The emergence of X4 human immunodeficiency virus type 1 (HIV-1) strains in HIV-1-infected individuals has been associated with CD4$^+$ T-cell depletion, HIV-mediated CD8$^+$ cell apoptosis, and an impaired humoral response. The bicyclam AMD3100, a selective antagonist of CXCR4, selected for the outgrowth of R5 virus after cultivation of mixtures of the laboratory-adapted R5 (BaL) and X4 (NL4-3) HIV strains in the presence of the compound. The addition of AMD3100 to peripheral blood mononuclear cells infected with X4 or R5X4 clinical isolates displays the syncytium-inducing phenotype resulted in a complete suppression of X4 variants and a concomitant genotypic change in the V2 and V3 loops of the envelope gp120 glycoprotein. The recovered viruses corresponded genotypically and phenotypically to R5 variants in that they could no longer use CXCR4 as coreceptor or induce syncytium formation in MT-2 cells. Furthermore, the phenotype and genotype of a cloned R5 HIV-1 virus converted to those of the R5X4 virus after prolonged culture in lymphoid cells. However, these changes did not occur when the infected cells were cultured in the presence of AMD3100, despite low levels of virus replication. Our findings indicate that selective blockade of the CXCR4 receptor prevents the switch from the less pathogenic R5 HIV to the more pathogenic X4 HIV strains, a process that heralds the onset of AIDS. In this article, we show that it could be possible to redirect the evolution of HIV so as to impede the emergence of X4 strains or to change the phenotype of already-existing X4 isolates to R5.

Human immunodeficiency virus type 1 (HIV-1) strains isolated from newly infected individuals are predominantly macrophage tropic (MT) and non-syncytium inducing (NSI) and require CC-chemokine receptors such as CCR5 as entry co-factors in combination with CD4 (1, 16) (referred to as R5 HIV strains [2]). T-tropic (TT) strains are rapidly replicating, non-syncytium-inducing (SI) strains that use the CXCR4 receptor (referred to as X4 strains [2]); they appear much later, after the primary infection, and their emergence is associated with a rapid decline of CD4$^+$ T cells that heralds the breakdown of the immune system and the onset of AIDS (9, 16, 19, 32, 33, 35). SI X4 viruses appear to exert their deleterious effect on the immune system not only by direct cytopathic effects on CD4$^+$ T cells but also by the indirect killing of CD8$^+$ T cells that is mediated by CXCR4 (22). Furthermore, it has also been shown that lymphoid cells infected with R5 strains retain their immunocompetence but that, conversely, infection by X4 strains blocks the immune response to specific antigens (20). This implies that the immunodeficiency hallmarking the progression of AIDS is due, at least in part, to the emergence of the more pathogenic SI X4 strains (3). Therefore, it can be inferred that strategies directed to prevent the emergence of X4 strains would be beneficial to HIV-infected individuals.

It has been recently shown that the bicyclam AMD3100 is a highly potent inhibitor of X4 HIV strains, and its mode of action resides in a selective antagonism of CXCR4 (15, 28), the receptor for the CXC-chemokine stromal cell-derived factor 1 (SDF-1) (5). AMD3100 competes with the binding of SDF-1 to its receptor, shuts off the intracellular Ca$^{2+}$ mobilization induced by SDF-1, and does not trigger an intracellular signal by itself. In this article, we show that the evolution of HIV-1 can be directed so as to prevent the emergence of the more pathogenic X4 strains over the less pathogenic R5 strains by blockade of the CXCR4 receptor.

**MATERIALS AND METHODS**

**Compounds, viruses, and cells.** The bicyclam AMD3100 [1,1’-(1,4-phenylene)-bis(1,1,2,3,4-tetraazacyclotetradecane) octahydrochloride dihydrate] was synthesized at Johnson Matthey as described previously (6). SDF-1x was purchased from R&D Systems (London, United Kingdom). Zidovudine (AZT) was purchased from Sigma (St. Louis, Mo.). The HIV-1 strains NL4-3 and BaL and the CD4$^+$ lymphocytic cell lines SUP-T1 and MT-2 were obtained through the Medical Research Council AIDS reagent program. U87-C4 cells expressing either CCR5 or CXCR4 were obtained from the National Institutes of Health AIDS Research and Reference Reagent Programs.

