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Frequent Intrapatient Recombination between Human Immunodeficiency Virus Type 1 R5 and X4 Envelopes: Implications for Coreceptor Switch[▽]

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Emergence of human immunodeficiency virus type 1 (HIV-1) populations that switch or broaden coreceptor usage from CCR5 to CXCR4 is intimately coupled to $CD4^+$ cell depletion and disease progression toward AIDS. To better understand the molecular mechanisms involved in the coreceptor switch, we determined the nucleotide sequences of 253 V1 to V3 *env* clones from 27 sequential HIV-1 subtype B isolates from four patients with virus populations that switch coreceptor usage. Coreceptor usage of clones from dualtropic R5X4 isolates was characterized experimentally. Sequence analysis revealed that 9% of the clones from CXCR4-using isolates had originated by recombination events between R5 and X4 viruses. The majority (73%) of the recombinants used CXCR4. Furthermore, coreceptor usage of the recombinants was determined by a small region of the envelope, including V3. This is the first report demonstrating that intrapatient recombination between viruses with distinct coreceptor usage occurs frequently. It has been proposed that X4 viruses are more easily suppressed by the immune system than R5 viruses. We hypothesize that recombination between circulating R5 viruses and X4 viruses can result in chimeric viruses with the potential to both evade the immune system and infect CXCR4-expressing cells. The broadening in cell tropism of the viral population to include CXCR4-expressing cells would gradually impair the immune system and eventually allow the X4 population to expand. In conclusion, intrapatient recombination between viruses with distinct coreceptor usage may contribute to the emergence of X4 viruses in later stages of infection.

Human immunodeficiency virus type 1 (HIV-1) is the fastest evolving human pathogen. The accelerated evolution of HIV-1 is a consequence of genetic drift due to the error-prone viral reverse transcriptase (28), immune system-mediated selection leading to high viral turnover (5), and recombination between two virion-associated RNA genomes during reverse transcription (14). Within the infected host, the combination of recombination, selection, and genetic drift gives rise to complex quasispecies populations. It has been estimated that HIV-1 may be subjected to as many as three to nine recombination events per round of replication (25, 51). Recombination may lead to major genome rearrangements and is important in the generation and diversification of subpopulations. Indeed, recombination has been found repeatedly in studies of HIV evolution and genetics. Recombination events between viruses of different subtypes of the major (M) group have resulted in a number of stable circulating recombinant forms (20, 21, 29, 47). Furthermore, recombination between viruses isolated from different anatomical sites from one individual has been reported (19, 34). Establishment of recombinant viruses within an infected individual may lead to serious consequences, for example, due to the rapid spread of drug resistance in the virus population (18) and accelerated progression toward AIDS (26).

HIV-1 enters target cells through interactions between the viral glycoproteins (gp120 and gp41), the cellular receptor CD4, and a coreceptor, most often CCR5 or CXCR4 (1). CCR5-using (R5) viruses are often present in the early phase of infection, whereas CXCR4-using (X4) viruses usually appear (or become detectable) only at later stages. The broadening of coreceptor usage to include CXCR4 is associated with accelerated loss of CD4 cells and faster progression to AIDS (41). After the appearance of X4 viruses, the R5 and X4 populations most often coexist in the host. The cellular and molecular mechanisms responsible for virus coreceptor switch during the course of infection are still unclear. Several hypotheses have been proposed that may explain the late appearance of X4 viruses (38). The transmission-mutation hypothesis suggests that R5 viruses are preferentially transmitted and gradually mutate into X4 viruses, whereas the target-cell-based hypothesis emphasizes that a gradual shift in the availability of CCR5- and CXCR4-expressing cell populations is responsible for the appearance of X4 viruses. Finally, the immune system-based hypothesis suggests that X4 viruses are better recognized by the immune system and subsequently suppressed. X4 populations may emerge as a consequence of gradual immune system dysfunction.

During a study of intrapatient HIV-1 evolution, we identified several cases of recombination between coexisting R5 and X4 viruses. A hot spot for recombination was identified in the C2 region of *env*, and sequence analysis showed that a small part of the envelope, including the V3 region, determined coreceptor usage for both R5 and X4 recombinants. On the basis of these findings, we hypothesize that double infection followed by recombination between coexisting R5 and X4 viruses could generate less well immune system-controlled X4 variants which

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could be of great importance for the emergence of X4 viruses later in infection.

