled HIV-1 CD4i antibodies for binding to HIV-2_{7312A} envelope glycoprotein with and without sCD4. Fig. 3 A shows that the HIV-1–elicited CD4i antibodies that were found in Table II to neutralize HIV-2_{7312A} most efficiently (19e, 17b, 31H, and 21c) also bound the HIV-2_{7312A} glycoprotein most efficiently in a CD4-dependent manner, whereas those antibodies that neutralized poorly, bound poorly. To further evaluate the breadth of HIV-1 CD4i monoclonal antibody binding, we tested three antibodies (19e, 21c, and 17b) for reactivity against additional primate lentiviruses (Fig. 3 B). The HIV-1 CD4i monoclonal anti-

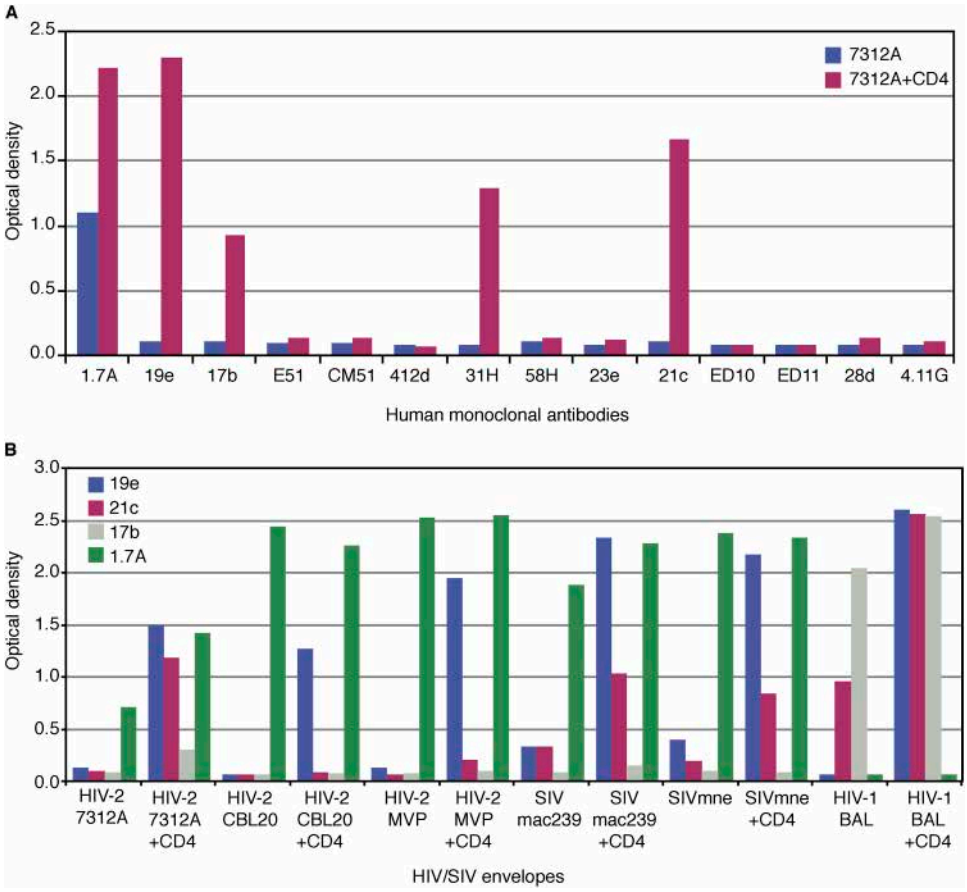

Figure 3. Screening of CD4i monoclonal antibodies for binding to HIV-2_{7312A} (A) and to additional HIV and SIV (B) gp120–sCD4 complexes. 1.7A is a human HIV-2 gp120-specific monoclonal antibody, whereas all other monoclonal antibodies are CD4i antibodies derived from HIV-1–infected humans.

bodies bound not only HIV-2_{7312A} Env–sCD4 complexes, but also HIV-2_{CBL20}, HIV-$2_{MVP15132}$, SIVmac239, SIVmne, and as a control, HIV-1_{BAL}. It is again noteworthy that gp120–sCD4 complexes from different HIV-2 and SIV strains were recognized variably by the three HIV-1 CD4i monoclonal antibodies, with 19e exhibiting the strongest reactivity to all viral envelopes, followed by 21c, and then 17b. These findings, together with the neutralization results, indicate that the CD4i chemokine receptor binding surfaces of HIV-2 strains 7312A, UC-1, ST/SXB1, CBL20, and MVP15132, as well as SIVmac239 and SIVmne, all share substantial antigenic cross-reactivity with each other and with HIV-1.

Site-directed mutagenesis of the HIV-2 bridging sheet alters HIV-1 CD4i antibody recognition

HIV-2 neutralization by HIV-1 CD4i monoclonal and polyclonal antibodies is best explained by antibodies binding to the conserved chemokine coreceptor binding surface, including the bridging sheet. To evaluate this hypothesis directly, we performed site-directed mutagenesis on the HIV-2 bridging sheet region (36). The primary amino acid sequence of the bridging sheet of HIV-1 and the corresponding sequence of HIV-2 is conserved but not identical (Fig. 4). Substitutions were made at three positions in the HIV-2_{7312A} sequence at or near the binding footprints of monoclonals 17b, 21c, and 19e in the corresponding HIV-1 sequence (8, 9, 31). The effects of these mutations on the susceptibility of the respective viruses to neutralization by HIV-1 monoclonal and polyclonal antibodies were substantial (Fig. 1 C and Table II). Mutations V434M and H419R (HXB2 numbering system; Fig. 4) made the HIV-2 sequence at these positions the same as HIV-1, and thus would be expected to enhance HIV-1 CD4i–antibody binding. The V434M substitution led to an 80-fold enhancement of 17b neutralization, at least 10-fold enhancement of X5 neutralization, 6-fold increase in E51 and 31H neutralization, and 2–3-fold enhancement of ED49 and 19e neutralization. Neutralization enhancement was not global, however, because there was a concomitant 85-fold decrease in 21c susceptibility and no change in susceptibility to the HIV-2 monoclonal 1.7A, which binds a conserved epitope distant from the bridging sheet (Table II). Similarly, the H419R mutation led to a 2- to 80-fold enhancement in neutralization by 17b, 31H, 19e, ED47, and ED49, but little or no change in susceptibility to E51, 21c, or

1.7A. In addition to mutations expected to enhance HIV-1 CD4i antibody binding, we also tested a Q422L mutant, which had been shown in HIV-1 to reduce CD4i–antibody binding (e.g., 17b), while allowing the envelope to otherwise retain its normal receptor binding and entry functions (31). The Q422L mutation in 7312A resulted in complete loss of 17b neutralization (>150-fold change), complete loss in 31H neutralization (>7-fold change), and a 3-fold decrease in 21c neutralization, but had little effect on 19e-, ED49-, or 1.7A-mediated neutralization. Enhanced susceptibility of the V434M and H419R mutants to neutralization was also observed with most of the HIV-1 patient plasmas tested (Table II).

