Cross-Subtype Neutralization Sensitivity despite Monoclonal Antibody Resistance among Early Subtype A, C, and D Envelope Variants of Human Immunodeficiency Virus Type 1

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The human immunodeficiency virus type 1 (HIV-1) variants that are transmitted to newly infected individuals are the primary targets of interventions, such as vaccines and microbicides, aimed at preventing new infections. Newly acquired subtype A, B, and C variants have been the focus of neutralization studies, although many of these viruses, particularly of subtypes A and B, represent viruses circulating more than a decade ago. In order to better represent the global diversity of transmitted HIV-1 variants, an additional 31 sexually transmitted Kenyan HIV-1 env genes, representing several recent infections with subtype A, as well as subtypes A/D, C, and D, were cloned, and their neutralization profiles were characterized. Most env variants were resistant to neutralization by the monoclonal antibodies (MAbs) b12, 4E10, 2F5, and 2G12, suggesting that targeting the epitopes of these MAbs may not be effective against variants that are spreading in areas of endemicity. However, significant cross-subtype neutralization by plasma was observed, indicating that there may be other epitopes, not yet defined by the limited available MAbs, which could be recognized more broadly.

Most effective viral vaccines are thought to provide protection primarily by stimulating neutralizing antibodies (NAbs) to clear cell-free virus (25, 27). Because protection by NAbs requires recognition of common viral epitopes, the extreme genetic diversity of human immunodeficiency virus type 1 (HIV-1) presents a particular challenge to NAB-based vaccine approaches. Therefore, a critical starting point for studies of immune-mediated protection against HIV-1 is a collection of newly transmitted HIV-1 variants, particularly from areas of endemicity, such as sub-Saharan Africa, in order to determine whether vaccines are appropriately targeted to common epitopes from these relevant transmitted strains.

During HIV-1 transmission, a bottleneck allows only one or a few variants to be transmitted to a newly infected individual (6, 9, 16, 29, 34, 37, 39), and the sensitivity of these early transmitted strains to antibody-mediated neutralization is therefore of particular interest. Newly transmitted HIV-1 variants have demonstrated significant heterogeneity in their neutralization phenotypes both within and between subtypes (2, 3, 6–8, 11, 13–15, 22, 30, 32, 36). Panels of sexually transmitted HIV-1 envelope variants (based on the envelope gene, env) have been characterized, including subtype B variants from North America, Trinidad, and Europe, subtype C variants from South Africa and Zambia, and subtype A variants from Kenya collected between 1994 and 1996 (2, 14, 15). Here, we characterize an additional 31 envelope variants from 14 subjects with sexually transmitted HIV-1 who were infected in Kenya, where subtypes A, C, and D circulate, between 1993 and 2005 (24, 31).

The env genes were cloned from samples drawn 14 to 391 (median, 65) days postinfection from individuals enrolled in a prospective cohort of high-risk women in Mombasa, Kenya (19–21). Demographic characteristics of the subjects are summarized in Table 1; the timing of first infection was determined by both HIV-1 serology and HIV RNA testing as described previously (12). All of the subjects were presumably infected by male-to-female transmission and displayed a range of plasma viral loads at the time of env gene cloning (Table 1). For most individuals, full-length env genes were cloned from uncultured peripheral blood mononuclear cell (PBMC) DNA, though for two individuals, clones were obtained from DNA following short-term coculture with donor PBMCs (Table 1). env genes were cloned by single-copy nested PCR with primers and PCR conditions as described previously (4, 17). We tested env genes for their ability to mediate infection by transfecting env plasmid DNA into 293T cells along with an env-deficient HIV-1 subtype A proviral plasmid, Q23Δenv, to make pseudoviral particles (17). More than 80 env clones were obtained from 16 subjects; less than one-half were functional on the basis of the infectivity of pseudoviral particles in a single-round infection of TZM-bl cells (AIDS Research and Reference Reagent Program, National Institutes of Health), as observed previously for env genes cloned from proviral sequences (17); a lower fraction of functional env genes have been reported from plasma (18). We focused on the proviral sequences here because they presumably best represent the sequence closest to that of the transmitted strains. The 31 functional env variants are described in Table 1.