**Determination of viral fitness by replication competition of defined mixtures of viruses.** Phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) (10$^5$ in 1-mL volumes) were infected with 25 ng of a mixture of the HIV strains NL4-3 and BaL (the percentage of each strain being 0, 20, 40, 60, 80, or 100% of the total p24 count) in the presence of AMD3100. The cells were incubated for 24 h and then washed twice in phosphate-buffered saline (PBS) and resuspended in medium containing AMD3100 (1 μg/mL). After a 5-day incubation at 37°C, DNA was isolated from infected cells for DNA sequencing. In similar experiments, PHA-stimulated PBMC infected with a predetermined mixture of 99% NL4-3 and 1% BaL in the absence and presence of AMD3100 (1 μg/mL) were cultured and passaged every 7 days in uninfected PHA-stimulated PBMC. After 28 days in culture, p24 antigen was measured in the culture supernatant and DNA was isolated from infected cells for DNA sequencing.

**Virus growth in the presence of AMD3100.** PHA-stimulated PBMC were infected with low-passage clinical HIV-1 isolates in the presence of AMD3100. HIV replication was measured (every 7 to 8 days) by a p24 antigen detection method (Coulter). The p24 antigen-positive supernatant was further passaged in fresh PBMC. After four passages (28 days) in the presence of the drug, recovered...
virus was used for the phenotype assay in MT-2 cells, and DNA was isolated for PCR amplification, DNA sequencing, and cloning.

**In vitro emergence of the SI phenotype.** The third variable region (the V3 loop) of the envelope of HIV contains a major neutralization epitope and determinants of cell tropism (23), SI capacity, replication rate (11), and coreceptor use (8). The recombination of a V3 loop DNA sequence corresponding to a R5 strain into the DNA sequence of a X4 strain is sufficient to modify the coreceptor use of the resulting virus from X4 to R5 (8). Furthermore, the phenotype of NSI slow-replicating HIV-1 converts to SI fast-replicating strains after prolonged culture in SUP-T1 cells. Mutations within the V3 loop have been shown to be responsible for the conversion into the SI phenotype (11, 12). Therefore, the evolution of HIV strains from R5 phenotype into the X4 or R5-X4 phenotype can also be monitored by genotypic changes that lead to amino acid changes in the V3 loop. The viral clone 106.1 (11, 12, 24) of the NSI slow-replicating phenotype was cultured in SUP-T1 cells in the absence or presence of AMD3100 (1 μg/ml). Every 5 or 6 days, the numbers of syncytia in the cultured cells were scored, and cells were passaged in fresh medium with or without compound. Once syncytia were scored positive in the untreated sample, the AMD3100 culture was continued for 55 more passages (i.e., until 405 days after the initial infection). DNA was isolated from infected cells for DNA sequencing.

Cloning and phylogenetic analysis of HIV-1 env. PCR fragments of the env gene from proviral DNA were cloned in the pCR-Script SK(+) cloning vector (Stratagene, La Jolla, Calif.) by following the manufacturer’s instructions and the procedure described elsewhere (18). Clones were isolated for DNA sequencing, and phylogenetic analysis was done by the neighbor-joining method using the Clustal X (34) software. Bootstrap resampling was used to assess the strength of support for each branch of the phylogenetic trees.