Prevalence and titers of CD4i-neutralizing antibodies in patients infected by diverse HIV-1 subtypes

Plasma samples from 189 individuals infected by HIV-1 clade A, B, C, D, F, G, or H, or by circulating recombinant form (CRF)01, CRF02, or CRF11, were tested for CD4i Nabs against HIV-2. In preliminary studies, we tested a subset of 69 of these specimens for reactivity against the wild-type HIV-2 strain 7312A and its derivative 7312A/V434M. This pilot study showed that the frequency of detection of HIV-2 cross-reactive CD4i Nabs was modestly higher for the V434M virus (94%) compared with 7312A (87%). Based on the enhanced sensitivity of HIV-$2_{7312A/V434M}$, we used this virus to test all 189 patient plasma specimens for CD4i Nabs (Table III). CD4i Nabs were detected in 174 (92%) of patients, with median IC_{50} titers of 0.0004 (1:2,500) and mean titers of 0.004 (1:250). Titers of CD4i Nab in plasma from clade D and CRF11 patients, considered separately or as a group, were significantly greater than for patients in the remaining groups ($P < 0.0001$). We considered the possibility that, despite the overall similarity in neutralization patterns observed for the HIV-2 strains depicted in Table II, divergent HIV-2 strains might detect CD4i Nabs in some of the patients' plasmas that tested negative against HIV-$2_{7312A/V434M}$. Thus, we retested the 15 negative samples, first by Western immunoblot to confirm HIV-1 positivity, and then by neutralization assay against three different HIV-2 strains: UC-1, ST/SXB1, and 7312A. All 15 samples were Western immunoblot positive against HIV-1 proteins. Four samples were found to have CD4i Nabs against one or more of these viruses in titers ranging from 1:25 to 1:750. Thus, overall, out of 189 HIV-1–infected patients tested, 178 (94%) had detectable neutralizing CD4i antibodies against HIV-2.

Role of CD4i antibodies in natural HIV-1 infection

Previous studies have shown that HIV-1 CD4i antibodies are largely excluded by steric hindrance from the virus–cell interface after CD4 engagement, and as a consequence, CD4i antibodies generally neutralize HIV-1 inefficiently (12, 28). However, this steric restriction could be overcome experimentally by using CD4i antibody fragments (Fab or sFv) or by disassociating (spatially or temporally) envelope–CD4 engagement from envelope–coreceptor engagement (12, 28). Given these constraints on CD4i antibody-mediated neutralization, we sought to examine what role CD4i antibodies might play in vivo. Sodroski et al. (37) first postulated that CD4i antibodies might constrain virus to CD4 dependence by selecting against envelope mutations that lead to spontaneous exposure of the viral coreceptor binding surface (38, 39). Our results support this hypothesis by showing in naturally infected humans that CD4i antibodies are prevalent, high-titer, and so broadly cross-reactive that they neutralize even HIV-2. However, to test more directly if CD4i antibodies might be active in constraining HIV-1 to CD4 dependence in vivo, we examined sequential uncultured plasma specimens from four HIV-1–infected patients (133M, WEAU0575, SUMA0874, BORI0637) for evidence of viruses that contain mutations in envelope that result in greater spontaneous exposure of the receptor binding surfaces. 74 full-length, functional gp160 envelope clones were derived by PCR amplification of plasma virion RNA and used to pseudotype *env*-deficient HIV-1 virus for entry in JC53BL-13 cells. Two clones from patient SUMA0874 (S736-68 and S736-75) were found to be uniquely sensitive to neutralization by sCD4 (IC_{50} <0.05 μg/ml), indicating that they might exhibit greater spontaneous exposure of receptor-binding surfaces than is generally observed in primary HIV-1 strains (40). These same two clones were also distinguished from all others that we examined by an isoleucine (I) to threonine (T) substitution at position 309 (HXB2 numbering system) immediately 5′ of the GPGR crown of the V3 loop (Fig. S1, available a http://www.jem.org/cgi/content/full/jem.20042510/DC1), a position reported by Quinnan et al. (41) to confer CD4-independent infectivity and enhanced susceptibility to neutralization in an unrelated primary HIV-1 strain. We, therefore, first tested clones S736-68 and S736-75, along with other SUMA clones lacking the I309T mutation (including S736-68m/TI), for CD4-independent fusion and infectivity in Cf2Th-synCCR5 cells, a canine thymocyte cell line that expresses human CCR5 but lacks CD4 on its surface (42). The S736-68 and S736-75 envelopes, but not isogenic envelopes lacking the I309T mutation, supported CD4-independent virus fusion and entry, and this was abolished by treatment with 17b and other HIV-1 CD4i antibodies (unpublished data). We next tested the S736-68 envelope clone, along with a site-directed mutant that restored the more common isoleucine at position 309 (S736-68m/TI), for their susceptibility to sCD4, to an anti-CD4 monoclonal antibody, to the CD4i monoclonal 17b, and to autologous SUMA plasma in JC53BL-13 cells (Fig. 5). The S736-68 pseudotyped virus was far more sensitive compared with the isogenic S736-68m/TI mutant to neutralization by sCD4, 17b, and autologous plasma, and it was less sensitive to inhibition by anti-CD4 antibody. Similar findings were made with S736-75. These data suggest that the S736-68 and S736-75 envelopes, like those from some T cell line adapted viruses, have a spontaneously exposed chemokine coreceptor binding site