MATERIALS AND METHODS

Patient material. Twenty-seven HIV-1 isolates from four patients (1865, 2239, 2242, and 2282) were selected on the basis of coreceptor usage evolution from a cohort of 53 HIV-1-infected individuals (15). The four patients were previously classified as switch virus patients (17), since viruses isolated early in infection used only CCR5, whereas the virus population isolated later in infection used both CXCR4 and CCR5 (patients 2239, 2242, and 2282) or CCR3 (patient 1865) (Table 1).

Generation of chimeric viruses. Subconfluent 293T cells were transfected with 3 μg of 43XCΔV, a NheI-linearized vector containing a full-length pNL4-3 genome with the region from V1 to V3 (V1-V3) deleted (46), and with 1 μg amplified V1-V3 fragment (see below) using the calcium phosphate precipitation method. Cells were washed with phosphate-buffered saline 16 h after transfection. After 48 h, the supernatant, containing chimeric virus, was removed, cleared by centrifugation, and stored at −80°C.

Determination of coreceptor usage. Human kidney embryonic cell line 293T cells and human glioma U87.CD4 cells, stably expressing CD4 and one of the chemokine receptors (CCR5 or CXCR4) (7) were maintained as described previously (30). Twenty-four hours prior to infection, 10^5 U87.CD4 cells/well were seeded in 48-well plates. For infection, 200 μl of chimeric virus was added. Cells were washed three times with Dulbecco modified Eagle medium 16 h postinfection. Six days postinfection, the cultures were analyzed for syncytium formation and p24 by using an enzyme-linked immunosorbent assay kit (Biomérieux, Boxtel, The Netherlands).

Amplification, cloning, and sequencing. Viral RNA was extracted and purified from peripheral blood mononuclear cell culture supernatants, using Nukleospin RNA virus kit (Machery-Nagel, Germany) according to the manufacturer's instructions. Purified RNA was reverse transcribed using Superscript II (Invitrogen), and the V1-V3 region was amplified from cDNA using the Expand High Fidelity PCR system (Roche) and primers E20 and E115 (46) as described by the protocol supplied by Roche. The amplified products, approximately 900 bp (nucleotides 6002 to 6903 in HXB2; GenBank accession number AF033819), were cloned using the TOPO-TA cloning system (Invitrogen). From each isolate, 10 colonies were picked, and viral V1-V3 DNA was amplified as described above. Clones were named as follows: the patient identification number, month of isolation, and clone number (patient-month:clone number). In the case of patient 2242, two samples were taken 63 and 85 months postinfection. The second isolates for each month for this patient are designated 2242-63:2 and 2242-85:2.

Purified V1-V3 DNA was sequenced using an ABI PRISM Big Dye Termination kit (Applied Biosystems) according to the manufacturer's instructions using primers E20 (46), 793SEQ4 (5′-CAGCAGTGAGTTGATACTACTGG-3′), and JA168 and JA169 (24). Sequences were determined using ABI Prism 3100 (Applied Biosystems).

Phylogenetic analysis. Sequences were assembled, and contigs were analyzed with CodonCode Aligner version 1.4.3 (CodonCode Corporation), aligned with ClustalX (45) and manually edited using GeneDoc. Sequences from each patient were treated as individual data sets, and Modeltest (36) was used to identify the nucleotide substitution model that fit the data best. Maximum-likelihood trees were constructed with PAUP* 4.0 (Sinauer Associates, Inc. Publishers) using heuristic searches. Statistical support of the trees was obtained by 100 bootstrap replicates using the LUNARC computer cluster (http://www.lunarc.lu.se) at Lund University, Sweden.

Recombination analysis. First, the data sets for each patient were split into two regions (V1/V2 and V3), and the phylogenetic trees were constructed for each data set (39). A clone was considered a recombinant if it clustered with different groups of sequences separated by significant bootstrap values (90% or more) in the two trees (see Fig. 2A and B). Putative parental sequences were identified as the sequences most similar to the recombinant in these trees. Second, we identified recombination breakpoints and parental sequences with BootScan analysis (27) using a window size of 200 bp and a 20-bp sliding step. The two putative parental sequences were considered true parental sequences if they clustered together with the recombinant in more than 90% of the permuted trees (see Fig. 2C). If both parental sequences were identified, the recombination breakpoint could be identified, the data set was split at that position, and trees were generated as described above (see Fig. 2D and E). Finally, the recombinant and parental sequences were inspected manually (see Fig. 3).

Nucleotide sequence accession numbers. Nucleotide sequences were deposited in GenBank under the following accession numbers: DQ516085 to DQ516124 (patient 1865), DQ516125 to DQ516172 (patient 2239), DQ516173 to DQ516266 (patient 2242), and DQ516267 to DQ516338 (patient 2282).