**DNA sequence analysis.** The gp120 proviral genome was isolated by PCR amplification of total cellular DNA purified from infected cells. For sequencing of the V3 loop, preparative PCR was performed with 5 to 20 μg of total DNA purified by the QIAGEN blood kit and with 0.1 μg of each of the primers TACAAGTACACATGGAAAT and ATTACAGTAGAAAATTCC. Then, a second preparative PCR, which amplifies the V3 loop region of gp120, was done with primers TGGCAGTCTAGCAGAAGAAG and TCTGGGTCCCCTCCTGG. For sequencing of the V2 loop, primers AATTAACCCCACTCTGTGTAGTTTA and TGATACTATGGAATT and ATTACAGTAGAAAATTCC were used for the second preparative PCR. DNA sequencing was performed directly on the purified PCR product following the protocols provided by the ABI PRISM cycle sequencing kit, and sequences were analyzed with an ABI PRISM genetic sequencer. The Navigator and Factura DNA analysis software packages (Perkin-Elmer) were used to identify and quantify ambiguous regions of the DNA sequence that are produced when a mixture of two sequences is detected.

**Determination of virus phenotype (MT-2 assay).** MT-2 cells were infected with different HIV-1 isolates. Cells cultures were monitored for syncytium formation for up to 14 days postinfection.

**Coreceptor use by different clinical isolates.** U87-CD4 cells expressing either CCR1, CCR2b, CCR3, CCR5, or CXC4R (4 × 10^5) were infected with 10 ng of p24 antigen of the corresponding virus strain and incubated for 24 h. Cells were then washed twice with PBS, and fresh Dulbecco’s modified Eagle’s medium was added. Cells were incubated for four more days, and p24 antigen in the culture supernatant was measured.

**RESULTS**

**Viral fitness determined by replication competition with defined X4-R5 virus mixtures.** The effect of AMD3100 on the replication of mixtures of two laboratory-adapted HIV isolates, the X4 isolate NL4-3 and the R5 isolate BaL, was evaluated based on the sequence of the V3 region of gp120 from proviral DNA isolated from PBMC that had been infected with these virus mixtures. The nucleic acid sequence of a fragment of the HIV-1 V3 region of gp120 from proviral DNA isolated from cells infected with either NL4-3 or BaL, or from mixtures of these two virus strains was determined. Provirial DNA sequence determination may serve as a marker of the viral fitness of each strain (21). As expected, the DNA sequence corresponding to either NL4-3 or BaL was found if the cells were infected solely with the NL4-3 or BaL strain, respectively. When the cells were infected with a mixture of these strains, DNA sequence analysis showed that the proviral DNA sequence could not be aligned with either the NL4-3 or the BaL sequence but rather corresponded to a mixture of both sequences (data not shown). Six sample sequencing chromatograms are shown in Fig. 1. DNA sequences (positions 50 to 54) correspond to amino acids I and R/N (amino acids 16 and 17) in the V3 loop of gp120. This region is located before the insertion QR in the V3 loop coding region (Fig. 1).

**Effect of AMD3100 on the outgrowth of X4 and R5 from X4-R5 virus mixtures.** The results presented above indicate
that the replication of the X4 strain NL4-3 is suppressed in the presence of AMD3100. To further assess the influence of AMD3100 on the replicative ability of X4 and R5 virus strains in X4-R5 virus mixtures, a mixture composed of 99% NL4-3 and 1% BaL was used to infect PHA-stimulated PBMC that were then cultured for 28 days (four passages) in the presence or absence of AMD3100. NL4-3 virus replication was inhibited by AMD3100, and NL4-3 proviral DNA became undetectable after 21 days in culture (data not shown). Both NL4-3 and BaL were detectable in the virus progeny at 28 days of an initial virus mixture containing 99% NL4-3 and 1% BaL (as demonstrated by sequencing the V3 loop region of the proviral DNA recovered after 28 days in culture). However, when this virus mixture was cultured for 28 days in the presence of AMD3100, only the BaL strain could be recovered (Fig. 2).

