JOURNAL OF VIROLOGY, Aug. 2007, p. 8533–8542 Vol. 81, No. 16
0022-538X/07/$08.00+0 doi:10.1128/JVI.02816-06

Susceptibility of Recently Transmitted Subtype B Human Immunodeficiency Virus Type 1 Variants to Broadly Neutralizing Antibodies[▽]

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Received 20 December 2006/Accepted 10 May 2007

The ability of the broadly neutralizing human immunodeficiency virus type 1 (HIV-1) specific human monoclonal antibodies (MAbs) b12, 2G12, 2F5, and 4E10 to neutralize recently transmitted viruses has not yet been explored in detail. We investigated the neutralization sensitivity of subtype B HIV-1 variants obtained from four primary HIV infection cases and six transmission couples (four homosexual and two parenteral) to these MAbs. Sexually transmitted HIV-1 variants isolated within the first 2 months after seroconversion were generally sensitive to 2F5, moderately resistant to 4E10 and b12, and initially resistant but later more sensitive to 2G12 neutralization. In the four homosexual transmission couples, MAb neutralization sensitivity of HIV in recipients did not correlate with the MAb neutralization sensitivity of HIV from their source partners, whereas the neutralization sensitivity of donor and recipient viruses involved in parenteral transmission was more similar. For a fraction (11%) of the HIV-1 variants analyzed here, neutralization by 2G12 could not be predicted by the presence of N-linked glycosylation sites previously described to be involved in 2G12 binding. Resistance to 2F5 and 4E10 neutralization did also not correlate with mutations in the respective core epitopes. Overall, we observed that the neutralization resistance of recently transmitted subtype B HIV-1 variants was relatively high. Although 8 of 10 patients had viruses that were sensitive to neutralization by at least one of the four broadly neutralizing antibodies studied, 4 of 10 patients harbored at least one virus variant that seemed resistant to all four antibodies. Our results suggest that vaccine antigens that only elicit antibodies equivalent to b12, 2G12, 2F5, and 4E10 may not be sufficient to protect against all contemporary HIV-1 variants and that additional cross-neutralizing specificities need to be sought.

Current immunization strategies against human immunodeficiency virus type 1 (HIV-1) are unable to induce humoral immune responses of sufficient breadth and potency to protect against infection (20, 28). It has also become increasingly apparent that even antibody responses during natural infection are generally of limited breadth (12, 30, 38), as suggested also by the recently recognized high incidence of HIV-1 superinfection (44). Clearly, a vaccine will have to elicit better responses than those measured in natural HIV infection and, considering the large variability of circulating HIV-1 variants, should induce broadly neutralizing antibodies (6). Four broadly neutralizing monoclonal antibodies (MAbs) (immunoglobulin G1b12 [IgG1b12], 2G12, 2F5, and 4E10) currently exist, although additional MAbs of somewhat more limited neutralization breadth have also been described (e.g., 447-52D [10], IgG1 X5 [31], and D5 [29]). The four most broadly neutralizing MAbs were isolated from HIV-infected individuals, which has led to the hypothesis that it may be possible to elicit these antibodies of similar specificity and breadth by vaccination. MAbs b12, 2G12, 2F5, and 4E10 generally exhibit broad cross-clade neutralization of HIV-1 in vitro (3, 5, 22, 27, 38), although a small number of viruses have been identified that are not neutralized by any of the four MAbs at the concentrations tested (5). All four MAbs have been shown to protect against viral challenge in vivo in animal models when administered alone or in combination (1, 15, 17, 25, 26, 35, 43, 50). Furthermore, passive transfer of 2G12, 2F5, and 4E10 in patients during structured treatment interruption resulted in a significant delay in viral rebound in some patients compared to viral rebound in the absence of antibodies (47), indicating that viral suppression was due to the antiviral activity of the administered antibodies.

Although MAbs b12, 2G12, 2F5, and 4E10 have been tested against viruses from both early and late stages of infection (3, 22, 38), these antibodies have not been tested vigorously for their ability to neutralize early transmitted virus variants. In the present study, we analyzed the neutralization sensitivity of recently transmitted subtype-B HIV-1 variants to these four broadly neutralizing antibodies. Since sensitivity to neutralizing antibodies of recently transmitted HIV-1 variants may be determined by the virus phenotype in the donor and/or route of transmission (12, 14, 52), we also analyzed the sensitivity of viruses from the donors that were involved in homosexual or parenteral transmission. Finally, we performed sequence analysis to correlate epitope sequence variation of the viruses under study with their sensitivity for the respective antibodies.

MATERIALS AND METHODS

Patients and viruses. Clonal virus variants from four individuals with primary HIV-1 infection after homosexual transmission were isolated at different time

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▽ Published ahead of print on 23 May 2007.

points around the moment of seroconversion until up to 2 years of seropositive follow-up. In addition, viruses were obtained from four homosexual transmission couples. Donor-recipient pairs H19545 [donor (D)1]-H18860 [recipient (R)1], H11686 (D2)-H19342 (R2), H18814 (D3)-H18766 (R3), and H13994 (D4)-H18839 (R4) participated in the Amsterdam Cohort Studies on HIV-1 infection and AIDS (http://www.amsterdamcohortstudies.org). D1-R1 and D3-R3 entered the cohort studies with a discordant serological status for HIV-1 antibodies. The transmission event was during active follow-up in the cohort studies and noticed by seroconversion of the recipient during routine 3-monthly visits. From donor-recipient pairs 2 and 4, initially only the recipients participated in the cohort studies and seroconverted for HIV-1 antibodies during active follow-up. Their respective HIV-1-positive sexual partners were asked to participate in the cohort studies after the transmission event.

In addition, we studied two parenteral transmission cases between whom accidental (H19296 [PD5] and p127 [PR5]) (19) or deliberate (p199 [PD6] and H10988 [PR6]) (51) transfer of HIV-1 contaminated blood was documented. Clonal virus variants were isolated from both donor and recipient around the moment of seroconversion of the recipient.

Clonal virus variants were previously obtained in multiple micrococultures of limiting amounts of HIV-infected patient peripheral blood mononuclear cells (PBMC) and phytohemagglutinin (PHA)-stimulated PBMC, as described previously (42, 49). Viruses were considered clonal when less then one-third of the microcultures of a given patient cell concentration produced progeny virus. Virus stocks were prepared on PHA-stimulated PBMC, and the number of passages was kept to a minimum since this may change the phenotype of the virus. Virus production was monitored by using an in-house p24 enzyme-linked immunosorbent assay (48). Upon sufficient virus production, virus stocks were frozen, and the titers of the stocks were quantified by determination of the 50% tissue culture infectious dose in PHA-stimulated PBMC. For virus titration and neutralization assays the same batch of PHA-stimulated PBMC was used. Phylogenetic analysis classified all viruses as subtype B (data not shown).

Cells. Experiments were performed with a pool of PHA-stimulated PBMC obtained from five healthy blood donors with a CCR5 wild-type genotype ($CCR5^{+/+}$) determined by PCR as described before (13). PBMC were isolated from buffy coats by Ficoll density centrifugation. For stimulation, 5×10^6 cells/ml were cultured for 2 days in Iscove modified Dulbecco medium supplemented with 10% fetal bovine serum (Perbio, Logan, Utah), penicillin (100 U/ml; Gibco, Paisly, Scotland, United Kingdom), streptomycin (100 μg/ml; Gibco), cyproxin (5 μg/ml; Bayer, Mijdrecht, The Netherlands), and PHA (5 μg/ml; Remel Europe, Dartford, England, United Kingdom). Subsequently, PBMC (10^6/ml) were grown in the absence of PHA, in medium supplemented with 10 U/ml of recombinant interleukin-2 (Cetus Corp., Emeryville, CA) and Polybrene (5 μg/ml; hexadimethrine bromide; Sigma, Zwijndrecht, The Netherlands).