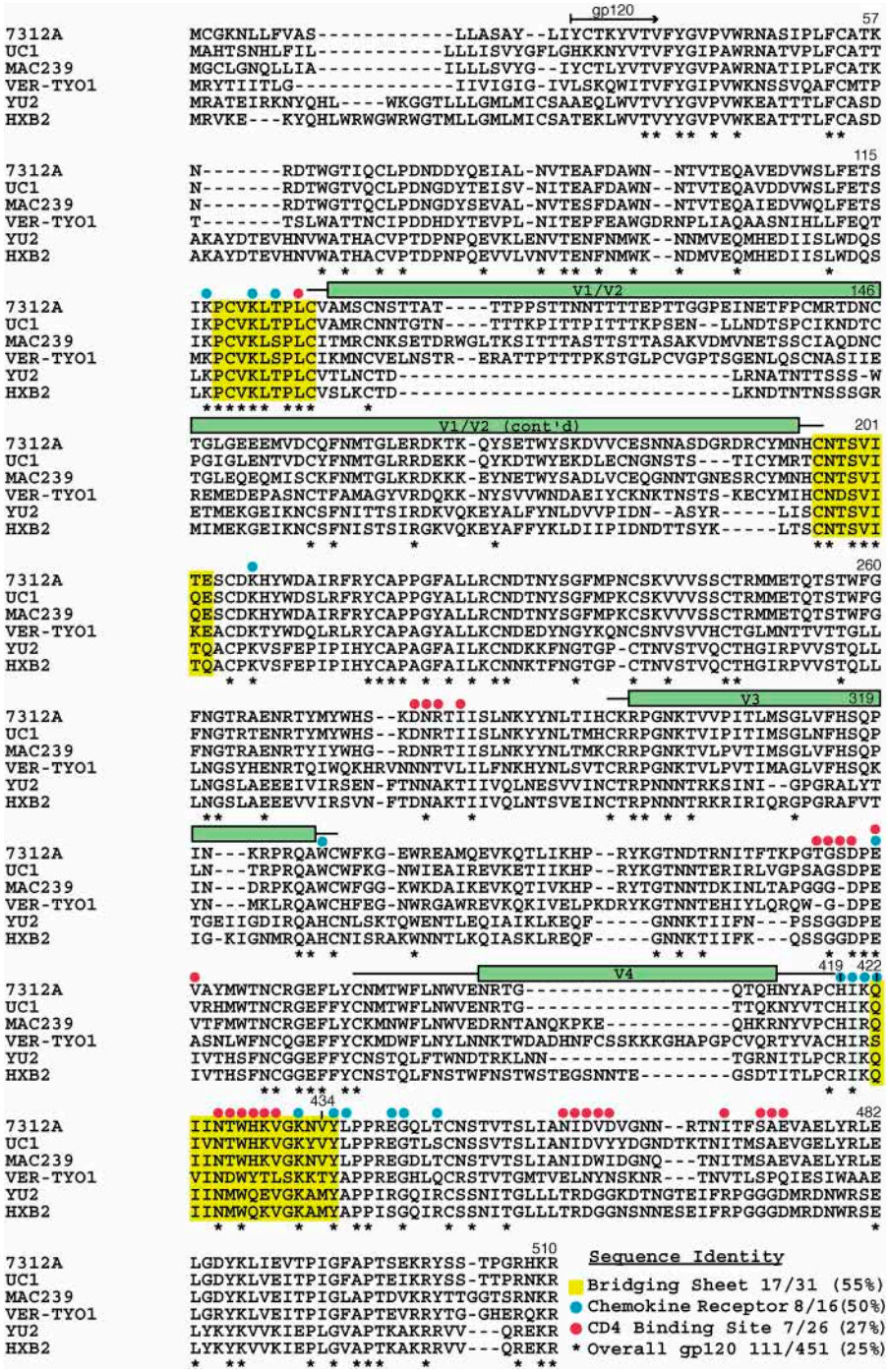

Figure 4. Envelope gp120 alignments for HIV-2 (7312A and UC1), SIV (Mac239 and Ver-Tyo1), and HIV-1 (YU2 and HXB2). Bridging sheet, variable loops, amino acid identities, and site-directed mutations (H419R, Q422L, and V434M) are indicated. The signal peptide-gp120 cleavage position for HIV-1 is shown. Variable loops (V1/V2, V3, and V4) have conventionally been defined by disulfide-linked cysteine residues at their bases as depicted. However, the actual limits of variable loops have been resolved structurally in the HXB2–CD4–17b crystal complex (reference 8), and these sequences are indicated by green bars. It is possible that structural details diverge in the more distantly related HIV/SIV sequences. The amino acids contributing to the bridging sheet are highlighted in yellow. Blue dots indicate residues contributing to chemokine coreceptor binding based on site-directed mutagenesis studies (references 29, 30). Additional amino acids within the stem of V3, including 298R, 301N, 303T, 323I, 325N, 326M, and 327R, may contribute to gp120 interaction with CCR5 (reference 76). Red dots indicate HIV-1 contact residues for CD4 based on crystal structure analyses (reference 8). Asterisks below the sequence indicate conservation of amino acid identity across all five virus strains. Overall gp120 sequence identity was calculated based on amino acid residues exclusive of the initiator methionine of the (cleaved) signal peptide and a gap-stripped alignment of the sequences shown. Except for SIVverTYO1, sequences were obtained from the HIV Sequence Compen-

Table III. Prevalence and titers of CD4i-neutralizing antibodies against HIV-$2_{7312A/V434M}$ in plasma of HIV-1–infected subjects

HIV-1 plasma	*n*	Positive	CD4i Nab titers[a]		
			Mean	SD	Median
		(%)			
Clade A	39	35 (90)	0.0029	0.0052	0.0007
Clade B	25	24 (96)	0.0047	0.0105	0.0003
Clade C	23	22 (96)	0.0051	0.0118	0.0004
Clade D	7	7 (100)	0.00007	0.00006	0.00007
Clade F	6	5 (83)	0.0008	0.0005	0.001
Clade G	5	3 (60)	0.0061	0.0092	0.0015
Clade H	2	2 (100)	0.002	0.0028	0.002
CRF01	1	1 (100)	0.0003	–	0.0003
CRF02	77	72 (94)	0.0053	0.0106	0.0008
CRF11	4	3 (75)	0.00005	0.00002	0.00004
Total	189	174 (92)	0.004	0.0093	0.0004

[a]Reciprocal IC_{50} titers of CD4i-neutralizing antibodies against HIV-$2_{7312A/V434M}$ pretreated with 15 nM sCD4.

and is less dependent on CD4 binding for entry compared with most primary viruses. Thus, exposure of the coreceptor binding surface on primary HIV-1 viral envelopes occurs spontaneously in vivo, but such viruses are exquisitely sensitive to neutralization by antibodies including those targeting CD4i epitopes.

Breadth of antigenic cross-reactivity in the HIV and SIV coreceptor binding sites

To examine the breadth of antigenic cross-reactivity in the coreceptor binding sites of HIV-1, HIV-2, SIVsm, and SIVagm, we preincubated strains of each virus with CD4i monoclonal antibodies or plasma from infected subjects (with and without sCD4) and assayed for virus neutralization or fusion inhibition. The results showed that natural infection by these lentiviruses elicits antibodies that neutralize the homologous virus as well as the evolutionarily divergent viruses. Fig. S2 (available at http://www.jem.org/cgi/content/full/jem.20042510/DC1) depicts potent neutralization of the four viruses by HIV-1–elicited monoclonal and polyclonal CD4i antibodies. These results extend the findings of Berger et al., who observed that HIV-1 subtypes A, B, C, D, E, and F were all susceptible to neutralization by the HIV-1 CD4i monoclonal antibody 17b (28).

DISCUSSION

Although much is known about the HIV-1 envelope glycoprotein (7–17, 24–33), the present study provides new insight into its immunogenicity and antigenic conservation. Previous studies suggested that the conformationally dependent coreceptor binding surface on HIV-1 was only weakly immunogenic and CD4i antibodies were relatively uncommon (31–33). This paper indicates quite the opposite to be the case. We find the vast majority (94%) of HIV-1–infected patients infected by any 1 out of 10 different clades or CRFs harbor HIV-specific CD4i Nabs with IC_{50} titers ranging from 1:20 to >1:100,000. The mean CD4i Nab titer against HIV-$2_{7312A/V434M}$ among 189 subjects was 1:250 and the median titer was 1:2,500. 114 subjects had Nab titers ≥1:1,000, the highest reaching 1:143,000. In a related study, we found that 8 out of 10 healthy, uninfected human volunteers immunized with ALVAC vCP1452 HIV-1 gp140 alone or in combination with soluble monomeric HIV-1 gp120 (AIDSVAX B/B) developed HIV-1 CD4i-neutralizing antibodies against HIV-2_{7312A}, compared with 0 out of 5 control subjects who were vaccinated with placebo (unpublished data). To explain the elicitation of CD4i Nabs by soluble HIV-1 gp120 or expressed gp140, we suspect that envelope glycoprotein is bound to cell surface–associated CD4, undergoes conformational change, and elicits a CD4i antibody response.