TABLE 1. Coreceptor usage of sequential HIV-1 isolates and of V1-V3 clones from dualtropic isolates

Patient	Time from infection (mo)[a]	Phenotype of isolate	No. of clones using coreceptor(s)[b]:			No. of inactive clones[c]
			CCR5	CCR5 and CXCR4	CXCR4	
1865	49	R5	10			
	55	R5	9			
	61	R3X4			10	
	70	R3X4			10	
2239	25	R5	8			
	45	R5	10			
	68	R5X4	1	0	7	1
	79	R5X4	0	0	10	0
	88	R5X4	4	0	4	2
2242	18	R5	10			
	45	R5	8			
	56	R5	9			
	63	R5	10			
	63	R5	9			
	64	R5	10			
	76	R5X4	1	0	0	9
	84	R5X4	7	0	1	2
	85:1[d]	R5X4	5	0	3	1
	85:2[e]	R5X4	5	0	3	2
2282	10	R5	10			
	21	R5	8			
	24	R5	7			
	41	R5	10			
	47	R5X4	3	3	0	3
	62	R5X4	3	5	0	1
	63	R5X4	2	2	4	1
	70	R5X4	1	3	4	2

[a] Time from infection was calculated as the midpoint between the last negative sample and the first positive sample.
[b] Coreceptor usage of chimeric viruses was determined by infection of U87.CD4 cells expressing CCR5 or CXCR4.
[c] Chimeric viruses that did not infect CCR5- or CXCR4-expressing cells.
[d] First sample, 85 months postinfection.
[e] Second sample, 85 months postinfection.

RESULTS

Coreceptor usage. We determined the coreceptor usage of 8 to 10 V1-V3 clones from R5X4 isolates of patients 2239, 2242, and 2282 (Table 1). Amplified V1-V3 fragments and an HIV-1 backbone were used to reconstruct chimeric viruses. The coreceptor usage of the chimeric viruses was determined by infecting cell lines expressing CD4 and either CCR5 or CXCR4. In our isolates, the R5X4 phenotype was the result of a mixture of R5 and X4 viruses in patients 2239 and 2242, whereas patient 2282 also had dualtropic R5X4 viruses (Table 1). The clones from previously characterized R5 (all patients) and R3X4 (patient 1865) isolates (16) were considered R5 and X4 clones, respectively.

Phylogenetic analysis. We determined the nucleotide sequences of 253 V1-V3 clones and constructed maximum-likelihood trees to study the relationships of sequences within each