Neutralization assays. Viruses were tested for their relative neutralization sensitivity to sCD4 (Progenics, Tarrytown, NY), IgG1b12 (a generous gift from D. R. Burton (7), and 2F5, 2G12, and 4E10 (32, 36, 40, 41, 45, 48) (all three purchased from Polymun Scientific, Vienna, Austria); antibodies b12 and 2G12 bind epitopes on gp120, whereas 2F5 and 4E10 bind to epitopes located in the membrane-proximal external region of gp41. From each virus isolate, an inoculum of 20 50% tissue culture infective doses in a total volume of 100 μl was incubated in triplicate with decreasing concentrations of the antibodies or sCD4 (starting at a concentration of 12.5 μg/ml) in 96-well microtiter plates. After 1 h of incubation at 37°C, 10^5 PHA-stimulated PBMC (50 μl) were added. On days 7 and 14, virus production in supernatant was analyzed by p24 production in an in-house p24 enzyme-linked immunosorbent assay. The percent neutralization was calculated as the mean reduction in p24 production of triplicate cultures in the presence of the neutralizing agent compared to cultures with virus only. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression. Neutralization sensitivity of a random selection of viruses was similar in two independent experiments, confirming reproducibility of our assay (data not shown).

DNA isolation, PCR, and sequence analyses. DNA was isolated from infected PBMC as described previously (4). PCR was used to amplify the complete HIV-1 *env* gene with the Expand High Fidelity PCR system (Roche Diagnostics, Mannheim, Germany). Primer combinations and PCR conditions were as described previously (2). PCR products were purified by using ExoSAP-IT (USB, Cleveland, Ohio) according to the manufacturer's protocol. For all viruses the HIV-1 envelope gene was sequenced. The sequencing conditions were 5 min at 94°C, followed by 30 cycles of 15 s at 94°C, 10 s at 50°C, and 2 min at 60°C, with a final 10-min extension at 60°C. Sequencing was performed by using BigDye Terminator v1.1 Cycle Sequencing kit (ABI Prism, Applied Biosystems, Warrington, United Kingdom), according to the manufacturer's protocol on an ABI 3130 automated sequencer (Applied Biosystems). Nucleotide sequences of all virus clones per individual were aligned by using CLUSTAL W in the software package of BioEdit (18) or DAMBE (53) and edited manually. Potential N-linked glycosylation sites were identified by using N-Glycosite (54). Envelope sequence positions are indicated as relative to the HXB2 reference sequence.

Statistical analysis. Differences in antibody IC_{50} values for neutralization of early and late HIV-1 variants were evaluated with the Mann-Whitney U test in SPSS 13.0 software (SPSS, Inc., Chicago, IL). A result was considered significant when the P value was <0.05.

RESULTS

Neutralization sensitivity of recently transmitted HIV-1 variants. HIV-1 variants that were isolated in the phase of primary HIV-1 infection after homosexual transmission from patients B, D, F, and W and during subsequent follow-up (patients B, D, and W only) were tested for their neutralization sensitivity to sCD4 and the broadly neutralizing antibodies b12, 2G12, 2F5 and 4E10 (Table 1).

With the exception of viruses from patient F, all HIV-1 variants isolated around the moment of seroconversion were resistant to neutralization by sCD4 and the anti-gp120 MAb b12. The other gp120-directed MAb, 2G12, was only able to neutralize a few of the very early virus variants from patient D.

Of the anti-gp41 antibodies, 2F5 exhibited the highest potency and neutralized the majority of both early and late HIV-1 variants from patients D and F and with somewhat reduced potency the variants from patient B. Antibody 4E10 only neutralized early HIV-1 variants from patients D and F but with only low potency. All early virus variants from patient W and three early virus variants from patient B were not neutralized by any of the four antibodies up to the highest antibody concentration tested here (i.e., 12.5 μg/ml).

For patients B, D, and W and for both sCD4 and each of the MAbs under study, differences in IC_{50} values required for neutralization of HIV-1 variants isolated within 2 months after seroconversion and for HIV-1 variants from the same patient isolated at least 2 months after seroconversion were evaluated in a Mann-Whitney U test. HIV-1 variants isolated later in the course of infection from patient W were significantly more sensitive to neutralization by all four MAbs, as well as sCD4 ($P < 0.01$). Virus variants isolated later in infection from patient D showed a significantly increased neutralization sensitivity for sCD4 and b12 ($P = 0.05$). For patient B, no significant change in neutralization sensitivity was observed during the study period.

Neutralization sensitivity of donor and recipient virus clones after homosexual transmission. The vast majority of recently transmitted virus variants that were isolated during primary infection from individuals B, D, F, and W were resistant to neutralization by sCD4, b12, and 2G12; moderately to completely resistant to 4E10; and more sensitive to 2F5 neutralization. We subsequently studied whether the neutralization sensitivity of viruses in recently infected individuals may relate to the neutralization sensitivity of HIV-1 variants in the donor by using clonal virus variants from four homosexual transmission couples. Phylogenetic analysis demonstrated that virus variants from donors and recipients grouped together, indicating that transmission between partners was very likely (data not shown). Viruses from three of four recipients were isolated within 3 weeks from seroconversion, whereas the time point of virus isolation from the donor varied from 23 weeks

TABLE 1. Ability of sCD4 and broadly neutralizing antibodies to neutralize viruses from patients with primary infection