The observation that CD4i antibodies elicited by HIV-1 infection potently neutralized multiple strains of HIV-2 came as a surprise. Although most primary human and simian lentiviruses use CCR5 as a coreceptor for cell attachment and entry (23), functionally important amino acids in the HIV-1 envelope coreceptor binding region identified by mutagenesis experiments (8, 29, 30) are only partially conserved in HIV-2, SIVmac, and SIVagm (Fig. 4). Moreover, conserved receptor binding would not necessarily be expected to be reflected in conserved receptor antigenicity (43–45). Thus, the finding that HIV-1 CD4i monoclonal antibodies such as 19e and 21c could bind viral glycoproteins as divergent as those from HIV-1, HIV-2, SIVsm, SIVmac, and SIVmne in a CD4-dependent fashion (Fig. 3, A and B), and that monoclonal and polyclonal antibodies from HIV-1–infected humans routinely neutralized sCD4-triggered HIV-2 (Tables II and III), was quite unexpected. We even found that sCD4-treated SIVverTyo1 from African green monkey (Fig. 4) is susceptible to CD4i neutralization by some HIV-1–infected patient samples in titers as high as 1:1,000 (Fig. S2). Together, these observations highlight the extraordinary degree of antigenic conservation linked to coreceptor binding exhibited by diverse HIV-1 and HIV-2 lineages, and at the same time, an ability of the human humoral immune system to exploit these constraints.

It is of interest to consider the cooperative interactions that may be occurring among sCD4, the HIV-2 envelope glycoprotein, and CD4i antibody that result in potent virus neutralization. We have ruled out the possibility that HIV-1–elicited CD4i antibodies neutralize HIV-2 by binding directly to CD4 because a scorpion toxin-based CD4 mimetic that differs sub-

dium 2002 (reference 18). We determined experimentally the nucleotide sequence of the SIVverTYO1 clone used in our studies (λ phage SAH12) and found that it differed from the reported sequence of the same clone in the Compendium at positions 171 (-), 172 (N), 402 (D), 418 (C), and 427 (W). Numbering is according to the HXB2 sequence.

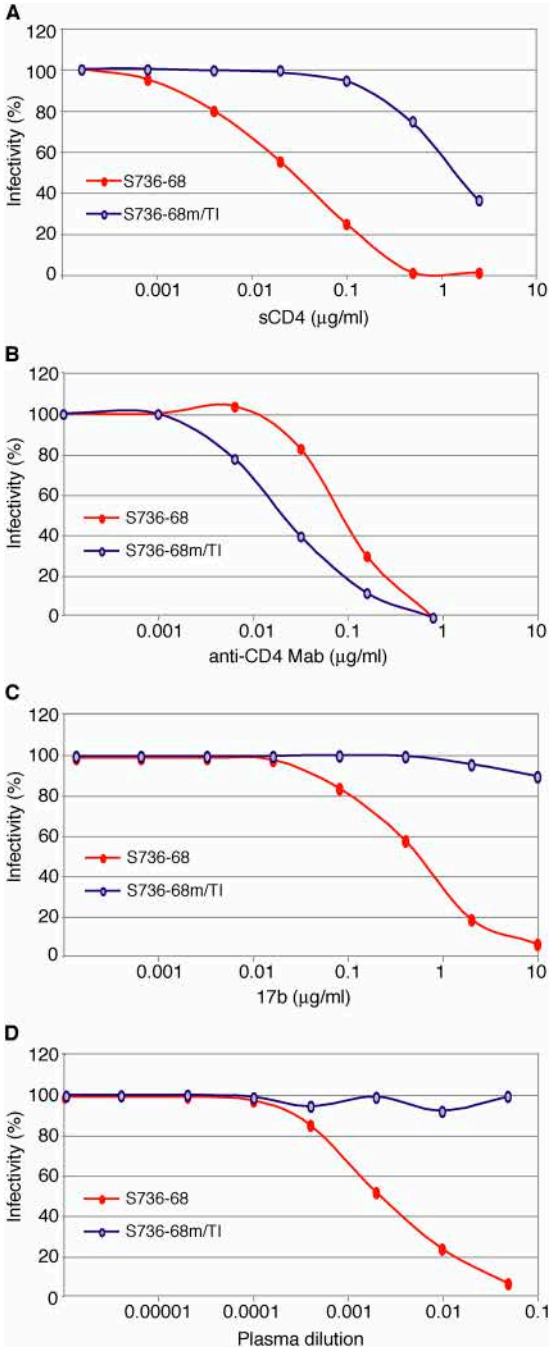

Figure 5. Neutralization of S736-68 and S736-68m/TI infectivity in JC53BL-13 cells (reference 3) by sCD4 (A), anti-CD4 monoclonal antibody RPA-T4 (B), CD4i monoclonal antibody 17b (C), and autologous patient plasma from day 278 after acute infection by HIV-1 (D).

stantially in amino acid sequence from CD4 also results in conformational changes in HIV-2 gp120 leading to binding and neutralization by different monoclonal and polyclonal CD4i antibodies (reference 46 and unpublished data). If sCD4 does not interact directly with CD4i antibodies, then it must enhance the susceptibility of virus to neutralization by inducing conformational change and exposure of CD4i epitopes, but in a cooperative manner because the magnitude of HIV-2 neutralization we observe is far greater than would be expected on the basis of additive stoichiometry. Of note, Berger et al. (47) have demonstrated cooperative interactions between different gp120 protomers within a trimer complex of HIV-1.

A role for CD4i antibodies in natural HIV-1 infection may become apparent. Our data, together with other results (37, 41), suggest that HIV-1 variants with exposed coreceptor binding surfaces and varying degrees of CD4 independence, are generated spontaneously in vivo where they are almost certainly neutralized by CD4i or other HIV-1–specific antibodies. In fact, four studies have now shown that single amino acid substitutions in the HIV-1 glycoprotein, either at the base of V1/V2 (3, 48) or in the V3 loop (the present text and reference 41), are sufficient to confer on the virus varying degrees of CD4 independence, spontaneous exposure of the coreceptor binding site, and enhanced susceptibility to CD4i Nabs. Principles of viral dynamics suggest that such mutations must be occurring in vivo on a virtually continuous basis, as has been documented for comparable mutations leading to antiretroviral drug resistance (49). Thus, CD4i antibodies may influence HIV-1 natural history and pathogenesis to a greater extent than is currently recognized by constraining virus to CD4 dependence. Consistent with this interpretation, Gabuzda et al. have shown that HIV-1 virus within the central nervous system (where circulating antibodies are relatively excluded) has less dependence on cell surface–bound CD4 for attachment and entry (50). CD4i antibodies could also influence the frequency of R5/X4 coreceptor switching (51) and target viruses with short or otherwise constrained envelope variable loop sequences (52).