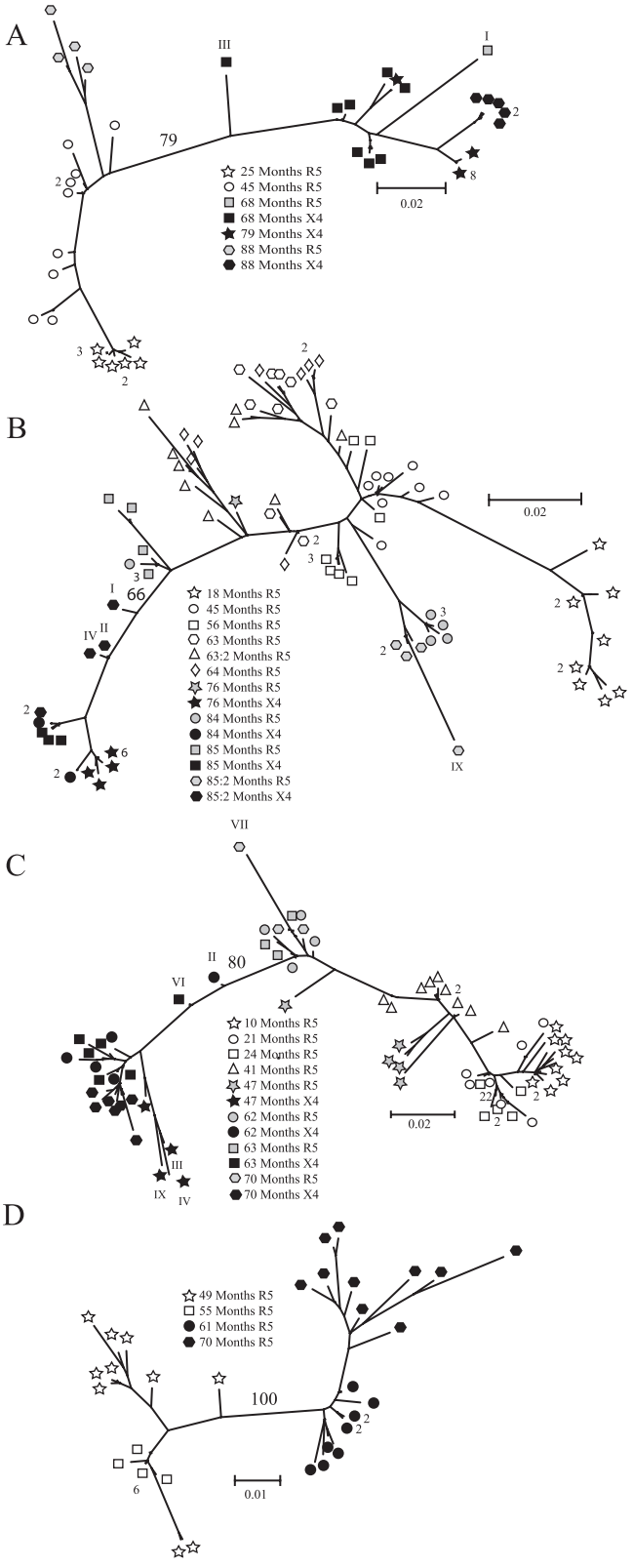

FIG. 1. Phylogenetic relationship of HIV-1 V1-V3 clones from patients (A) 2239, (B) 2242, (C) 2282 and (D) 1865. Bootstrap values (as percentages) are indicated on branches separating R5 and X4 populations. Sequences that differed by 3 nucleotides or less are represented by one terminal branch, and the number of clones that are represented at a branch is indicated. Deviant clones (see Results) are indicated with roman numerals. Different symbols represent the coreceptor usage of the isolate and sampling time postinfection. Open symbols show clones derived from R5 isolates, gray symbols indicate phenotypically characterized R5 clones from R5X4 isolates, and black symbols represent phenotypically characterized X4 clones from R5X4 isolates or clones from R3X4 isolates.

patient (Fig. 1A to D). In all four patients, the sequences were separated according to coreceptor usage. The bootstrap values for branches separating R5 and X4 clones were 100%, 79%, 66%, and 80% for patients 1865, 2239, 2242, and 2282, respectively. Closer inspection of the phylogenetic trees revealed several deviant sequences that either (i) were scattered between the R5 and X4 populations (2239-68 III; 2242-85:2 I, II; 2282-62 II; 2282-63 VI; Fig. 1A to C), (ii) had long branch lengths (2242-85:2 IX; 2282-47 III, IV, IX; 2282-70 VII; Fig. 1A to C), or (iii) clustered together with sequences that represented different phenotypes, i.e., one R5 clone (2239-68 I; Fig. 1A) clustered with X4 clones.

It has been reported that intrapatient recombination occurs frequently (14, 25, 51) and that recombinant clones often deviate from other sequences in phylogenetic trees (42) in a fashion similar to our observations. We therefore anticipated that the deviant sequences represented potential recombinants, and they were subjected to further analysis.

Recombination analysis. Since recombinants have acquired genetic material from at least two sources, they should cluster with different groups of sequences when trees are constructed from subsets of the data (39). To identify recombinants, we analyzed our data sets in three different ways. First, we generated one tree for the V1/V2 region and one for the V3 region from sequences from each patient. As exemplified by patient 2282 in Fig. 2A and B, six clones (47 III, IV, IX; 62 II; and 63 VI and 70 VII) clustered with different groups of sequences in the two trees. The cluster identity of the clones was supported by significant bootstrap values in both the V1/V2 and V3 trees, supporting that these sequences were the result of recombination events. To confirm these findings, we performed BootScan analysis. As seen in Fig. 2C, clone 62 II clustered together with clone 62 X in the 5′ end and with clone 63 III in the 3′ end. The recombination breakpoint was determined at nucleotide position 328 in the V1-V3 region analyzed (nucleotide 6473 in HXB2; GenBank accession number AF033819). Finally, to confirm the BootScan analysis, the data set was split at this position, and two trees were generated. As expected, clone 62 II clustered together with different sequences in the two trees (Fig. 2D and E).