Virus variant	No. of days after seroconversion	IC_{50} (μg/ml)				
		sCD4	b12	2G12	2F5	4E10
B.1.A6	−2	>12.50	>12.50	>12.50	8.28	>12.50
B.1.B5		>12.50	>12.50	>12.50	9.77	>12.50
B.1.G11		>12.50	>12.50	>12.50	>12.50	>12.50
B.2.A12	0	>12.50	>12.50	>12.50	1.01	>12.50
B.2.B7		>12.50	>12.50	>12.50	0.32	>12.50
B.2.G11		>12.50	>12.50	>12.50	>12.50	>12.50
B.2.E6		>12.50	>12.50	>12.50	2.87	>12.50
B.3.F3	6	>12.50	>12.50	>12.50	6.36	3.75
B.3.H5		>12.50	>12.50	>12.50	>12.50	>12.50
B.3.A12		>12.50	>12.50	>12.50	4.71	11.65
B.3.D7		>12.50	>12.50	>12.50	4.21	9.33
B.4.B1	13	>12.50	>12.50	>12.50	0.43	>12.50
B.5.B8	20	>12.50	>12.50	>12.50	0.86	>12.50
B.7.D11	55	>12.50	>12.50	2.82	0.59	>12.50
B.7.H8		>12.50	>12.50	>12.50	1.62	7.67
B.11.A8	349	>12.50	>12.50	>12.50	11.54	>12.50
B.11.E10		>12.50	>12.50	>12.50	>12.50	>12.50
B.11.G11		>12.50	>12.50	>12.50	11.67	>12.50
B.13.C2	636	>12.50	10.32	8.89	4.09	3.69
B.13.E4		>12.50	>12.50	>12.50	7.31	>12.50
B.13.G11		>12.50	>12.50	>12.50	8.22	>12.50
D.1.H7	1	>12.50	11.62	1.27	1.81	5.37
D.1.B8		>12.50	>12.50	>12.50	1.28	>12.50
D.1.G1		>12.50	>12.50	0.34	0.89	7.32
D.3.A10	30	>12.50	2.82	>12.50	0.81	>12.50
D.3.G1		>12.50	>12.50	>12.50	0.50	7.10
D.7.A5	547	0.33	<0.20	0.22	0.32	<0.20
D.7.F8		>12.50	4.11	3.19	0.92	11.44
D.7.G3		1.78	1.15	4.61	<0.20	0.61
F.1	1	1.43	1.16	>12.50	0.36	7.84
F.2.I	7	>12.50	4.52	>12.50	0.29	11.62
F.2.II		>12.50	10.45	>12.50	1.12	11.35
F.3.A12	10	>12.50	11.68	>12.50	0.49	10.17
F.3.F6		>12.50	>12.50	>12.50	0.44	7.52
F.3.G9		>12.50	1.54	>12.50	0.33	>12.50
F.4.I	14	>12.50	>12.50	>12.50	0.34	4.69
F.4.II		>12.50	6.27	>12.50	0.51	5.48
F.5.A8	17	ND	1.77	>12.50	1.04	5.76
F.5.B2		>12.50	3.01	>12.50	0.38	2.53
F.5.G8		>12.50	>12.50	>12.50	0.44	>12.50
F.7.F6	31	>12.50	8.34	>12.50	0.24	>12.50
F.7.G11		>12.50	10.77	>12.50	0.41	>12.50
F.7.G4		8.92	6.60	>12.50	0.41	12.20
F.9.I	59	0.92	8.57	>12.50	0.42	10.16
F.9.B7		>12.50	0.20	>12.50	0.27	<0.20
F.9.F5		>12.50	0.61	>12.50	0.38	2.61
W.I.16	−5	>12.50	>12.50	>12.50	>12.50	>12.50
W.I.3		>12.50	>12.50	>12.50	>12.50	>12.50
W.I.6		>12.50	>12.50	>12.50	>12.50	>12.50
W.II.1	−3	>12.50	>12.50	>12.50	>12.50	>12.50
W.II.4		>12.50	>12.50	>12.50	>12.50	>12.50
W.II.5		>12.50	>12.50	>12.50	>12.50	>12.50
W.III.2	0	>12.50	>12.50	>12.50	>12.50	>12.50
W.III.6		>12.50	>12.50	>12.50	>12.50	>12.50
W.III.7		>12.50	>12.50	>12.50	>12.50	>12.50
W.IV.1	4	>12.50	>12.50	>12.50	>12.50	>12.50
W.IV.2		>12.50	>12.50	>12.50	>12.50	>12.50
W.IV.4		>12.50	>12.50	>12.50	>12.50	>12.50
W.V.E6	268	>12.50	0.54	0.50	0.37	0.63
W.V.E7		>12.50	>12.50	0.83	2.24	1.56
W.VII.B6	1,918	>12.50	0.58	<0.20	>12.50	1.15

TABLE 2. Ability of sCD4 and broadly neutralizing antibodies to neutralize viruses from homosexual transmission couples for sCD4 and broadly neutralizing antibodies

Virus variant[a]	No. of wks after seroconversion of recipient	IC_{50} (μg/ml)				
		sCD4	b12	2G12	2F5	4E10
D1.6F1	−23	>12.50	7.94	4.30	0.63	3.92
D1.6G5		10.93	0.47	>12.50	<0.20	2.31
D1.6F3		>12.50	>12.50	>12.50	>12.50	>12.50
R1.6F5	2	>12.50	>12.50	>12.50	>12.50	>12.50
R1.6G9		>12.50	>12.50	>12.50	2.08	0.33
R1.48H8	452	>12.50	>12.50	>12.50	8.90	0.28
R1.48C4		5.92	0.74	0.41	0.34	0.58
R1.48D4		9.40	>12.50	0.36	0.37	<0.20
R1.48E1		>12.50	>12.50	0.39	2.31	6.23
D2.3A6	77	>12.50	0.56	>12.50	9.62	11.93
D2.3B10		>12.50	>12.50	>12.50	1.83	>12.50
D2.3C3		>12.50	1.87	3.70	2.10	>12.50
D2.3D12		>12.50	>12.50	9.54	1.97	>12.50
D2.3F4		>12.50	>12.50	>12.50	2.61	>12.50
R2.8A6	3	>12.50	1.06	>12.50	3.01	<0.20
R2.8D6		>12.50	1.93	>12.50	>12.50	>12.50
R2.8C6		>12.50	<0.20	>12.50	>12.50	6.86
R2.8D2		>12.50	<0.20	>12.50	>12.50	3.42
R2.8F6		>12.50	0.25	>12.50	>12.50	0.31
R2.54G11	415	>12.50	>12.50	0.43	8.43	>12.50
R2.54H8		>12.50	>12.50	0.58	7.86	6.86
D3.14A5	0	>12.50	0.89	0.32	2.55	2.92
D3.14B2		>12.50	0.68	0.58	>12.50	1.60
D3.14E3		>12.50	1.30	0.31	0.57	0.63
D3.14G1		>12.50	0.50	<0.20	0.60	4.12
R3.3A9	3	>12.50	>12.50	>12.50	>12.50	10.36
R3.3D9		>12.50	>12.50	>12.50	>12.50	>12.50
D4.3E9	102	>12.50	>12.50	<0.20	11.08	1.12
D4.3H4		>12.50	>12.50	<0.20	<0.20	<0.20
D4.3H9		>12.50	>12.50	<0.20	0.54	4.69
D4.5A11	126	11.65	0.53	<0.20	2.17	5.21
D4.5C8		7.84	<0.20	<0.20	>12.50	>12.50
D4.5F10		>12.50	<0.20	<0.20	<0.20	5.09
D4.5G3		12.33	<0.20	<0.20	ND[b]	1.90
D4.5H9		7.42	<0.20	<0.20	ND	<0.20
R4.15A9	89	>12.50	>12.50	>12.50	>12.50	>12.50
R4.15D5		>12.50	>12.50	>12.50	>12.50	>12.50
R4.15F10		>12.50	>12.50	<0.20	>12.50	>12.50
R4.15F9		>12.50	0.38	>12.50	>12.50	>12.50
R4.15H6		>12.50	1.08	>12.50	>12.50	>12.50
R4.43A11	340	>12.50	10.97	2.45	>12.50	8.96
R4.43B12		>12.50	>12.50	>12.50	>12.50	>12.50
R4.43E1		>12.50	>12.50	>12.50	>12.50	>12.50

[a] D, donor; R, recipient.
[b] ND, not determined.

before to 102 weeks after the moment of seroconversion in the recipient. The earliest virus variants from R4 were isolated relatively late (89 weeks after SC), and it cannot be excluded that their neutralization sensitivity is determined by adaptation in R4 as the new host rather than the virus phenotype in the donor.