The full-length, functional env genes were sequenced and aligned to generate a maximum likelihood phylogenetic tree.
oratory HIV database, as described previously (26). Viral env with reference sequences from the Los Alamos National Lab-
ners were infected with a spectrum of genetic diversity was observed overall (Fig. 1). However, as observed previously in this cohort (16, 28, 29), some women, such as subject QF495, were infected with more heterogeneous viral popula-
tions with average pairwise differences of 1.4% and 0.88% between variants, respectively (Table 1 and Fig. 1).

The deduced amino acid sequences revealed that all func-
tional variants had an uninterrupted open reading frame in env except for variant QB099.391I.ENV.C8, which had a frame-
shift mutation within the cytoplasmic tail of gp41. There was significant heterogeneity in the length of the protein variable loops, particularly V1/V2, which ranged from 57 amino acids (aa) to 113 aa (Table 1). The V3, V4, and V5 loops also varied in length, though less dramatically (Table 1). Variants from the same subject were generally similar in their variable-loop lengths. Moderate variation was also observed in the number and position of potential N-linked glycosylation sites (PNGS) (Table 1).

Previous analyses indicated that early subtype C env proteins had shorter variable loops than did early subtype B env proteins (13), suggesting that there are different env protein features between subtypes. Thus, to compare variable-loop lengths and the numbers of PNGS between subtypes using this expanded group of early env variants, we evaluated the 31 newly cloned variants plus an additional 15 subtype A variants (2), 19 subtype B variants (14), and 18 subtype C variants (15) from other early virus panels. In order to avoid bias, when more than one env variant was available from a subject, the average loop length or PNGS number for that subject’s env proteins was used. We did not observe significant differences in V1/V2 length, V5 length, or the numbers of PNGS between subtypes by the Kruskal-Wallis equality-of-populations rank test (Table 2). However, there were significant differences between the V3 and V4 loop lengths of the subtypes after adjusting for multiple comparisons (Table 2). The differences in V3 length appeared to be a result of shorter V3 loops in subtype D env proteins than in early subtype B ($P = 0.006$) or C ($P < 0.001$) env proteins (Table 2). The differences in V4 length were caused by shorter V4 loops in subtype C env

### TABLE 1. Demographic characteristics, diversities, gp120 variable-region lengths, numbers of PNGS, and accession numbers of cloned env variants