Using this approach, we identified recombinants in all patients except for patient 1865, which was the only patient that had R5 and X4 variants that were phylogenetically separated by significant bootstrap values in the phylogenetic analysis (Fig. 1D). We identified 11 recombinants, representing 8.8% of the total 125 clones from CXCR4-using (R5X4 and R3X4) isolates (Table 1). Sequence analysis revealed that 10 of the recombinants had originated by recombination events between R5 and X4 viruses, where the majority of the recombinants used CXCR4 (8 of the 11 recombinants) (Table 2). Five of the recombinants originated from recombination between pheno-

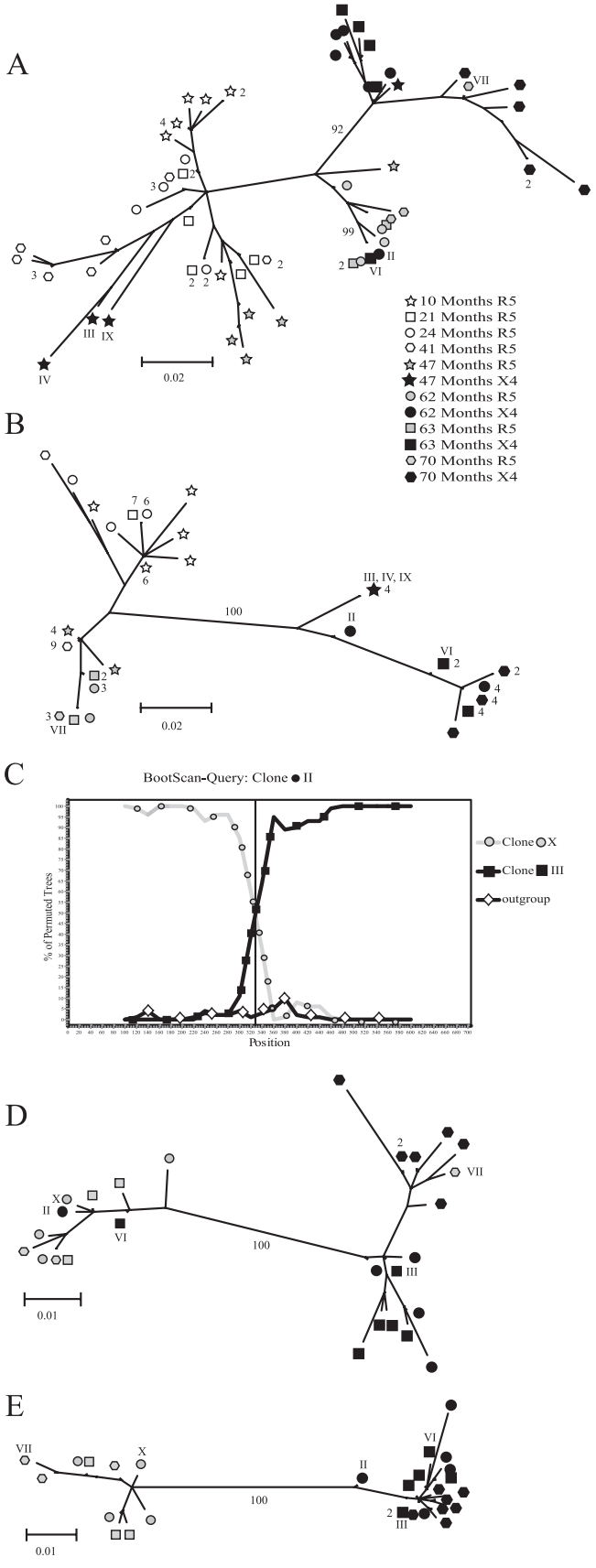

TABLE 2. Recombinant clones

Patient	Clone name of recombinant[a]	5′ Parental sequence name[b]	3′ Parental sequence name[c]	Breakpoint[d]
2239	68 I (R5)	68 VII (X4)	NI (R5)	NI
	68 III (X4)	NI (R5)	68 IV (X4)	NI
2242	85:2 I (X4)	85 VIII (R5)	84 VII (X4)	6594 (C2)
	85:2 II (X4)	85 VIII (R5)	85 V (X4)	6683 (V3)
	85:2 IX (R5)	85:2 IV (R5)	85 VIII (R5)	6418 (C2)
2282	47 III (X4)	NI (R5)	47 V (X4)	NI
	47 IV (X4)	NI (R5)	47 V (X4)	NI
	47 IX (X4)	NI (R5)	47 V (X4)	NI
	62 II (X4)	62 X (R5)	63 III (X4)	6473 (C2)
	63 VI (X4)	63 I (R5)	63 V (X4)	6499 (C2)
	70 VII (R5)	70 I (X4)	70 X (R5)	6531 (C2)