The ability of sCD4 and the four antibodies to neutralize HIV-1 variants from homosexual transmission pairs is shown in Table 2. All early virus variants from recipients were resistant to neutralization by sCD4 and 2G12. Virus variants from their source partners were also resistant to neutralization by sCD4, but 2G12 neutralization sensitivity of these donor viruses varied from moderately to highly resistant (D1 and D2) to even highly sensitive (D3 and D4). Three recipients (R1, R3,

and R4) had b12-resistant HIV-1 variants at their earliest moment of virus isolation, whereas their respective source partners had highly sensitive virus variants (D3), a mixture of neutralization resistant and sensitive viruses (D1), or viruses that were completely resistant (D4) to b12 neutralization at the time point closest to the moment of transmission. The earliest viruses isolated from subject R2 were highly sensitive to b12 neutralization and his donor had a virus population consisting of b12-sensitive and -resistant variants.

With the exception of some early virus variants in R1 and R2, early recipient HIV-1 variants were resistant to 2F5, whereas 2F5 neutralization sensitivity of viruses from all source partners was relatively high. For D1-R1 and D2-R2, 4E10 neutralization sensitivity of recipient HIV-1 variants was in general higher than that of the HIV-1 variants from their partners. The opposite was observed for D3-R3 and D4-R4.

Although it is arguable that time points of virus isolation from the donor may be too far from the moment of transmission, neutralization sensitivity patterns of recipient viruses did not seem to be determined by the neutralization sensitivity of HIV-1 variants in the donor. Instead, recipient HIV-1 variants early in the course of infection tended to be more resistant to antibody neutralization than HIV-1 variants from their respective source partners. However, this observation was not consistent for all antibodies tested and varied also depending on the recipient.

Neutralization sensitivity of donor and recipient virus clones after parenteral transmission. Our observation that the neutralization sensitivity of viruses after homosexual transmission is low and may not be related to the neutralization sensitivity of viruses from the donor prompted us to investigate whether this phenomenon is specific for homosexual transmission or whether it is also observed in parenteral transmission, a route in which the mucosa is not involved. For this, we studied the neutralization sensitivity of HIV-1 variants from two parenteral transmission pairs (Table 3).

Viruses from donor PD5 that were isolated 8 months before the moment of transmission were mainly resistant to neutralization by sCD4 and all four antibodies. All clonal virus variants from recipient PR5 isolated 1.5 months after transmission also resisted neutralization by sCD4, 2G12, 2F5, and 4E10 but were sensitive to b12. Recipient virus variants obtained 5.5 months after transmission only showed increased neutralization sensitivity to sCD4 ($P = 0.05$).

Donor PD6 and recipient PR6 harbored both R5 and X4 virus variants (Table 3). The R5 and X4 viruses from the donor that were isolated 4.5 months after the moment of virus transmission were mainly resistant to 2G12 neutralization and sensitive to neutralization by 2F5 and 4E10. Compared to donor virus variants, recipient viruses were more sensitive to 2G12 neutralization compared to donor viruses and equally sensitive to 2F5 and 4E10 neutralization. Donor X4 viruses were more sensitive to neutralization by b12 and sCD4 than R5 viruses ($P = 0.02$ and $P = 0.03$, respectively), and a similar pattern was observed for early recipient X4 and R5 virus variants ($P = 0.03$ and $P = 0.05$, respectively) but only for b12 later in infection ($P = 0.05$).

In summary, HIV-1 variants from parenteral donor-recipient pair PD5-PR5 were similarly resistant to neutralization by sCD4, 2G12, 2F5, and 4E10 but not b12. HIV-1 variants from parenteral donor-recipient pair PD6-PR6 were equally sensitive to neutralization by sCD4, b12, 2F5, and 4E10 but not 2G12. These results are in contrast to the observations above for homosexual transmission pairs and may imply that the neutralization sensitivity of parenterally transmitted HIV-1 variants can in part be determined by the neutralization sensitivity of the HIV-1 variants in the donor.

TABLE 3. Sensitivity of viruses from parenteral transmission couples for sCD4 and broadly neutralizing antibodies

Virus variant[a]	No. of mos after exposure	Coreceptor use	IC_{50} (μg/ml)				
			sCD4	b12	2G12	2F5	4E10
D-H19296.D8	−8		>12.50	>12.50	>12.50	>12.50	7.10
PD5.E5			>12.50	>12.50	>12.50	>12.50	>12.50
PD5.E8			>12.50	>12.50	>12.50	>12.50	>12.50
PD5.F2			>12.50	>12.50	>12.50	>12.50	>12.50
PD5.F3			>12.50	>12.50	>12.50	9.70	6.70
PD5.G5			>12.50	>12.50	>12.50	11.60	>12.50
PD5.D6			>12.50	>12.50	>12.50	4.10	>12.50
PD5.F7			>12.50	3.40	>12.50	>12.50	>12.50
PD5.H11			>12.50	9.60	>12.50	>12.50	>12.50
PR5.O4.A2	1.5		12.40	<0.20	>12.50	>12.50	>12.50
PR5.O4.A3			>12.50	0.80	>12.50	>12.50	>12.50
PR5.O4.A5			>12.50	1.20	>12.50	>12.50	>12.50
PR5.O4.B1			6.80	0.70	>12.50	>12.50	>12.50
PR5.O4.C7			>12.50	1.50	>12.50	>12.50	>12.50
PR5.O4.D1			>12.50	1.50	>12.50	>12.50	>12.50
PR5.O4.F4			>12.50	0.80	>12.50	>12.50	>12.50
PR5.O8.B6	5.5		1.20	8.70	>12.50	>12.50	>12.50
PR5.O8.C5			6.60	4.60	>12.50	>12.50	>12.50
PR5.O8.D4			2.40	6.20	>12.50	>12.50	>12.50
PR5.O8.F11			>12.50	2.80	>12.50	>12.50	>12.50
PD6.A10	4.5	R5	>12.50	6.33	>12.50	0.40	5.05
PD6.C11		R5	>12.50	4.55	10.40	4.22	<0.20
PD6.D9		R5	>12.50	5.92	>12.50	6.97	0.83
PD6.E3		R5	>12.50	4.53	>12.50	7.88	6.43
PD6.F11		R5	3.62	0.63	ND	0.59	0.92
PD6.F2		R5	>12.50	<0.20	>12.50	<0.20	6.51
PD6.A9		X4	7.75	<0.20	>12.50	0.57	>12.50
PD6.C10		X4	3.31	0.68	>12.50	5.07	4.60
PD6.C7		X4	0.78	<0.20	ND[b]	6.12	<0.20
PD6.D7		X4	>12.50	<0.20	8.16	8.24	0.57
PD6.E8		X4	<0.20	<0.20	>12.50	>12.50	0.74
PD6.F10		X4	8.26	<0.20	>12.50	2.24	0.71
PR6.A10	3.5	R5	>12.50	6.92	0.18	3.68	>12.50
PR6.A2		R5	0.22	3.64	<0.20	0.70	0.62
PR6.A7		R5	8.63	3.34	1.27	0.94	0.38
PR6.E10		X4	1.38	<0.20	<0.20	0.30	3.41
PR6.E6		X4	0.29	0.28	ND	<0.20	<0.20
PR6.F3		X4	>12.50	0.18	>12.50	ND	>12.50
PR6.G8		X4	4.58	0.30	0.20	0.39	ND
PR6.11.A5	9	X4	>12.50	0.27	<0.20	0.99	0.97
PR6.11.B3		X4	12.00	0.40	7.20	0.65	5.61
PR6.11.C1		X4	8.99	1.96	0.62	0.27	4.57
PR6.12.A4	11	R5	>12.50	11.64	<0.20	<0.20	<0.20
PR6.12.C6		R5	>12.50	6.73	>12.50	<0.20	1.04
PR6.12.D4		R5	11.78	4.69	ND	2.12	6.37

[a] D, donor; R, recipient; P, parenteral.
[b] ND, not determined.