| Subject | Virus subtype | Sample date (mo/day/yr) | dpi* | Plasma VL* | Source† | Individual env clone | Pairwise difference (%)‡ | Variable-loop length (aa) | No. of PNGS | GenBank accession no. |
|---------|---------------|-------------------------|------|------------|---------|----------------------|--------------------------|--------------------------|-------------|----------------------|
| QF726   | A             | 04/16/96                | 70   | 61,940     | ucPBMC  | QB726.70M.ENV.B3     | 0.16                     | 63 35 36 10             | 22 4 4      | FJ866111            |
| QF495   | A             | 05/16/06                | 23   | 217,050    | ucPBMC  | QF495.23M.ENV.A1     | 0.12                     | 107 35 37 11            | 30 4 4      | FJ866113            |
| QG984   | A             | 07/12/04                | 21   | 30,300     | ucPBMC  | QG984.21M.ENV.A3     | NA                       | 69 34 36 11             | 24 3 3      | FJ866117            |
| QH209   | A             | 10/13/05                | 14   | 28,600     | ucPBMC  | QH209.14M.ENV.A2     | NA                       | 72 35 29 11             | 24 4 4      | FJ866119            |
| QH343   | A             | 09/08/05                | 21   | 40,750,000 | ucPBMC  | QH343.21M.ENV.A10    | 0.19                     | 77 35 32 15             | 26 4 4      | FJ866119            |
| QA790   | A/D           | 06/10/96                | 204  | 48,100     | ccPBMC  | QA790.204I.ENV.A4    | 0.36                     | 77 35 33 11             | 25 4 4      | FJ866124            |
| QA790   | A/D           | 06/10/96                | 204  | 48,100     | ccPBMC  | QA790.204I.ENV.C1    | 0.36                     | 77 35 33 11             | 26 4 4      | FJ866125            |
| QA790   | A/D           | 06/10/96                | 204  | 48,100     | ccPBMC  | QA790.204I.ENV.E2    | 0.36                     | 77 35 33 11             | 26 4 4      | FJ866126            |
| QA790   | A/D           | 06/10/96                | 204  | 48,100     | ccPBMC  | QA790.204I.ENV.C1    | 0.36                     | 77 35 33 11             | 26 4 4      | FJ866125            |
| QA790   | A/D           | 06/10/96                | 204  | 48,100     | ccPBMC  | QA790.204I.ENV.E2    | 0.36                     | 77 35 33 11             | 26 4 4      | FJ866126            |
| QB099   | C             | 02/10/95                | 391  | 27,280     | ucPBMC  | QB099.391M.ENV.B1    | 0.43                     | 65 35 29 10             | 25 4 4      | FJ866131            |
| QC406   | C             | 07/08/97                | 70   | 692,320    | ucPBMC  | QC406.70M.ENV.F3     | NA                       | 65 35 20 11             | 22 5 4      | FJ866133            |
| QA013   | D             | 10/11/95                | 70   | 5,277,700  | ccPBMC  | QA013.70I.ENV.H1     | 0.16                     | 60 34 29 12             | 25 4 4      | FJ866134            |
| QA465   | D             | 08/19/93                | 59   | 37,750     | ucPBMC  | QA465.59M.ENV.A1     | 0.24                     | 65 35 30 11             | 28 4 4      | FJ866135            |
| QB857   | D             | 10/16/97                | 110  | 14,640     | ucPBMC  | QB857.23I.ENV.B3     | NA                       | 68 34 32 11             | 26 4 4      | FJ866138            |
| QD435   | D             | 04/06/99                | 100  | 17,470     | ucPBMC  | QD435.100M.ENV.A4    | 0.88                     | 69 34 29 12             | 26 4 4      | FJ866139            |

**Notes:**
- *dpi,* days postinfection as defined by RNA testing (12).
- *VL,* viral load on the sample date in which env genes were cloned.
- *ucPBMC,* uncultured PBMCs; *ccPBMC,* cocultured PBMCs.
- *Pairwise distance between the full-length env variants from a given subject. NA, not applicable because there was only one variant available from the subject.*
- *env variants from these two subjects were cloned from >6 months postinfection, as noted, and should not be considered true early env variants.*

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proteins in comparison to both subtype A and B env proteins ($P < 0.001$; Table 2).

We then assessed the neutralization sensitivity of the pseudoviruses to antibodies in plasma from HIV-1-infected individuals and to HIV-1-specific MAbs by using the TZM-bl neutralization assay as described previously (2, 23, 38). Median inhibitory concentrations (IC$_{50}$s) were defined as the reciprocal dilution of plasma or concentration of MAb that resulted in 50% inhibition of infection (2, 38).