The discovery that sCD4-triggered HIV-2 is susceptible to binding and neutralization by HIV-1–elicited CD4i antibodies has practical application in studies of HIV-1 natural history and vaccine assessment. A number of investigative groups have attempted to stabilize the HIV-1 envelope glycoprotein in a CD4-bound configuration to use it as an immunogen designed to elicit antibodies against viral receptor surfaces or other intermediate envelope structures (53–55). But methods to selectively identify and titer Nabs specific for such epitopes have been limited. Here, we show that neutralization of sCD4-treated HIV-2 represents an extremely sensitive and specific assay to detect HIV-1–elicited CD4i antibodies. Investigators have also targeted the membrane-proximal external region (MPER) of HIV-1 gp41 for vaccine development (56–66) because conserved epitopes in this region are capable of eliciting broadly reactive Nabs in natural infection (56–58, 65). But again, neutralization assays are lacking that allow for the sensitive and specific detection of MPER epitope-specific Nabs (17). We considered the possibility that HIV-2 could act as a "molecular scaffold" on which to present these and other HIV-1 epitope-specific antigens in the context of a functional envelope glycoprotein that does not otherwise cross-react with HIV-1–neutralizing antibodies. In recent studies, we have identified and modi-

fied by site-directed mutagenesis HIV-2 strains that can be used to detect and titer neutralization by the HIV-1 gp41 MPER-specific human monoclonal antibodies 4E10 and 2F5 with high sensitivity and specificity (unpublished data). Thus, the strategy described in this paper of using HIV-2 envelope glycoproteins in the context of infectious virions or as isolated proteins to detect HIV-1 epitope-specific neutralizing antibodies may find application in the assessment of candidate vaccines and in studies of HIV-1 natural history.

MATERIALS AND METHODS

Plasma specimens. Pre-existing coded plasma samples from 189 HIV-1–infected subjects and 15 uninfected normal control individuals were analyzed. Human subjects gave informed consent and protocols received (University of Alabama at Birmingham) institutional review board approvals.

Cell entry and neutralization assays. Plasma samples and monoclonal antibodies were assayed for Nab activity using a modification of recently described HIV entry and fusion assays (3, 32, 67). These assay systems employ the HeLa cell-derived JC53BL-13 cell line (National Institutes of Health AIDS Research and Reference Reagent Program catalogue no. 8129, TZM-b1), which has been genetically modified to constitutively express CD4, CCR5, and CXCR4, and the canine thymocyte cell line Cf2Th-synCCR5, which expresses human CCR5 but not CD4 (42).

Virus stocks. HIV-2 proviral clones pJK7312A (GenBank/EMBL/DDBJ accession no. L36874), pJK7312A/V434M, pJK7312A/H419R, pJK7312A/Q422L, and pJSP4-27 (ST/SXB1; references 22, 68–70) were used to transfect 293T cells. HIV-2 UC-1 *env* (22, 35) and HIV-1 133M *env*, cloned in pSM and pCR3.1, respectively, were cotransfected with pSG3deltaEnv or pJK7312AdeltaEnv to create infectious pseudovirions, as described previously (3). HIV-1 *env* genes cloned in pcDNA3.1 were cotransfected with an HIV-1 reporter virus (pNLENG1-ES-IRES) containing an enhanced green fluorescence gene (67) for virus entry studies in Cf2Th-synCCR5 cells.

Binding and competition assays. Biotinylated monoclonal antibodies (31–33, 71–76) were tested for binding to HIV-2, SIV, or HIV-1 gp120 envelope glycoproteins (34, 35, 68–70, 77–79) captured on microtiter plates coated with mAb 2.6C or EH21, as previously described (31, 32). Before the addition of biotin-labeled antibodies, gp120 was preincubated with 1–10 μg/ml sCD4 (R&D Systems) or a mock preparation and with or without competing plasma specimens.

Monoclonal antibodies. mAbs are described in supplemental Materials and methods (available at http://www.jem.org/cgi/content/full/jem.20042510/DC1).

Molecular cloning, sequencing, and mutagenesis. Full-length gp160 envelope genes were amplified by nested PCR from plasma HIV-1 RNA, cloned, and sequenced as previously described (3, 49). Sequences are deposited in GenBank/EMBL/DDBJ (accession nos. AY223761-90, AY223720-54, AY858550).

Statistical analyses. Linear regression, Pearson correlations, Fisher's exact test, and Wilcoxon rank sum test were performed on primary and log transformed datasets. Calculations were performed in SAS.

Online supplemental material. Fig. S1 shows the complete amino acid sequences for 31 gp160 envelope clones derived from plasma virus from subject SUMA0874. Four additional gp160 sequences corresponding to site-directed mutants of wild-type clones S736-68 and S736-73 containing substitutions at positions 308 or 309 (HXB2 numbering system) are designated S736-68m/TI, S736-68m/PI, S736-73m/TT, and S736-73m/PI. Fig. S2 depicts sCD4-dependent neutralization of different HIV and SIV viruses by HIV-1–elicited CD4i antibodies. Included in supplemental Materials and methods are detailed descriptions of all materials and methods. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20042510/DC1.

We thank the participants and staff of the Birmingham, Lusaka, and Kigali HIV study sites; D. Burton for providing monoclonal antibody reagents; S. Hu for providing purified SIVmne gp160; C. Weiss for providing pUC-1env; G. Air and S. Soong for helpful discussions; and W. Abbott for artwork and technical assistance.

This work was supported by the National Institute for Allergy and Infectious Diseases/National Institutes of Health (NIH) Acute Infection and Early Disease Research Program initiative (no. AI41530), the UAB Center for AIDS Research (no. AI27767), and grants from the NIH (nos. AI35467, AI24030). We also thank Bristol-Meyers Squibb, Glaxo-Smith-Kline-Agouron, and Merck for ongoing support of acute HIV-1 infection studies.

The authors have no conflicting financial interests.