Correlation between *env* sequence and neutralization sensitivity. We next studied the *env* sequences of all HIV-1 variants to assess a potential correlation between neutralization sensitivity and mutations in the antibody core epitopes. First, variations in the five potential N-linked glycosylation sites (PNGS) that have been implicated as important in the formation of the 2G12 epitope (8, 40, 41, 48) were analyzed and compared to 2G12 IC_{50} values (Table 4). Viruses from patients B and F, donors D2 and PD5, and recipients R3 and PR5 lacked one or more of the PNGS involved in the 2G12 epitope. In agreement, these viruses were resistant to 2G12 neutralization.

TABLE 4. 2G12 neutralization sensitivity and epitope sequences

Virus variant	2G12 IC_{50} (μg/ml)	2G12 epitope stretch[a]				
		295	332	339	386	392
B.1.A6	>12.50	–*	+	–†	+	+
B.1.B5	>12.50	–*	+	–†	+	+
B.1.G11	>12.50	–*	+	–†	+	+
B.2.A12	>12.50	–*	+	–†	+	+
B.2.B7	>12.50	–*	+	–†	+	+
B.2.E6	>12.50	–*	+	–†	+	+
B.2.G11	>12.50	–*	+	–†	+	+
B.3.A12	>12.50	–*	+	–†	+	+
B.3.D7	>12.50	–*	+	–†	+	+
B.3.F3	>12.50	–*	+	–†	+	+
B.3.H5	>12.50	–*	+	–†	+	+
B.4.B1	>12.50	–*	+	–†	+	+
B.5.B8	>12.50	–*	+	–†	+	+
B.7.D11	>12.50	–*	+	–†	+	+
B.7.H8	>12.50	–*	+	–†	+	+
B.11.A8	>12.50	–*	+	+	+	+
B.11.E10	>12.50	–*	+	+	+	+
B.11.G11	>12.50	–*	+	+	+	+
B.13.E4	>12.50	–	+	–	+	–
B.13.G11	>12.50	–*	+	+	+	+
D.1.H7	1.27	+	+	+	+	+
D.1.B8	>12.50	+	+	+	+	+
D.1.G1	0.34	+	+	+	+	+
D.3.A10	>12.50	+	+	+	+	+
D.3.G1	>12.50	+	+	+	+	+
D.7.A5	0.22	+	+	+	+	+
D.7.F8	3.19	+	+	+	+	+
D.7.G3	4.61	+	+	+	+	+
F.2	>12.50	+	+	–	–	+
F.3.F6	>12.50	+	+	–	–	+
F.3.A12	>12.50	+	+	–	–	+
F.3.G9	>12.50	+	+	–	–	+
F.4	>12.50	+	+	–	–	+
F.4.II	>12.50	+	+	–	–	+
F.5.A8	>12.50	+	+	–	–	+
F.5.B2	>12.50	+	+	–	–	+
F.5.G8	>12.50	+	+	–	–	+
F.7.F6	>12.50	+	+	–	–	+
F.7.G11	>12.50	+	+	–	–	+
F.7.G4	>12.50	+	+	–	–	+
F.9.F5	>12.50	+	+	–	–	+
F.9.B7	>12.50	+	+	–	–	+
W.I.3	>12.50	–	+	+	–	+
W.I.6	>12.50	–	+	+	–	+
W.I.16	>12.50	–	+	+	–	+
W.II.1	>12.50	–	+	+	–	+
W.II.4	>12.50	–	+	+	–	+
W.II.5	>12.50	–	+	+	–	+
W.III.2	>12.50	–	+	+	–	+
W.III.6	>12.50	–	+	+	–	+
W.III.7	>12.50	–	+	+	–	+
W.IV.1	>12.50	–	+	+	–	+
W.IV.2	>12.50	–	+	+	–	+
W.IV.4	>12.50	–	+	+	–	+
W.V.E6	0.50	+	+	+	+	+
W.V.E7	0.83	+	+	+	+	+
W.VI.B6	<0.20	+	+	+	+	+

Virus variant	2G12 IC_{50} (μg/ml)	2G12 epitope stretch[a]				
		295	332	362	386	392
D1.6F1	4.30	+	+	+	+	+
D1.6F3	>12.50	+	+	+	+	+
R1.6F5	>12.50	+	+	+	+	+
R1.6G9	>12.50	+	+	+	+	+
R1.48.E1	0.39	+	+	–	+	+
R1.48C4	0.41	+	+	–	+	+
R1.48D4	0.36	+	+	–	+	+
D2.3A6	>12.50	+	+	+	+	+
D2 3F4	>12.50	+	+	+	+	+
D2.3.D12	9.54	+	+	+	+	+
D2.3C3	3.70	+	+	+	+	+
R2.8A6	>12.50	–	+	+	+	+
R2.8D6	>12.50	–	+	+	+	+
R2.8C6	>12.50	–	+	+	+	+
R2.8D2	>12.50	–	+	+	+	+
R2.8F6	>12.50	–	+	+	+	+
R2.54H8	0.58	+	+	+	+	+
R2.54G11	0.43	+	+	+	–	+
D3.14A5	0.32	+	+	+	+	+
D3 14E3	0.31	+	+	+	+	+
D3.14G1	<0.20	+	+	+	+	+
D3.14B2	0.58	+	+	+	–	+
R3.3A9	>12.50	+	+	+	+	+
R3.3D9	>12.50	+	+	+	+	+
D4.3E9	<0.20	+	+	+	+	+
D4.3H4	<0.20	+	+	+	+	+
D4.5A11	<0.20	+	+	+	+	+
D4.5C8	<0.20	+	+	+	+	+
D4.5F10	<0.20	+	+	+	+	+
D4.5G3	<0.20	+	+	+	+	+
D4.5H9	<0.20	+	+	+	+	+
R4 15A9	>12.50	+	+	+	+	+
R4 15D5	>12.50	+	+	+	+	+
R4 15F10	<0.20	+	+	+	+	+
R4 15F9	>12.50	+	+	+	+	+
R4 15H6	>12.50	+	+	+	+	+
R4 43A11	2.45	+	+	+	+	+
R4 43B12	>12.50	+	+	+	+	+
R4 43E1	>12.50	+	+	+	+	+