[a] Phenotypically characterized coreceptor usage is indicated in parentheses.
[b] Phenotypically characterized coreceptor usage is indicated in parentheses. Coreceptor usage of parental sequences that were not identified (NI) was based on significant separation from X4 clones in V1/V2 phylogenetic trees.
[c] Coreceptor usage is indicated in parentheses. The coreceptor usage of the parental sequence that was not identified (NI) was based on the coreceptor usage of the recombinant clone.
[d] Recombination breakpoint identified using BootScan and manual inspection. The numbers are the nucleotide positions corresponding to the location in HXB2 (GenBank accession number AF033819). Envelope regions where the breakpoints were identified are indicated in parentheses. NI, not identified.

typically characterized R5 and X4 clones, five from recombination between phenotypically characterized X4 clones and predicted R5 clones (on the basis of significant separation from X4 clones in V1/V2 phylogenetic tree, see Materials and Methods), and one, 2242-85:2 IX, originated from recombination event between two phenotypically characterized R5 clones (Table 2). For 6 of the 11 recombinants, both parental sequences were identified. The recombination breakpoints were located in the C2 or V3 region. For the remaining recombinant sequences, we were not able to identify the putative parental sequences (see Materials and Methods for definition of parental sequences) which corroborated identification of breakpoints.

Sequence analysis. Identification of identical parental sequences reconstituting the recombinant is expected if the recom-

FIG. 2. Schematic illustration of the recombination analysis, exemplified by clones from patient 2282. Phylogenetic trees were constructed from (A) the V1/V2 regions and (B) the V3 regions. Clones that clustered with different groups of sequences were considered recombinants if the groups were separated by a significant bootstrap value (≥90%) in the two trees. (C) Recombinants were analyzed by BootScan analysis for identification of the recombination breakpoints. (D and E) The data set was split at the breakpoint (nucleotide 328) and two trees were constructed to confirm the results. Different symbols represent the coreceptor usage of the clones and sampling time postinfection. Open symbols show clones from R5 isolates, gray symbols indicate phenotypically characterized R5 clones from R5X4 isolates, and black symbols represent phenotypically characterized X4 clones from R5X4 isolates. Recombinants (II, VI, and VII) and parental (III and X) clones are indicated with roman numerals. Sequences that differed by 3 nucleotides or less are represented by one terminal branch, and the number of clones that are represented at a branch is indicated. Bootstrap values that separated groups and were used for identification of recombinants are indicated.