Submitted: 9 December 2004
Accepted: 11 March 2005

REFERENCES

1. Albert, J., B. Abrahamsson, and K. Nagy. 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS*. 4:107–112.
2. Moog, C., H.J. Fleury, I. Pellegrin, A. Kirn, and A.M. Aubertin. 1997. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. *J. Virol.* 71:3734–3741.
3. Wei, X., J.M. Decker, S. Wang, H. Hui, J.C. Kappes, X. Wu, J.F. Salazar-Gonzalez, M.G. Salazar, J.M. Kilby, M.S. Saag, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature*. 422:307–312.
4. Richman, D.D., T. Wrin, S.J. Little, and C.J. Petropoulos. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. USA*. 100:4144–4149.
5. Pilgrim, A.K., G. Pantaleo, O.J. Cohen, L.M. Fink, J.Y. Zhou, J.T. Zhou, D.P. Bolognesi, A.S. Fauci, and D.C. Montefiori. 1997. Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term-nonprogressive infection. *J. Infect. Dis.* 176:924–932.
6. Montefiori, D.C., T.S. Hill, H.T. Vo, B.D. Walker, and E.S. Rosenberg. 2001. Neutralizing antibodies associated with viremia control in a subset of individuals after treatment of acute human immunodeficiency virus type 1 infection. *J. Virol.* 75:10200–10207.
7. Parren, P.W., J.P. Moore, D.R. Burton, and Q.J. Sattentau. 1999. The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity. *AIDS*. 13:S137–S162.
8. Kwong, P.D., R. Wyatt, J. Robinson, R.W. Sweet, J. Sodroski, and W.A. Hendrickson. 1998. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature*. 393:648–659.
9. Wyatt, R., P.D. Kwong, E. Desjardins, R.W. Sweet, J. Robinson, W.A. Hendrickson, and J.G. Sodroski. 1998. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature*. 393:705–711.
10. Wyatt, R., and J. Sodroski. 1998. The HIV-1 envelope glycoproteins: fusogens, antigens and immunogens. *Science*. 280:1884–1888.
11. Kwong, P.D., M.L. Doyle, D.J. Casper, C. Cicala, S.A. Leavitt, S. Majeed, T.D. Steenbeke, M. Venturi, I. Chaiken, M. Fung, et al. 2002. HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites. *Nature*. 420:678–682.
12. Labrijn, A.F., P. Poignard, A. Raja, M.B. Zwick, K. Delgado, M. Franti, J. Binley, V. Vivona, C. Grundner, C.C. Huang, et al. 2003. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. *J. Virol.* 77:10557–10565.
13. Burton, D.R., R.C. Desrosiers, R.W. Doms, W.C. Koff, P.D. Kwong, J.P. Moore, G.J. Nabel, J. Sodroski, I.A. Wilson, and R.T. Wyatt. 2004. HIV vaccine design and the neutralizing antibody prob-

lem. *Nat. Immunol.* 5:233–236.
14. Zolla-Pazner, S. 2004. Identifying epitopes of HIV-1 that induce protective antibodies. *Nat. Rev. Immunol.* 4:199–210.
15. Broliden, P.A., A. von Gegerfelt, P. Clapham, J. Rosen, E.M. Fenyo, B. Wahren, and K. Broliden. 1992. Identification of human neutralization-inducing regions of the human immunodeficiency virus type 1 envelope glycoproteins. *Proc. Natl. Acad. Sci. USA.* 89:461–465.
16. Scala, G., X. Chen, W. Liu, J.N. Telles, O.J. Cohen, M. Vaccarezza, T. Igarashi, and A.S. Fauci. 1999. Selection of HIV-specific immunogenic epitopes by screening random peptide libraries with HIV-1-positive sera. *J. Immunol.* 162:6155–6161.
17. Opalka, D., A. Pessi, E. Bianchi, G. Ciliberto, W. Schleif, M. McElhaugh, R. Danzeisen, R. Geleziunas, M. Miller, D.M. Eckert, et al. 2004. Analysis of the HIV-1 gp41 specific immune response using a multiplexed antibody detection assay. *J. Immunol. Methods.* 287:49–65.
18. Sequence Compendium, H.I.V. 2002. Kuiken, C.L., B. Foley, E. Freed, B. Hahn, B. Korber, P.A. Marx, F. McCutchan, J.W. Mellors, and S. Wolinsky, eds. Los Alamos National Laboratory, Los Alamos, NM. LA-UR 03-3564.
19. Weiss, R.A., P.R. Clapham, J.N. Weber, D. Whitby, R.S. Tedder, T. O'Connor, S. Chamaret, and L. Montagnier. 1988. HIV-2 antisera cross-neutralize HIV-1. *AIDS.* 2:95–100.
20. Bottiger, B., A. Karlsson, P.A. Andreasson, A. Naucler, C.M. Costa, E. Norrby, and G. Biberfeld. 1990. Envelope cross-reactivity between human immunodeficiency virus types 1 and 2 detected by different serological methods: correlation between cross-neutralization and reactivity against the main neutralizing site. *J. Virol.* 64:3492–3499.
21. Thomas, E.R., C. Shotton, R.A. Weiss, P.R. Clapham, and A. McKnight. 2003. CD4-dependent and CD4-independent HIV-2: consequences for neutralization. *AIDS.* 17:291–300.
22. Deng, H.K., D. Unutmaz, V.N. KewalRamani, and D.R. Littman. 1997. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature.* 388:296–300.
23. Zhang, Y., B. Lou, R.B. Lal, A. Gettie, P.A. Marx, and J.P. Moore. 2000. Use of inhibitors to evaluate coreceptor usage by simian and simian/human immunodeficiency viruses and human immunodeficiency virus type 2 in primary cells. *J. Virol.* 74:6893–6910.
24. Sattentau, Q.J., J.P. Moore, F. Vignaux, F. Traincard, and P. Poignard. 1993. Conformational changes induced in the envelope glycoproteins of the human and simian immunodeficiency viruses by soluble receptor binding. *J. Virol.* 67:7383–7393.
25. Wu, L., N.P. Gerard, R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Borsetti, A.A. Cardoso, E. Desjardin, W. Newman, et al. 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature.* 384:179–183.
26. Trkola, A., T. Dragic, J. Arthos, J.M. Binley, W.C. Olson, G.P. Allaway, C. Cheng-Mayer, J. Robinson, P.J. Maddon, and J.P. Moore. 1996. CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5. *Nature.* 384:184–187.