Virus variant	2G12 IC_{50} (μg/ml)	2G12 epitope stretch[a]				
		295	332	339	386	392
PD5.D8	>12.50	+	+	–	+	+
PD5.E5	>12.50	+	+	–	+	+
PD5.E8	>12.50	+	+	–	+	+
PD5.F3	>12.50	+	+	–	+	+
PD5.D6	>12.50	+	+	–	+	+
PD5.F7	>12.50	+	+	–	+	+
PD5.H11	>12.50	+	+	–	+	+
PD5.F2	>12.50	+	+	–	+	+
PD5.G5	>12.50	+	+	–	+	+
PR5.O4.A2	>12.50	+	+	–	+	+
PR5.O4.A3	>12.50	+	+	–	+	+
PR5.O4.A5	>12.50	+	+	–	+	+
PR5.O4.B1	>12.50	+	+	–	+	+
PR5.O4.C7	>12.50	+	+	–	+	+
PR5.O4.D1	>12.50	+	+	–	+	+
PR5.O4.F4	>12.50	+	+	–	+	+
PR5.O8.B6	>12.50	+	+	–	+	+
PR5.O8.C5	>12.50	+	+	–	+	+
PR5.O8.F11	>12.50	+	+	–	+	+
PR5.O8.D4	>12.50	+	+	–	+	+
PD6.A10	>12.50	+	+	–	+	–
PD6.C11	10.40	+	+	–	–	+
PD6.D9	>12.50	+	+	–	+	+
PD6.E3	>12.50	+	+	–	+	+
PD6.F2	>12.50	+	+	–	+	+
PD6.A9	>12.50	+	+	–	–	+
PD6.C10	>12.50	+	+	–	–	+
PD6.D7	8.16	+	+	–	–	+
PD6.E8	>12.50	+	+	–	–	+
PD6.F10	>12.50	+	+	–	–	+
PR6.A10	0.18	+	+	–	+	+
PR6.A2	<0.20	+	+	–	+	+
PR6.A7	1.27	+	+	–	+	+
PR6.E10	<0.20	+	+	–	–	+
PR6.F3	>12.50	+	+	–	–	+
PR6.G8	0.20	+	+	–	–	+
PR6.11.A5	<0.20	+	+	–	+	+
PR6.11.B3	7.20	+	+	–	+	+
PR6.11.C1	0.62	+	+	–	+	+
PR6.12.A4	<0.20	+	+	–	+	+
PR6.12.C6	>12.50	+	+	–	+	+

[a] Numbers represent amino acid positions, relative to HXB2; +, presence of PNGS; −, absence of PNGS. *, Presence of a PNGS at position 293; †, presence of a PNGS at position 337.

However, viruses from recipient PR6 that also missed one or two PNGS involved in the 2G12 epitope were highly sensitive to 2G12 neutralization. In the late-stage viruses from patient W, recipients R1 and R2, and donors D1, D3, and D4, the PNGS involved in the 2G12 epitope were present which correlated with 2G12 neutralization sensitivity of these viruses. In viruses from recipients R1, R3, and R4 and from donors D1 and D2, the PNGS involved in the 2G12 epitope were also present, but these viruses resisted 2G12 neutralization. Thus, we here observed that sensitivity to 2G12 neutralization, for most of the primary viruses, could not readily be predicted by the presence or absence of 2G12-related PNGS, a finding similar to observations with in vitro-generated 2G12-resistant HIV-1 variants (33).

In Table 5, virus variants are ranked on IC_{50} values for 2F5 and 4E10, and epitope sequences are given for each virus. Despite variations in sensitivity to 2F5 neutralization, no mutations were observed in the crucial DKW residues of the 2F5 epitope in any of the viruses under study (55), which is in agreement with previous observations (3).

Differences in 4E10 neutralization sensitivity were also observed between viruses from the same individual, but mutations in the 4E10 epitope did not involve residues W671, F672, and W680, which have previously been described to be crucial for 4E10 binding and neutralization (55). In agreement with this, the mutations we observed in the 4E10 epitope did not correlate with the sensitivity of the HIV-1 variants to 4E10-mediated neutralization. These data suggest that the absence of 2F5 and 4E10 neutralization susceptibility in naturally occurring HIV-1 strains can result from changes in the Env glycoprotein outside the 2F5 and 4E10 epitopes, preventing antibody access or binding.

DISCUSSION

It is generally assumed that an effective vaccine against HIV-1 will need to elicit broadly neutralizing antibodies (6). Although a vaccine formulation that can elicit such antibodies is still not available, the existence of the broadly neutralizing antibodies b12, 2G12, 2F5, and 4E10 suggest that humans, in principle, can make such cross-neutralizing antibodies. However, the formulation of potential vaccine antigens that may elicit antibodies of a specificity and breadth similar to b12, 2G12, 2F5, and 4E10 has been unsuccessful thus far. Nevertheless, it is important to understand the neutralizing efficacy of broadly neutralizing antibodies against recently transmitted HIV-1 variants since this may predict coverage of protection by a vaccine that is based on their epitopes. Thus far, studies on neutralization sensitivity of recently transmitted HIV-1 variants have mainly focused on autologous serum neutralization (14, 21, 25, 39) and the efficacy of broadly neutralizing antibodies on vertically, heterosexually, and homosexually recently transmitted HIV-1 has only recently been studied (16, 22, 23, 38).

Here, we studied the sensitivity of recently transmitted subtype B HIV-1 virus variants for neutralization by sCD4 and the broadly neutralizing antibodies b12, 2G12, 2F5, and 4E10. From some individuals, virus variants from even before seroconversion were available. Although a vast majority (~85%) of the subtype B viruses studied here were sensitive to neutralization by at least one of four MAbs studied, 4 of 10 patients harbored at least one virus variant that seemed resistant to all four antibodies. Recently, transmitted viruses were generally neutralized by 2F5 in 6 of 10 recipients and by 4E10 in 5 of 10 recipients, although most recipients with sensitive viruses also had virus variants that resisted 2F5 and 4E10 neutralization. Early viruses were less well neutralized by sCD4, b12, and 2G12, not even when antibody concentrations of up to 25 μg/ml were used (data not shown). Although one could argue that the antibody concentrations tested here are still lower than the potential neutralizing antibody concentrations in vivo, our data show that a significant proportion of recently transmitted viruses resist neutralization by at least some of the broadly neutralizing antibodies, in agreement with studies by others (22, 38). These studies have shown that antibodies 2F5 and 4E10, which recognize adjacent epitopes within the membrane-proximal external region of gp41, have the largest breadth with respect to HIV-1 neutralization (3, 22, 39). We observed here that these antibodies are able to neutralize ~35% of the recently transmitted virus variants that were isolated within the first 2 months after seroconversion. Antibody 2F5 showed more potent neutralization of the different viruses in our panel than antibody 4E10. The potency of 4E10 seems to depend on the neutralization assay used. Indeed, the neutralizing ability of 4E10 seems much better in a pseudovirus assay than in a PBMC-based assay like we used here (3, 9, 22). Recently, Louder et al. showed that PBMC grown viruses carry far more envelope spikes than pseudotyped viruses, which may explain, at least in part, differences in neutralization sensitivity between PBMC-grown viruses and pseudoviruses (24). The level of expression of coreceptor molecules on the target cell may further influence neutralization sensitivity (3, 24).

Since the viruses in our study originate from the same geographic region, they may be closely related and, therefore, potentially not representative of circulating subtype B HIV-1 variants worldwide. However, all viruses in our study (all were subtype B) and the reference subtype B viruses used in the study of Li et al. (22) interspersed in phylogenetic analysis (data not shown). This indicates that the viruses from the different patients studied here are not closely related and may indeed be representative of circulating subtype B HIV-1 variants.

Interestingly, resistance to 4E10 and 2F5 neutralization did not correlate with sequence variation within the antibody epitope. These results with 2F5 are in accordance with recent observations by others (3). Our data support the notion that sequence variation outside the epitopes may influence the neutralization sensitivity of a virus, for instance by changing the conformation of the antibody epitope or the accessibility of the epitope to antibody.