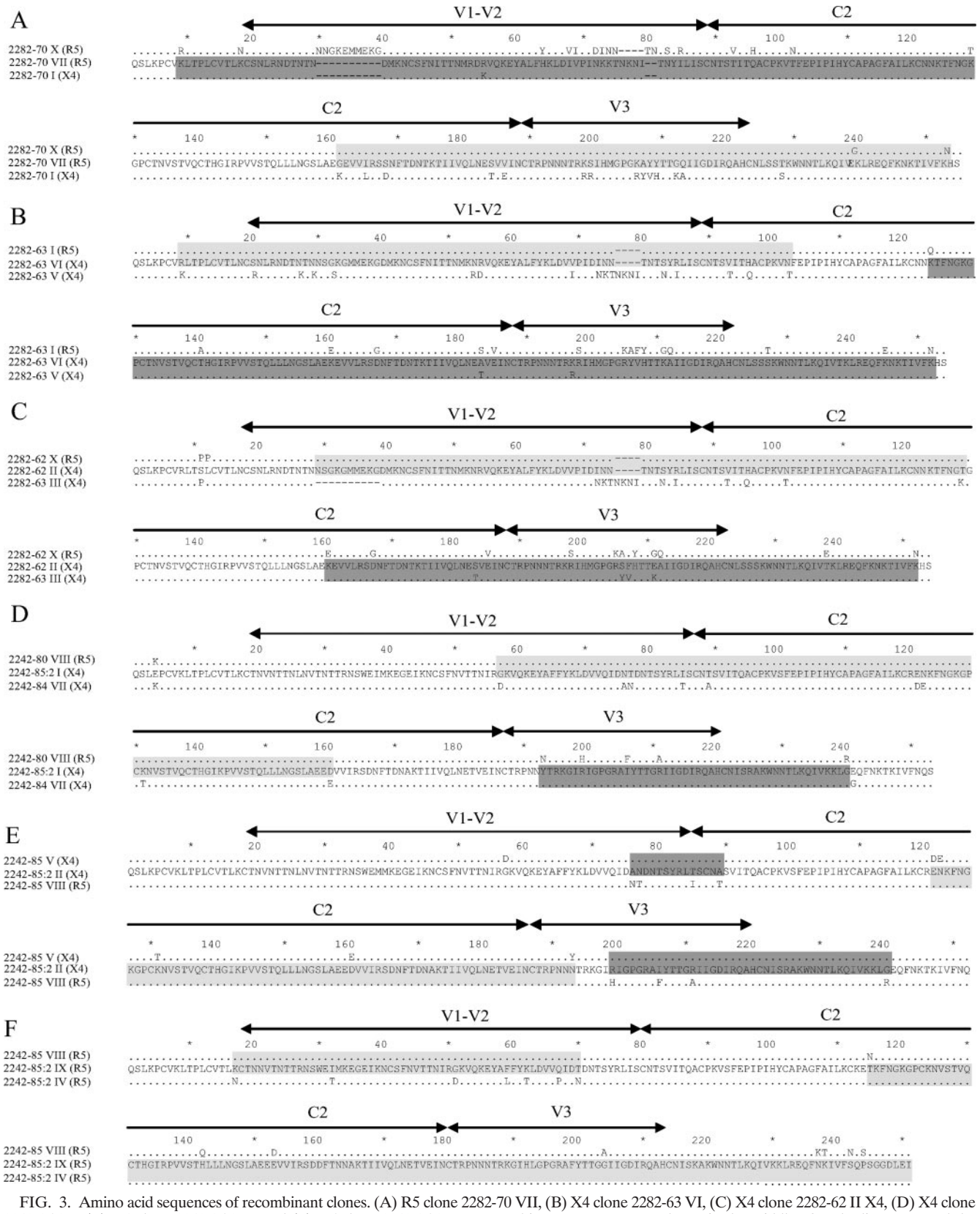

FIG. 3. Amino acid sequences of recombinant clones. (A) R5 clone 2282-70 VII, (B) X4 clone 2282-63 VI, (C) X4 clone 2282-62 II X4, (D) X4 clone 2242-85:2 I, (E) X4 clone 2242-85:2 II, and (F) R5 clone 2242-85:2 IX. Recombinant sequences are shown in the middle of each alignment, and the parental sequences are shown above and below each recombinant sequence. Shaded regions indicate where the recombinants are most similar to one of the parental sequences. Regions shaded in light gray indicate similarity between the recombinant sequence and the R5 parental sequence, and regions highlighted in dark gray show regions of similarity between the recombinant and the X4 parent. The locations of the V1-V2, C2, and V3 regions are indicated. Dots represent identical amino acids between the recombinant and parental sequences. Recombinant clone 2242-85:2 II was most likely a result of a double-crossover event as indicated. The coreceptor usage is indicated in parentheses.

binants were generated during PCR, since the parental sequences statistically should be in excess. In our study, the parental sequences never reconstituted the nucleotide sequence of their recombinant offspring (Fig. 3), indicating that the recombinants had accumulated additional mutations after the recombination event. In addition, three recombinants (2242-85:2 I, II, and IX) had parental sequences that belonged to phylogenetic clusters that encompassed only clones isolated from a time point that differed from the isolation time point of the recombinant (Fig. 3D to F and Table 2). These observations supported that the recombinants had originated in vivo (37).

Identification of recombination breakpoints between V1/V2 and V3 allowed us to analyze the impact on coreceptor usage of these two regions. For R5 recombinant clone 2282-70 VII, the V3 region and part of the C2 region was derived from the R5 parental sequence, whereas the remaining part of C2 and V1/V2 was contributed by the parental X4 sequence (Fig. 3A). The opposite was observed for the recombinant X4 clones 2282-63 VI (Fig. 3B) and 2282-62 II (Fig. 3C). In the case of the recombinant X4 clone 2242-85:2 I, only a small part, including V3, was derived from the X4 parental sequence, whereas the rest of the sequence was donated by the R5 parent (Fig. 3D). In fact, only five amino acids of the entire recombinant sequence (four located in V3) were X4 specific.

All recombinants had the same phenotype as the parental sequences that donated the 3′ part (including V3) of the recombinant sequences (Fig. 3 and Table 2). Our results suggest that the V1/V2 region does not impact on coreceptor usage and that the V3 region determines coreceptor usage for our recombinant clones (Fig. 3).