The env variants demonstrated a range of neutralization sensitivities to plasma samples, from neutralization resistant (defined as $< 50\%$ neutralization with a 1:50 dilution of plasma) to neutralization sensitive with an IC$_{50}$ of 333 (Fig. 2). Some clones, such as QF495.23M.ENV.A1, were relatively sensitive to all the plasma pools, with IC$_{50}$s from 100 to 333, whereas other clones, such as QH343.21M.ENV.A10, were relatively resistant to these plasma pools, with IC$_{50}$s from $< 50$ to 85 (Fig. 2). The plasma pools did differ in their neutralizing potencies. The Kenya pool, with a median IC$_{50}$ across all viruses tested, was significantly less likely to neutralize these transmitted variants than were the subtype A, C, and D plasma pools, which had median IC$_{50}$s of 110, 105, and 123, respectively ($P$ values of $< 0.0001$, 0.0001, and 0.001, respectively, by paired $t$-test on log-transformed IC$_{50}$s). The basis for these differences in neutralizing activity is not clear, although the location, timing, and level of immunodeficiency at the time of sample collection could have contributed to the differences in NAb levels between the pools.
The env variants were significantly more susceptible to their subtype-matched plasma pool, with a higher mean IC<sub>50</sub> for subtype-matched plasma samples than for unmatched plasma samples (138 versus 108, P = 0.0081, paired t test). However, a significant amount of cross-subtype neutralization was observed, as every env variant that was susceptible to the subtype-matched plasma pool was also susceptible to at least one of the other plasma pools (Fig. 2). Thus, although potency was enhanced when the plasma antibodies were produced in response to infection with the same subtype of HIV-1, there were shared neutralization determinants between subtypes, as has been observed previously (reviewed in reference 3).

To identify potential correlates of neutralization sensitivity to the antibodies within these plasma pools, we included these 31 env variants and an additional 15 subtype A env variants we previously characterized from the same cohort with the same plasma pools (2). We did not observe a change in neutralization sensitivity during the evolution of the HIV-1 epidemic in Kenya, as no correlation was observed between neutralization sensitivity and the calendar date from which the env variants were isolated. In addition, no correlation was observed between the neutralization sensitivity of a variant to the plasma pools and the duration of estimated infection within that individual. Finally, there was no significant correlation between the neutralization sensitivity and variable-loop length or the number of PNGS. Thus, although changes in the variable-loop length or number of PNGS may alter the exposure of epitopes within the HIV-1 env protein, these changes do not appear to be the primary determinant of neutralization sensitivity.

Despite relatively universal sensitivity to at least one of the pooled plasma samples, these transmitted Kenyan env variants were generally resistant to the MAbs 2G12 (provided by Hermann Katinka, Polymun Scientific) and b12 (provided by Dennis Burton, The Scripps Research Institute), as well as 2F5 and 4E10 (obtained from the AIDS Research and Reference Reagent Program, National Institutes of Health) (Fig. 2), though these MAbs neutralized the subtype B env variant SF162, with IC<sub>50</sub> similar to those reported previously (1). Subtype D strains were the most susceptible to MAbs, with 4/8 variants neutralized with <20 μg/ml of 2F5 and 2/8 neutralized with <20 μg/ml of the other MAbs. This could reflect the fact that subtype D variants are more closely related to subtype B strains (Fig. 1) (see reference 10), and these MAbs were all derived from subtype B-infected individuals.

Among all 31 variants, 2F5 was the most broadly neutralizing, with 15/31 variants from 8/14 subjects neutralized with <20 μg/ml of this MAb. Some 2F5-resistant env variants, such as QH209.14M.ENV.A2 and QB857.110I.ENV.B3, had mutations in the canonical 2F5 binding epitopes, though other 2F5-resistant env variants such as QF495.23M.ENV.A3 and QA790.204I.ENV.A4 maintained the canonical 2F5 epitope. The results with the MAb 4E10 were similar; 4E10 neutralized only seven variants from 4 of the 14 subjects, and the presence of mutations in the 4E10 epitope, which were common, did not predict neutralization sensitivity (Fig. 2). For instance, the env variants QH343.21M.ENV.A10 and QH343.21M.ENV.B5 contained identical N671S and D674S mutations and QH343.21M.ENV.B5 was highly sensitive to 4E10, while QH343.21M.ENV.A10 was resistant (Fig. 2). Thus, for the 2F5 and 4E10 epitopes, the presumed epitopes appear to be shielded in a subset of these early non-subtype B env variants, as has been previously observed (Fig. 2) (1, 2, 5, 14).

The MAb b12 neutralized only two variants from two subtype D-infected individuals, with no neutralization of the subtype A, C, and A/D recombinant pseudoviruses. Only four variants from two subjects were neutralized by 2G12 at <20 μg/ml, and these were the only variants that maintained all five of the PNGS within the 2G12 epitope (Fig. 2). Overall, the median IC<sub>50</sub> of all the MAbs against these transmitted variants was >20 μg/ml. None of the variants was susceptible to all four MAbs (Fig. 2), unlike many of the early subtype B env variants characterized previously (14).