Given that the b12 epitope is a discontinuous conformational epitope overlapping the CD4 binding site (34), it is difficult to dissect the effects of multiple mutations on antibody binding; some mutations likely contribute to escape from responses in the infected host, whereas others may act as compensatory mutations.

Previous studies have revealed five PNGS in gp120 that are critical for 2G12 binding and neutralization (8, 40, 41). Here we observed a high proportion (85%) of recently transmitted virus variants that were highly resistant to 2G12 neutralization.

TABLE 5. 2F5 and 4E10 neutralization sensitivity and epitope sequences[a]

Virus variant	2F5 IC_{50} (μg/ml)	Epitope	Virus variant	4E10 IC_{50} (μg/ml)	Epitope	Virus variant	2F5 IC_{50} (μg/ml)	Epitope	Virus variant	4E10 IC_{50} (μg/ml)	Epitope	Virus variant	2F5 IC_{50} (μg/ml)	Epitope	Virus variant	4E10 IC_{50} (μg/ml)	Epitope
		ELDKWA			NWFDITNWLW			ELDKWA			NWFDITNWLW			ELDKWA			NWFDITNWLW
B.1.A6	>12.50	------	B.1.A6	>12.50	---S------	R2.8.D6	>12.50	------	D2.3.C3	>12.50	T---------	PD5.E8	>12.50	------	PD5.G5	>12.50	------Q---
B.1.B5	>12.50	------	B.1.B5	>12.50	---S------	R2.8.D2	>12.50	------	D2.3.D12	>12.50	S---------	PD5.H11	>12.50	------	PD5.E8	>12.50	------Q---
B.1.G11	>12.50	------	B.1.G11	>12.50	---S------	R2.8.F6	>12.50	------	D2.3.F4	>12.50	S---------	PD5.F2	>12.50	------	PD5.F2	>12.50	------Q---
B.2.A12	>12.50	------	B.2.A12	>12.50	---S------	D2.3.A6	9.62	------	R2.8.D6	>12.50	S---------	PD5.D8	>12.50	------	PD5.H11	>12.50	------Q---
B.2.G11	>12.50	------	B.2.B7	>12.50	---S------	R2.54.G11	8.43	------	R2.54.G11	>12.50	------Q---	PD5.G5	11.6	------	PD5.D8	7.1	------Q---
B.4.B1	>12.50	------	B.2.E6	>12.50	---S------	R2.54.H8	7.86	------	D2.3.A6	11.93	S---------	PD5.F3	9.7	------	PD5.F3	6.7	------Q---
B.5.B8	>12.50	------	B.2.G11	>12.50	---S------	R2.8.A6	3.01	------	R2.54.H8	6.86	------Q---						
B.7.D11	>12.50	------	B.3.A12	>12.50	---S------	D2.3.F4	2.61	------	R2.8.D2	3.42	S---------	PR5.O4.A3	>12.50	------	PR5.O4.A2	>12.50	------Q---
B.7.H8	>12.50	------	B.3.D7	>12.50	---S------	D2.3.C3	2.10	------	R2.8.F6	0.31	S---------	PR5.O4.A5	>12.50	------	PR5.O4.A3	>12.50	------Q---
B.11.A8	>12.50	------	B.3.F3	>12.50	---S------	D2.3.D12	1.97	------	R2.8.A6	0.20	S---------	PR5.O4.B1	>12.50	------	PR5.O4.A5	>12.50	------Q---
B.11.E10	>12.50	------	B.3.H5	>12.50	---S------							PR5.O4.C7	>12.50	------	PR5.O4.B1	>12.50	------Q---
B.11.G11	>12.50	------	B.4.B1	>12.50	---S------	R3.3.A9	>12.50	------	R3.3.D9	>12.50	----------	PR5.O8.B6	>12.50	------	PR5.O4.C7	>12.50	------Q---
B.13.E4	>12.50	------	B.5.B8	>12.50	---S------	R3.3.D9	>12.50	------	R3.3.A9	10.36	----------	PR5.O8.F11	>12.50	------	PR5.O4.D1	>12.50	------Q---
B.13.G11	>12.50	------	B.7.D11	>12.50	---S------	D3.14.A5	2.55	------	D3.14.A5	2.92	----------	PR5.O4.D1	>12.50	------	PR5.O4.F4	>12.50	------Q---
B.3.A12	1.48	------	B.7.H8	>12.50	---S------	D3.14.E3	0.57	------	D3.14.E3	0.63	----------	PR5.O4.A2	>12.50	------	PR5.O8.B6	>12.50	------Q---
B.3.F3	1.48	------	B.11.A8	>12.50	---S------							PR5.O8.D4	>12.50	------	PR5.O8.C5	>12.50	------Q---
B.2.E6	1.42	------	B.11.E10	>12.50	---S------	D4.5.C8	>12.50	------	D4.5.C8	>12.50	------K---	PR5.O8.C5	>12.50	------	PR5.O8.D4	>12.50	---N--Q---
B.3.D7	0.91	------	B.11.G11	>12.50	---S------	R4.15.A9	>12.50	------	R4.15.A9	>12.50	---N------	PR5.O4.F4	>12.50	------	PR5.O8.F11	>12.50	------Q---
B.3.H5	0.76	------	B.13.E4	>12.50	---S------	R4.15.D5	>12.50	------	R4.15.D5	>12.50	---S-SK---						
B.2.B7	0.66	------	B.13.G11	0.65	---S------	R4.15.F10	>12.50	------	R4.15.F10	>12.50	---N------	PD6.E8	>12.50	------	PD6.A9	>12.50	------Q---
						R4.15.F9	>12.50	------	R4.15.F9	>12.50	---N------	PD6.D7	8.24	------	PD6.F2	6.51	------Q---
D.1.H7	1.81	------	D.3.A10	>12.50	---T------	R4.15.H6	>12.50	------	R4.15.H6	>12.50	---N------	PD6.E3	7.88	------	PD6.E3	6.43	------Q---
D.1.B8	1.28	------	D.1.B8	>12.50	---T------	D4.3.E9	11.08	------	D4.5.A11	5.21	----------	PD6.D9	6.97	------	PD6.A10	5.05	------Q---
D.7.F8	0.92	------	D.7.F8	11.44	---T------	D4.5.A11	2.17	------	D4.5.F10	5.09	----------	PD6.C7	6.12	------	PD6.C10	4.60	------Q---
D.1.G1	0.89	------	D.1.G1	7.32	---T------	D4.3.H9	0.54	------	D4.3.H9	4.69	----------	PD6.C10	5.07	------	PD6.F11	0.92	------Q---
D.3.A10	0.81	------	D.3.G1	7.10	---T------	D4.3.H4	0.20	------	D4.3.E9	1.12	---N------	PD6.C11	4.22	------	PD6.D9	0.83	------Q---
D.3.G1	0.50	------	D.1.H7	5.37	---T------	D4.5.F10	0.20	------	D4.3.H4	0.20	----------	PD6.F10	2.24	------	PD6.E8	0.74	---N--Q---
D.7.A5	0.32	------	D.7.G3	0.61	---N------				D4.5.H9	0.20	----P-----	PD6.F11	0.59	------	PD6.F10	0.71	------Q---
D.7.G3	0.20	------	D.7.A5	0.20	---N------							PD6.A9	0.57	------	PD6.D7	0.57	------Q---
												PD6.A10	0.40	------	PD6.C11	0.20	------Q---
F.5.A8	1.04	------	F.3.G9	>12.50	------K---							PD6.F2	0.20	------	PD6.C7	0.20	------Q---
F.4.II	0.51	------	F.5.G8	>12.50	------K---												
F.3.A12	0.49	------	F.7.F6	>12.50	------K---							PR6.A10	3.68	------	PR6.A10	>12.50	------Q---
F.3.F6	0.44	K-----	F.7.G11	>12.50	------K---							PR6.12.D4	2.12	------	PR6.F3	>12.50	---N--Q---
F.5.G8	0.44	------	F.7.G4	>12.50	------K---							PR6.11.A5	0.99	------	PR6.12.D4	6.37	------Q---
F.7.G11	0.41	------	F.2	11.62	------K---							PR6.A7	0.94	------	PR6.11.B3	5.61	------Q---
F.7.G4	0.41	------	F.3.A12	10.17	------K---							PR6.A2	0.70	------	PR6.11.C1	4.57	------Q---
F.9.F5	0.38	------	F.3.F6	7.52	------K---							PR6.11.B3	0.65	------	PR6.E10	3.41	------Q---
F.5.B2	0.38	------	F.5.A8	5.76	------K---							PR6.G8	0.39	------	PR6.12.C6	1.04	------Q---
F.4	0.34	------	F.4.II	5.48	------K---							PR6.E10	0.30	------	PR6.11.A5	0.97	------Q---
F.3.G9	0.33	------	F.4	4.69	------K---							PR6.11.C1	0.27	------	PR6.A2	0.62	---N--Q---
F.2	0.29	------	F.9.F5	2.61	------K---							PR6.E6	0.20	------	PR6.A7	0.38	------Q---
F.9.B7	0.27	------	F.5.B2	2.53	------K---							PR6.12.A4	0.20	------	PR6.E6	0.20	------Q---
F.7.F6	0.24	------	F.9.B7	0.20	------K---							PR6.12.C6	0.20	------	PR6.12.A4	0.20	------Q---
W.I.3	>12.50	------	W.I.3	>12.50	------K---												
W.I.6	>12.50	------	W.I.6	>12.50	------K---												
W.I.16	>12.50	------	W.I.16	>12.50	------K---												
W.II.1	>12.50	------	W.II.1	>12.50	------K---												
W.II.4	>12.50	------	W.II.4	>12.50	------K---												
W.II.5	>12.50	------	W.II.5	>12.50	------K---												
W.III.2	>12.50	------	W.III.2	>12.50	------K---												
W.III.6	>12.50	------	W.III.6	>12.50	------K---												
W.III.7	>12.50	------	W.III.7	>12.50	------K---												
W.IV.1	>12.50	------	W.IV.1	>12.50	------K---												
W.IV.2	>12.50	------	W.IV.2	>12.50	------K---												
W.IV.4	>12.50	------	W.IV.4	>12.50	------K---												
W.VII.B6	>12.50	------	W.V.E7	1.56	------K---												
W.V.E7	2.24	------	W.VII.B6	1.15	----------												
W.V.E6	0.37	------	W.V.E6	0.63	------K---												