DISCUSSION

The study on sequence variation of the V1-V3 regions of viruses from four switch patients led to the finding that intrapatient recombination between R5 and X4 viruses occur frequently. Detection of R5/X4 recombinants is expected, since HIV-1 recombination is commonly observed, even in a single round of infection (25, 51). In 2002, Jung et al. used fluorescence in situ hybridization and found that splenocytes from two HIV-1-infected patients contained on average three or four proviruses per cell (14). It has also been shown that cells become double infected by both R5 and X4 chimeric viruses (4). These observations indicate that cells frequently become coinfected which has to occur for a recombination event to take place. Coinfection and recombination are expected to occur frequently in vivo because a substantial fraction of memory $CD4^+$ T cells express both CCR5 and CXCR4 (2, 22).

Recombinants may be generated in vitro during the process of PCR (37) or when the virus is propagated in human peripheral blood mononuclear cells. However, several observations make in vitro recombination an unlikely explanation for the origin of the recombinants reported here. First, the nucleotide sequences of the recombinants differed from the parental sequences, that is, identical parental sequences representing the recombinant were never found. This would have been expected if the recombinants were generated in vitro (37). Second, we identified only one of the two putative parental sequences for five of the recombinants (2239-68 I and III, 2282-47 III, IV, and IX). The remaining part of the recombinant had low similarity to other clones. Third, three recombinants had one of the their parental sequences in a phylogenetic cluster that contained only clones isolated from a time point that differed from the isolation time point of the recombinant (Fig. 3 and Table 2). Taking these observations into consideration (37) and the fact that both double infection (4, 14) and intrapatient recombination are commonly observed for HIV-1 in vivo (3, 14, 19, 34, 48), we feel confident that the majority of our recombinant sequences originated in vivo (37).

Recombination events between R5 and X4 within patients have to our knowledge been reported only three times previously (3, 19, 48). Results of these studies differ from ours because none of them addressed the impact of recombination on coreceptor usage and HIV-1 pathogenesis. Here, we determined both the genotype and coreceptor usage of V1-V3 clones from sequential isolates from four switch virus patients. Characterization of the patient material in this way allowed us to couple recombination events to coreceptor usage. The majority of breakpoints that we identified were located in the C2 region (Fig. 3 and Table 2) which is in agreement with a recent report where the C2 region was identified as a hotspot for recombination (9). Identification of the recombination breakpoints together with coreceptor usage data made it possible for us to perform a detailed analysis on how the V1-V3 region impacts on coreceptor usage. We presented evidence that a small part of the envelope, including the V3 region, alone determined coreceptor specificity of the recombinant sequences studied here. Several reports have previously suggested that the V3 region is the dominant determinant for coreceptor usage (6, 8, 13, 43). It has also been suggested that other regions of *env* are involved in determining coreceptor usage (12, 23, 31, 32). This highlights that HIV-1 coreceptor usage, and its determinants, is complex. This is supported by a recent study which demonstrated that the V1/V2 region can compensate for loss-of-fitness mutations in the V3 region (33). A possible explanation for our results is that the clones studied here, have well-adapted, biologically optimal X4 and R5 V3 regions. Such V3 regions would be independent of the V1/V2 region in the context of coreceptor usage (33).

The appearance and dominance of X4 viruses late in infection have been debated for many years without finding a biological explanation for this phenomenon. One hypothesis addressing the coreceptor switch involves immune control (38). This hypothesis is based upon the assumption that X4 viruses are better recognized by the immune system than R5 viruses and, consequently, are suppressed. In agreement with this, in 2003, Harouse et al. showed that rhesus macaques coinfected with R5 and X4 simian-human immunodeficiency hybrid viruses showed an increase in the X4 population and a decrease in the R5 population upon depletion of $CD8^+$ T cells (11). It has also been shown that the V1/V2 region is important for inducing neutralizing antibody response (10, 40, 44, 49, 50). A recent report also suggested that the V1/V2 region is a global regulator of the sensitivity of primary HIV-1 isolates to neutralizing antibodies (35). Furthermore, Ye et al. (50) showed that the conformational arrangement of V2 and V3 with respect to the CD4 receptor binding region of gp120 appears to be critical for the recognition by neutralizing antibodies. Thus, rearrangements in the C2 region could have a dramatic effect on the immune response directed toward the viral population.

Therefore, a recombination event between an immune-resistant R5 virus and an X4 virus in the C2 region could generate variants with the potential to evade the immune response and infect cells expressing CXCR4. The broadening in cell tropism of the viral population to include CXCR4-expressing cells would result in increased $CD4^+$ cell death and further impair the immune system, which would allow the suppressed X4 population to expand. We hypothesize that coinfection and recombination between R5 and X4 viruses may in part be responsible for the coreceptor switch late in infection.

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