27. Wyatt, R., J. Moore, M. Accola, E. Desjardin, J. Robinson, and J. Sodroski. 1995. Involvement of the V1/V2 variable loop structure in the exposure of human immunodeficiency virus type 1 gp120 epitopes induced by receptor binding. *J. Virol.* 69:5723–5733.
28. Salzwedel, K., E.D. Smith, B. Dey, and E.A. Berger. 2000. Sequential CD4-coreceptor interactions in human immunodeficiency virus type 1 Env function: soluble CD4 activates Env for coreceptor-dependent fusion and reveals blocking activities of antibodies against cryptic conserved epitopes on gp120. *J. Virol.* 74:326–333.
29. Rizzuto, C.D., R. Wyatt, N. Hernandez-Ramos, Y. Sun, P.D. Kwong, W.A. Hendrickson, and J. Sodroski. 1998. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. *Science.* 280:1949–1953.
30. Rizzuto, C., and J. Sodroski. 2000. Fine definition of a conserved CCR5-binding region on the human immunodeficiency virus type 1 glycoprotein 120. *AIDS Res. Hum. Retroviruses.* 16:741–749.
31. Xiang, S.H., N. Doka, R.K. Choudhary, J. Sodroski, and J.E. Robinson. 2002. Characterization of CD4-induced epitopes on the HIV type 1 gp120 envelope glycoprotein recognized by neutralizing human monoclonal antibodies. *AIDS Res. Hum. Retroviruses.* 18:1207–1217.
32. Xiang, S.H., L. Wang, M. Abreu, C.C. Huang, P.D. Kwong, E. Rosenberg, J.E. Robinson, and J. Sodroski. 2003. Epitope mapping and characterization of a novel CD4-induced human monoclonal antibody capable of neutralizing primary HIV-1 strains. *Virology.* 315:124–134.
33. Huang, C.C., M. Venturi, S. Majeed, M.J. Moore, S. Phogat, M.Y. Zhang, D.S. Dimitrov, W.A. Hendrickson, J. Robinson, J. Sodroski, et al. 2004. Structural basis of tyrosine sulfation and VH-gene usage in antibodies that recognize the HIV type 1 coreceptor-binding site on gp120. *Proc. Natl. Acad. Sci. USA.* 101:2706–2711.
34. Gao, F., L. Yue, A.T. White, P.G. Pappas, J. Barchue, A.P. Hanson, B.M. Greene, P.M. Sharp, G.M. Shaw, and B.H. Hahn. 1992. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature.* 358:495–499.
35. Barnett, S.W., M. Quiroga, A. Werner, D. Dina, and J.A. Levy. 1993. Distinguishing features of an infectious molecular clone of the highly divergent and noncytopathic human immunodeficiency virus type 2 UC1 strain. *J. Virol.* 67:1006–1014.
36. Reeves, J.D., and R.W. Doms. 2002. Human immunodeficiency virus type 2. *J. Gen. Virol.* 83:1253–1265.
37. Kolchinsky, P., E. Kiprilov, and J. Sodroski. 2001. Increased neutralization sensitivity of CD4-independent human immunodeficiency virus variants. *J. Virol.* 75:2041–2050.
38. Kolchinsky, P., T. Mirzabekov, M. Farzan, E. Kiprilov, M. Cayabyab, L.J. Mooney, H. Choe, and J. Sodroski. 1999. Adaptation of a CCR5-using, primary human immunodeficiency virus type 1 isolate for CD4-independent replication. *J. Virol.* 73:8120–8126.
39. Hoffman, T.L., C.C. LaBranche, W. Zhang, G. Canziani, J. Robinson, I. Chaiken, J.A. Hoxie, and R.W. Doms. 1999. Stable exposure of the coreceptor-binding site in a CD4-independent HIV-1 envelope protein. *Proc. Natl. Acad. Sci. USA.* 96:6359–6364.
40. Pugach, P., S.E. Kuhmann, J. Taylor, A.J. Marozsan, A. Snyder, T. Ketas, S.M. Wolinsky, B.T. Korber, and J.P. Moore. 2004. The prolonged culture of human immunodeficiency virus type 1 in primary lymphocytes increases its sensitivity to neutralization by soluble CD4. *Virology.* 321:8–22.
41. Zhang, P.F., P. Bouma, E.J. Park, J.B. Margolick, J.E. Robinson, S. Zolla-Pazner, M.N. Flora, and G.V. Quinnan Jr. 2002. A variable region 3 (V3) mutation determines a global neutralization phenotype and CD4-independent infectivity of a human immunodeficiency virus type 1 envelope associated with a broadly cross-reactive, primary virus-neutralizing antibody response. *J. Virol.* 76:644–655.
42. Mirzabekov, T., N. Bannert, M. Farzan, W. Hofmann, P. Kolchinsky, L. Wu, R. Wyatt, and J. Sodroski. 1999. Enhanced expression, native purification, and characterization of CCR5, a principal HIV-1 coreceptor. *J. Biol. Chem.* 274:28745–28750.
43. Colman, P.M. 1997. Virus versus antibody. *Structure.* 5:591–593.
44. Hewat, E., and D. Blaas. 2001. Structural studies on antibody interacting with viruses. *Curr. Top. Microbiol. Immunol.* 260:29–44.
45. Bizebard, T., C. Barbey-Martin, D. Fleury, B. Gigant, B. Barrere, J.J. Skehel, and M. Knossow. 2001. Structural studies on viral escape from antibody neutralization. *Curr. Top. Microbiol. Immunol.* 260:55–64.
46. Darbha, R., S. Phogat, A.F. Labrijn, Y. Shu, Y. Gu, M. Andrykovitch, M.Y. Zhang, R. Pantophlet, L. Martin, C. Vita, et al. 2004. Crystal structure of the broadly cross-reactive HIV-1-neutralizing Fab X5 and fine mapping of its epitope. *Biochemistry.* 43:1410–1417.
47. Salzwedel, K., and E.A. Berger. 2000. Cooperative subunit interactions within the oligomeric envelope glycoprotein of HIV-1: functional complementation of specific defects in gp120 and gp41. *Proc. Natl. Acad. Sci. USA.* 97:12794–12799.
48. Kolchinsky, P., E. Kiprilov, P. Bartley, R. Rubinstein, and J. Sodroski. 2001. Loss of a single N-linked glycan allows CD4-independent human immunodeficiency virus type 1 infection by altering the position of the gp120 V1/V2 variable loops. *J. Virol.* 75:3435–3443.
49. Wei, X., S.K. Ghosh, M.E. Taylor, V.A. Johnson, E.A. Emini, P. Deutsch, J.D. Lifson, S. Bonhoeffer, M.A. Nowak, B.H. Hahn, et al. 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature.* 373:117–122.