[a] Viruses are ranked from neutralization resistant to neutralization sensitive.

Interestingly, 12% of these 2G12 resistant viruses had all five PNGS. Although the association between 2G12 sensitivity and the presence of these PNGS was confirmed for a number of primary HIV-1 variants, we show here the existence of at least some primary HIV-1 variants for which these residues may be less critical for neutralization by 2G12. In agreement, in vitro-generated 2G12-resistant HIV-1 variants also still had all critical PNGS (33). Of the 2G12-resistant viruses, 88.5% lacked at least one of the five PNGS of the 2G12 epitope. Viruses that were isolated later in infection that had become sensitive to 2G12 neutralization had restored the 2G12 epitope. This observation is in accordance with a study by Dacheux et al. (11), who described the absence of PNGS involved in the 2G12 epitope early in infection and the restoration of these PNGS later in infection.

It has been suggested that the route of transmission and the subtype of the virus may determine the neutralization sensitivity of the transmitted HIV-1 variant (12, 14, 52). Although in our study, the number of patients was too low to draw any conclusions, we did observe that recipients of homosexual transmission tended to have HIV-1 variants that were more resistant to neutralization compared to the HIV-1 variants from their source partners, whereas HIV-1 variants from recipients of parenteral transmission tended to have a similar or even higher neutralization sensitivity than the HIV-1 variants from their respective donors. It is tempting to speculate that the mucosal barrier in sexual transmission may select for HIV-1 variants with an envelope configuration that coincides with resistance to neutralizing antibodies. Since this may become a highly relevant issue for vaccine efficacy, this observation warrants further study.

Although not confirmed by all studies to date (14), it has been hypothesized that during initial virus replication in a newly infected individual, before the appearance of neutralizing antibodies, neutralization-sensitive virus variants may be preferentially selected based on their relatively higher replication fitness in the absence of immune pressure compared to neutralization-resistant viruses (12, 39). This may have lead to the assumption that vaccine elicited neutralizing antibodies potentially could be more effective against recently transmitted viruses than initially assumed based on the neutralization resistance of primary HIV-1 in general. However, in the present study, recently sexually transmitted viruses were generally more resistant to neutralization by broadly neutralizing antibodies, and over time an increase in neutralization sensitivity was observed. As discussed above, the envelope configuration best adapted for sexual transmission may coincide with a neutralization-resistant phenotype. The subsequent increase in neutralization sensitivity may indeed be associated with reversion of these mutations, driven by gain of fitness, in the absence of neutralizing antibodies in the newly infected recipient. However, we have recently reported an absent correlation between resistance to broadly neutralizing antibodies and HIV-1 replication rate in vitro (37).

As stated above, 4 of 10 patients with recent HIV-1 infection studied here carried viruses that were resistant to all four broadly neutralizing antibodies tested in our study. This indicates that a vaccine that can elicit a combination of antibodies that resemble the activities of b12, 2G12, 2F5, and 4E10 may only provide partial protective immunity against currently circulating viruses. Our study thus emphasizes that any successful vaccine may have to elicit an even broader repertoire of neutralizing antibodies than the currently known broadly neutralizing antibodies. Efforts to isolate further cross-neutralizing antibodies to conserved epitopes on the viruses and to characterize their fine specificities are therefore urgently warranted.

ACKNOWLEDGMENTS

The Amsterdam Cohort Studies on HIV Infection and AIDS, a collaboration between the Amsterdam Health Service, the Amsterdam Medical Center of the University of Amsterdam, Sanquin Blood Supply Foundation, and the University Medical Center Utrecht, are part of The Netherlands HIV Monitoring Foundation and financially supported by The Netherlands National Institute for Public Health and the Environment.

This study is supported financially by the Dutch AIDS Fund, grant 7009, and a travel grant from The Netherlands Organization for Scientific Research.

We thank Fransje Koning, Jos Dekker, Cindy Koevoets, Fareed Samad, and Peter van Swieten for technical support. We thank Dennis Burton for his generous gift of MAb IgG1b12 and critical reading of the manuscript and Michael Zwick for helpful discussions.

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