In summary, these newly characterized HIV-1 env clones represent a range of neutralization sensitivities and can be used to supplement existing panels of transmitted variants, in particular, adding the first subtype D and A/D recombinant variants. Some differences between subtypes in env structure following transmission were noted, though these differences did not correlate with neutralization sensitivity. Although the significant levels of cross-subtype neutralization sensitivity observed with plasma samples indicate that some neutralization determinants were shared across subtypes, the epitopes for the
MAbs b12, 2G12, 2F5, and 4E10 did not appear to be among the shared determinants. Thus, despite the fact that significant attention has focused on using vaccination to develop antibodies that resemble these MAbs in their specificity, such antibodies may not neutralize the transmitted strains that are causing most new infections worldwide. These data therefore stress the importance of evaluating transmitted variants in endemic areas when designing immunogens and evaluating vaccine and microbicide strategies.

Nucleotide sequence accession numbers. The accession numbers for sequences deposited in GenBank are noted in Table 1; these env clones will be made available through the NIH AIDS Reference and Reagent Program.

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REFERENCES

1. Binley, J. M., T. Wrin, B. Korber, M. B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, C. J. Petropoulos, and D. R. Burton. 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. J. Virol. 78(13):13232–13252.
2. Blish, C., R. Nedelk, K. Mandalia, D. Mosier, and J. Overbaugh. 2007. HIV-1 subtype A envelope variants from early in infection have variable sensitivity to neutralization and to inhibitors of viral entry. AIDS 21:693–702.

3. Blish, C. A., W. M. Blay, N. L. Haigwood, and J. Overbaugh. 2007. Transmission of HIV-1 in the face of neutralizing antibodies.Curr. HIV Res. 5:578–587.

4. Blish, C. A., O. C. Dogan, N. R. Derby, M. A. Nguyen, B. Chohan, B. A. Richardson, and J. Overbaugh. 2008. Human immunodeficiency virus type 1 superinfection occurs despite relatively robust neutralizing antibody responses. J. Virol. 82:12094–12103.

5. Blish, C. A., M. A. Nguyen, and J. Overbaugh. 2008. Enhancing exposure of HIV-1 neutralization epitopes through mutations in gp41. PLoS Med. 5:e9.

6. Derdeyn, C., J. Rakwar, K. Mandaliya, F. Gao, B. Bolbet-Ruche, J. L. Mokili, M. Muddon, S. A. Denham, M. L. Hei, F. Kasolo, R. Musonda, B. H. Hahn, G. M. Shaw, B. T. Korber, S. Allen, and E. Hunter. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 303:2019–2021.

7. Gray, E. S., T. Meyers, G. Gray, D. C. Montefiori, and L. Morris. 2006. Insensitivity of pandemic HIV-1 subtype C viruses to broadly neutralizing monoclonal antibodies raised against subtype B. PLoS Med. 3:e255.

8. Long, E. M., H. L. Martin, J. K. Kreiss, S. M. Rainwater, L. Lavreys, S. A. Denham, F. Gao, B. Bolbet-Ruche, J. L. Mokili, M. Muddon, S. A. Denham, M. L. Hei, F. Kasolo, R. Musonda, B. H. Hahn, G. M. Shaw, B. T. Korber, S. Allen, and E. Hunter. 2004. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science 303:2019–2021.

9. Korber, B., B. Gaschen, K. Yusim, R. Thakallapally, C. Kesmir, and V. Derdeyn. 2003. Evolutionary and immunological implications of contemporary HIV-1 variability. Br. Med. Bull. 58:19–42.