**Determination of phenotype of clinical HIV isolates grown in the presence of AMD3100.** In vivo HIV infection is characterized by the existence of marked heterogeneity in viral populations (25). To better reproduce these conditions, we studied the effect of CXCR4 blockade on the replication of six clinical isolates, three that were defined as NSI and three that were characterized by the existence of marked heterogeneity in viral populations. The dominating virus population can be determined by the proportion of the virus mixture containing 99% NL4-3 and 1% BaL.

**Determination of genotype of clinical HIV isolates grown in the presence of AMD3100.** Proviral DNA of cells infected with clinical HIV isolates for up to 28 days in the presence or absence of AMD3100 was amplified and sequenced.

**TABLE 1. Low-passage clinical isolates of HIV-1 that were cultured in the presence of AMD3100: phenotype in MT-2 cells and sensitivity to AMD3100 and AZT**

<table>
<thead>
<tr>
<th>Clinical isolate*</th>
<th>EC_{50} b (µg/ml) following treatment with:</th>
<th>Phenotype in MT-2 cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMD3100</td>
<td>AZT</td>
</tr>
<tr>
<td>NSI strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDM (−)</td>
<td>&gt;1</td>
<td>0.005</td>
</tr>
<tr>
<td>MDM (+)</td>
<td>&gt;1</td>
<td>0.005</td>
</tr>
<tr>
<td>MCS (−)</td>
<td>&gt;1</td>
<td>0.004</td>
</tr>
<tr>
<td>MCS (+)</td>
<td>&gt;1</td>
<td>0.004</td>
</tr>
<tr>
<td>JGA (−)</td>
<td>&gt;1</td>
<td>0.005</td>
</tr>
<tr>
<td>JGA (+)</td>
<td>&gt;1</td>
<td>0.02</td>
</tr>
<tr>
<td>SI strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CST (−)</td>
<td>0.13</td>
<td>0.013</td>
</tr>
<tr>
<td>CST (+)</td>
<td>&gt;1</td>
<td>0.01</td>
</tr>
<tr>
<td>AOM (−)</td>
<td>0.11</td>
<td>0.005</td>
</tr>
<tr>
<td>AOM (+)</td>
<td>&gt;1</td>
<td>0.006</td>
</tr>
<tr>
<td>FCP (−)</td>
<td>0.14</td>
<td>0.005</td>
</tr>
<tr>
<td>FCP (+)</td>
<td>&gt;1</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Clinical isolates or supernatant recovered from PHA-stimulated PBMC of HIV-1-infected persons with PBMC from negative donors that scored positive for syncytia in the MT-2 test as early as 3 days postinfection when the cells were cultured in the presence or absence of AMD3100. **EC_{50}** concentration required to inhibit by 50% the p24 antigen production by HIV-1-infected PBMC: 1 × 10^6 cells were infected with 10 ng of p24 from each of the clinical isolates in the presence of varying concentrations of the corresponding drug. Data are presented as percentages of wild-type NL4-3 level as follows: 76 to 100% (+ + + +), 51 to 75% (+ + +), 25 to 50% (+ +), 1 to 24% (+).
were observed compared to the untreated virus DNA sequences. However, several mutations in the SI strains cultured in AMD3100-treated cells that were not present in the untreated samples were noted. Amino acid changes were found in those V3 loop regions (isolates AOM and CST) and V2 loop regions (isolates AOM and FCP) that have been associated with SI and NSI phenotype and HIV tropism (Fig. 3) (23, 29). PCR products corresponding to the V3 loop sequence were also cloned, and individual clones were sequenced. The consensus sequence derived from the alignment of clone sequences from each virus (data not shown) was identical to the proviral sequence that was determined by sequencing of the amplified pDNA shown in Fig. 3. Figure 4 shows the phylogenetic analyses of the V3 loop amino sequences from two patients' isolates of the SI phenotype that showed changes in the V3 amino acid composition after treatment with AMD3100. Cloned sequences corresponding to the untreated AOM or CST isolates and treated AOM or CST isolates were clustered in separate parts of the tree, indicating a clear shift in the composition of the viral population after treatment with AMD3100. Two clones of the untreated CST isolate clustered together with the treated CST clones, suggesting that the emerging population, although in a minor proportion, was already present in the untreated clinical isolate. Furthermore, sequences from the AMD3100-treated virus clustered together and closer to the V3 sequence of the R5 strain BaL but more distant from the V3 sequence of the X4 strain NL4-3.