50. Gorry, P.R., J. Taylor, G.H. Holm, A. Mehle, T. Morgan, M. Cayabyab, M. Farzan, H. Wang, J.E. Bell, K. Kunstman, et al. 2002. Increased CCR5 affinity and reduced CCR5/CD4 dependence of a neurovirulent primary human immunodeficiency virus type 1 isolate. *J. Virol.* 76:6277–6292.
51. Moore, J.P., S.G. Kitchen, P. Pugach, and J.A. Zack. 2004. The CCR5 and CXCR4 coreceptors–central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retroviruses.* 20:111–126.
52. Derdeyn, C.A., J.M. Decker, F. Bibollet-Ruche, J.L. Mokili, M. Muldoon, S.A. Denham, M.L. Heil, F. Kasolo, R. Musonda, B.H. Hahn, et al. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. *Science.* 303:2019–2022.
53. Xiang, S.H., P.D. Kwong, R. Gupta, C.D. Rizzuto, D.J. Casper, R. Wyatt, L. Wang, W.A. Hendrickson, M.L. Doyle, and J. Sodroski. 2002. Mutagenic stabilization and/or disruption of a CD4-bound state reveals distinct conformations of the human immunodeficiency virus type 1 gp120 envelope glycoprotein. *J. Virol.* 76:9888–9899.
54. Liao, H.X., S.M. Alam, J.R. Mascola, J. Robinson, B. Ma, D.C. Montefiori, M. Rhein, L.L. Sutherland, R. Scearce, and B.F. Haynes. 2004. Immunogenicity of constrained monoclonal antibody A32-human immunodeficiency virus (HIV) Env gp120 complexes compared to that of recombinant HIV type 1 gp120 envelope glycoproteins. *J. Virol.* 78:5270–5278.
55. Fouts, T.R., R. Tuskan, K. Godfrey, M. Reitz, D. Hone, G.K. Lewis, and A.L. DeVico. 2000. Expression and characterization of a single-chain polypeptide analogue of the human immunodeficiency virus type 1 gp120-CD4 receptor complex. *J. Virol.* 74:11427–11436.
56. Purtscher, M., A. Trkola, G. Gruber, A. Buchacher, R. Predl, F. Steindl, C. Tauer, R. Berger, N. Barrett, A. Jungbauer, and H. Katinger. 1994. A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. *AIDS Res. Hum. Retroviruses.* 10:1651–1658.
57. Buchacher, A., R. Predl, K. Strutzenberger, W. Steinfellner, A. Trkola, M. Purtscher, G. Gruber, C. Tauer, F. Steindl, A. Jungbauer, and H. Katinger. 1994. Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. *AIDS Res. Hum. Retroviruses.* 10:359–369.
58. Zwick, M.B., A.F. Labrijn, M. Wang, C. Spenlehauer, E.O. Saphire, J.M. Binley, J.P. Moore, G. Stiegler, H. Katinger, D.R. Burton, and P.W. Parren. 2001. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. *J. Virol.* 75:10892–10905.
59. Ho, J., K.S. MacDonald, and B.H. Barber. 2002. Construction of recombinant targeting immunogens incorporating an HIV-1 neutralizing epitope into sites of differing conformational constraint. *Vaccine.* 20:1169–1180.
60. Liang, X., S. Munshi, J. Shendure, G. Mark III, M.E. Davies, D.C. Freed, D.C. Montefiori, and J.W. Shiver. 1999. Epitope insertion into variable loops of HIV-1 gp120 as a potential means to improve immunogenicity of viral envelope protein. *Vaccine.* 17:2862–2872.
61. McGaughey, G.B., M. Citron, R.C. Danzeisen, R.M. Freidinger, V.M. Garsky, W.M. Hurni, J.G. Joyce, X. Liang, M. Miller, J. Shiver, and M.J. Bogusky. 2003. HIV-1 vaccine development: constrained peptide immunogens show improved binding to the anti-HIV-1 gp41 MAb. *Biochemistry.* 42:3214–3223.
62. Tian, Y., C.V. Ramesh, X. Ma, S. Naqvi, T. Patel, T. Cenizal, M. Tiscione, K. Diaz, T. Crea, E. Arnold, et al. 2002. Structure-affinity relationships in the gp41 ELDKWA epitope for the HIV-1 neutralizing monoclonal antibody 2F5: effects of side-chain and backbone modifications and conformational constraints. *J. Pept. Res.* 59:264–276.
63. Barnett, S.W., S. Lu, I. Srivastava, S. Cherpelis, A. Gettie, J. Blanchard, S. Wang, I. Mboudjeka, L. Leung, Y. Lian, et al. 2001. The ability of an oligomeric human immunodeficiency virus type 1 (HIV-1) envelope antigen to elicit neutralizing antibodies against primary HIV-1 isolates is improved following partial deletion of the second hypervariable region. *J. Virol.* 75:5526–5540.
64. Mascola, J.R., S.W. Snyder, O.S. Weislow, S.M. Belay, R.B. Belshe, D.H. Schwartz, M.L. Clements, R. Dolin, B.S. Graham, G.J. Gorse, et al. 1996. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. *J. Infect. Dis.* 173:340–348.
65. Binley, J.M., T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, et al. 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. *J. Virol.* 78:13232–13252.
66. Ofek, G., M. Tang, A. Sambor, H. Katinger, J.R. Mascola, R. Wyatt, and P.D. Kwong. 2004. Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. *J. Virol.* 78:10724–10737.
67. Levy, D.N., G.M. Aldrovandi, O. Kutsch, and G.M. Shaw. 2004. Dynamics of HIV-1 recombination in its natural target cells. *Proc. Natl. Acad. Sci. USA.* 101:4204–4209.
68. Robertson, D.L., B.H. Hahn, and P.M. Sharp. 1995. Recombination in AIDS viruses. *J. Mol. Evol.* 40:249–259.
69. Wu, X., H. Liu, H. Xiao, J. Kim, P. Seshaiah, G. Natsoulis, J.D. Boeke, B.H. Hahn, and J.C. Kappes. 1995. Targeting foreign proteins to human immunodeficiency virus particles via fusion with Vpr and Vpx. *J. Virol.* 69:3389–3398.
70. Kumar, P., H.X. Hui, J.C. Kappes, B.S. Haggarty, J.A. Hoxie, S.K. Arya, G.M. Shaw, and B.H. Hahn. 1990. Molecular characterization of an attenuated human immunodeficiency virus type 2 isolate. *J. Virol.* 64:890–901.
71. Thali, M., J.P. Moore, C. Furman, M. Charles, D.D. Ho, J. Robinson, and J. Sodroski. 1993. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. *J. Virol.* 67:3978–3988.
72. Choe, H., W. Li, P.L. Wright, N. Vasilieva, M. Venturi, C.C. Huang, C. Grundner, T. Dorfman, M.B. Zwick, L. Wang, et al. 2003. Tyrosine sulfation of human antibodies contributes to recognition of the CCR5 binding region of HIV-1 gp120. *Cell.* 114:161–170.
73. Immunology, H.I.V. and HIV/SIV Vaccine Databases 2003. Korber, B.T.M., C. Brander, B.F. Haynes, R. Koup, J.P. Moore, B.D. Walker, and D.I. Watkins, eds. Los Alamos National Laboratory, Los Alamos, NM. LA-UR 04-8162.
74. Cole, K.S., M. Alvarez, D.H. Elliott, H. Lam, E. Martin, T. Chau, K. Micken, J.L. Rowles, J.E. Clements, M. Murphey-Corb, et al. 2001. Characterization of neutralization epitopes of simian immunodeficiency virus (SIV) recognized by rhesus monoclonal antibodies derived from monkeys infected with an attenuated SIV strain. *Virology.* 290:59–73.
75. Robinson, J.E., K.S. Cole, D.H. Elliott, H. Lam, A.M. Amedee, R. Means, R.C. Desrosiers, J. Clements, R.C. Montelaro, and M. Murphey-Corb. 1998. Production and characterization of SIV envelope-specific rhesus monoclonal antibodies from a macaque asymptomatically infected with a live SIV vaccine. *AIDS Res. Hum. Retroviruses.* 14:1253–1262.
76. Cormier, E.G., D.N. Tran, L. Yukhayeva, W.C. Olson, and T. Dragic. 2001. Mapping the determinants of the CCR5 amino-terminal sulfopeptide interaction with soluble human immunodeficiency virus type 1 gp120-CD4 complexes. *J. Virol.* 75:5541–5549.
77. Beyl, W., L. Nehring, L. Gurtler, J. Eberle, and F. Deinhardt. 1987. AIDS verusacht durch HIV-2. *Munch. Med. Wochenschr.* 129:895–896.
78. Gao, F., L. Yue, P.M. Sharp, and B.H. Hahn. 1993. Genetic typing of HIV-2 from a Senegalese/German heterosexual transmission. *AIDS Res. Hum. Retroviruses.* 9:703–704.
79. Schulz, T.F., D. Whitby, J.G. Hoad, T. Corrah, H. Whittle, and R.A. Weiss. 1990. Biological and molecular variability of human immunodeficiency virus type 2 isolates from the Gambia. *J. Virol.* 64:5177–5182.