10. Kulkarni, S. S., A. Lapedes, H. Tang, S. Gnanakaran, M. G. Daniels, M. Zhang, T. Bhattacharya, M. Li, V. Polonis, F. E. McCutchan, L. Morris, D. Ellensberger, S. T. Butera, R. C. Rullinger, B. T. Korber, R. S. Paranjape, and D. C. Montefiori. 2009. Highly complex neutralization determinants on a monophyletic lineage of newly transmitted subtype C HIV-1 Env clones from India. Virology 385:505–520.

11. Lavreys, L., J. M. Baeten, B. Chohan, R. S. McClelland, W. M. Hassam, B. A. Richardson, K. Mandalia, J. O. Ndinya-Achola, and J. Overbaugh. 2006. Higher set point plasma viral load and more severe acute HIV type 1 (HIV-1) illness predict mortality among high-risk HIV-1-infected African women. Clin. Infect. Dis. 42:1333–1339.

12. Li, B., J. M. Decker, R. W. Johnson, F. Bolbet-Ruche, X. Wei, J. Mulenga, S. Allen, E. Hunter, B. H. Hahn, G. M. Shaw, J. L. Blackwell, and C. A. Derdeyn. 2006. Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1. J. Virol. 80:5211–5218.

13. Li, M., F. Gao, J. R. Mascola, L. Statamatos, V. R. Polonis, M. Koutsoukos, G. Voss, P. Goepfert, P. Gilbert, K. M. Greene, M. Bliska, D. L. Kofie, J. F. Salazar-Gonzalez, J. M. Decker, B. T. Korber, B. H. Hahn, and D. C. Montefiori. 2005. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. J. Virol. 79:10108–10125.

14. Li, M., J. F. Salazar-Gonzalez, C. A. Derdeyn, B. T. Korber, B. H. Hahn, and D. C. Montefiori. 2006. Genetic and neutralization properties of subtype C human immunodeficiency virus type 1 molecular env clones from acute and early heterosexually acquired infections in southern Africa. J. Virol. 80:11776–11790.

15. Long, E. M., H. L. Martin, Jr., J. K. Kreiss, S. M. Rainwater, L. Lavreys, D. J. Jackson, J. Rakwar, K. Mandalia, and J. Overbaugh. 2000. Gender differences in HIV-1 diversity at time of infection. Nat. Med. 6:671–75.

16. Long, E. M., S. M. J. Rainwater, L. Lavreys, K. Mandalia, and J. Overbaugh. 2002. HIV-1 type 1 variants transmitted to women in Kenya require the CCR5 co-receptor for entry, regardless of the genetic complexity of the infecting virus. AIDS Res. Hum. Retrovir. 18:567–576.

17. Mahalanabis, M., P. Jayaraman, T. Miura, F. Perrya, E. M. Chester, B. Richardson, B. Walker, and N. L. Haigwood. 2009. Continuous viral escape and selection of oligoclonal neutralizing antibodies during drug-naive human immunodeficiency virus controllers. J. Virol. 83:662–672.

18. Martin, H. L., D. J. Jackson, K. Mandalia, J. Bwayo, J. P. Rakwar, P. Nyange, S. Moses, J. O. Ndinya-Achola, K. Holmes, F. Plummer, E. Ngugi, and J. Kreiss. 1999. Identification of conserved epitopes in AIDs vaccine: an HIV-1 reference panel of subtype B envelope clones isolated from the plasma of recently infected individuals. J. Acquir. Immune Defic. Syndr. 46:1–11.

19. Wolinsky, S. M., C. M. Wike, T. B. Korber, C. Hutto, W. P. Parks, L. L. Rosenthal, R. Partido, K. J. Kunstman, and J. L. Munn. 1992. Selective transmission of human immunodeficiency virus type 1 variants from mothers to infants. Science 255:1134–1137.

20. Wu, X., A. B. Parish, B. A. Richardson, R. Ndutu, G. John-Stewart, D. Mbori-Ngacha, S. M. Rainwater, and J. Overbaugh. 2006. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. J. Virol. 80:835–844.

21. Zhu, T., H. Mo, N. Wang, D. S. N. Yam, C. Cao, R. A. Koup, and D. D. Ho. 1993. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. Science 261:1179–1181.