**Coreceptor use of clinical isolates after culture in AMD3100.** As seen in Fig. 5, U87-CD4 cells expressing CCR5 supported the replication of all HIV clinical isolates of the NSI phenotype, as evaluated by p24 antigen production after 5 days postinfection. However, no virus replication was detected in the CXCR4-transfected cells. Conversely, the SI strains were able to infect the CXCR4-transfected cells, and one SI isolate (CST) was able to replicate in both the CXCR4- and CCR5-transfected cells. After being cultured for 28 days in the presence of AMD3100, all the recovered virus strains replicated in CCR5-transfected cells but not in CXCR4-transfected cells regardless of the coreceptor used by the original virus isolate. The replication of the untreated or treated clinical isolates was marginal (<10% of the principal coreceptor used) in U87-CD4 cells expressing CCR1, CCR2b, or CCR3 (data not shown).

**Blockade of CXCR4 prevents the emergence of the SI phenotype.** Simulating what happens during the course of infection (that is, that R5 strains evolve in some individuals into X4 or R5X4 [dual-tropic] HIV strains), the phenotype of NSI slow-replicating HIV-1 converts to SI fast-replicating strains after prolonged culture in SUP-T1 cells (24, 35). Upon prolonged propagation in SUP-T1 cells, the NSI virus 168.1 tended to give...
rise to virus mutants with an SI phenotype and high replicative capacity. The viral clone 168.1 with an NSI slow-replicating phenotype was cultured in SUP-T1 cells in the absence or presence of AMD3100 (1 μg/ml). Every 4 or 5 days, the numbers of syncytia in the cultured cells were scored, and cells were passaged in fresh medium with or without compound. In the untreated cells, syncytia were first detected after 100 days in culture. At 200 days postinfection, clear cytopathic effect (CPE) and formation of syncytia were noted in the untreated culture. Conversely, no CPE or syncytium formation was detected in the AMD3100 treated cells even after 305 days after the first detection of syncytia in the untreated culture (405 days postinfection), despite low but continuous virus replication (Fig. 6).

To test if genotypic changes paralleled the change from NSI phenotype to SI phenotype, the gp120 proviral genome was isolated by PCR amplification of total cellular DNA purified from infected cells for sequencing of the V3 loop coding region. DNA sequence analysis of proviral DNA isolated from untreated cells where syncytia were observed detected the emergence of mutations in the V3 loop that have been shown to predict the SI phenotype (11, 12, 23) (Fig. 7). Amplified DNA from the untreated cells at day 100 postinfection, when the first syncytia were noted, showed the presence of a mixture of two amino acids, serine (S) or arginine (R), at position 11 of the V3 loop (data not shown). Proviral DNA amplified and sequenced from day 200 postinfection (100 days after the first detection of syncytia) revealed the emergence of mutations at position 6 of the V3 loop, from asparagine (N) to lysine (K); at position 11 from serine (S) to arginine (R); and from glycine (G) to arginine (R) at position 28 of the V3 loop (Fig. 7). Furthermore, there was a net increase in the overall charge of the V3 loop from 3+ to 5+. However, in the culture that was treated with AMD3100, no changes in the

FIG. 5. Coreceptor use of clinical HIV strains after treatment with AMD3100. U87-CD4 cells (5 × 10³ cells) expressing CCR5 (empty bars) or CXCR4 (filled bars) were infected with 10 ng of p24 antigen of the HIV-1 strains that were cultured in PBMC for 28 days in the presence (+) or absence (−) of AMD3100 (1 μg/ml). Cells were incubated for 24 h, washed twice in PBS, and resuspended in fresh medium. At 5 days postinfection, p24 antigen was detected in the cell-free supernatant. The phenotype of the parental HIV-1 strains was determined in MT-2 cells (see Table 1)—phenotype SI for isolates CST, AOM, and FCP and phenotype NSI for isolates MDM, MCS, and JGA. The HIV-1 NL4-3 and BaL strains are included for comparison.
V3 region of the recovered virus were noted even 305 days after the first detection of syncytia in the untreated culture (i.e., 405 days postinfection).

DISCUSSION

HIV-1 strains isolated from infected individuals are predominantly MT and NSI and mainly use CCR5 as a co-receptor for entry into CD4⁺ T cells (R5 strains). Over the course of the infection, TT SI variants that use the CXCR4 co-receptor appear (X4 strains). Their emergence has been implicated in CD4⁺ T-cell decline, CD8⁺ T-cell apoptosis (22), specific irreversible effects on B-cell activity (19), and the onset of AIDS and disease progression (9, 19, 30, 36).

A major strategy in the fight against AIDS may consist in the prevention of the emergence of the more-pathogenic CXCR4-using strains of HIV. AMD3100 is a potent anti-HIV agent that is targeted at the CXCR4 receptor (15, 28). AMD3100 blocks the intracellular signal induced by SDF-1 but does not induce a signal by itself; thus, it can be considered an antagonist of CXCR4. Its great potency against TT HIV variants (the ratio between the 50% cytotoxic concentration and the 50% effective concentration is 100,000) makes it an ideal candidate to prevent the emergence of X4 strains.

FIG. 6. Replication of NSI strains in SUP-T1 cells in the presence of AMD3100. SUP-T1 cells permit the growth of the HIV-1 168.1. Replication of HIV-1 168.1 was sustained for up to 200 days without AMD3100 (empty circles) or 405 days in the presence of 1 μg of AMD3100/ml (filled circles). Syncytia were noted in the untreated sample after 100 days of culture but were not detected in the treated sample for 405 days.

FIG. 7. V3 loop amino acid sequence of the parental NSI strain (168.1/NSI phenotype) and that obtained from proviral DNA isolated from cells at 200 days (168.1/SI phenotype) and 260 days (168.1/AMD3100) after initiation of the experiment from untreated and AMD3100-treated cells. The amino acid sequence of HIV-1 168.10 strain (11) of SI phenotype is included for comparison. -, homology; ., amino acid deletion.

<table>
<thead>
<tr>
<th>HIV-1 strain</th>
<th>V3 genotype</th>
<th>Charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>168.1/NSI phenotype</td>
<td>CTRPNNTRKSIHGPGARYGATGDIIGDRC</td>
<td>3+</td>
</tr>
<tr>
<td>168.1/SI phenotype</td>
<td>____________________________</td>
<td>5+</td>
</tr>
<tr>
<td>168.1/AMD3100</td>
<td>____________________________</td>
<td>3+</td>
</tr>
<tr>
<td>168.10</td>
<td>____________________________</td>
<td>6-</td>
</tr>
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</table>
We have shown in this article that cultivation of a heterogeneous population of HIV, composed of a laboratory-adapted TT (NL4-3) strain and an MT (Bal) strain, in the presence of AMD3100 leads to the selection of the MT over the TT strain (Fig. 1). Even when the initial virus population consisted of only 1% Bal (and 99% NL4-3), Bal completely took over the population after 21 days of subcultivation in PHA-stimulated PBMC. HIV-infected individuals harbor a swarm of closely related viruses, the so-called HIV quasispecies, in which R5 and X4 strains may coexist. From our results, it can be surmised that under selective pressure against the CXCR4 receptor, only MT strains will continue to replicate. That is, in a heterogeneous population, as is the case of a viral population in an infected individual, the fitness of MT quasispecies will be greater than that of TT ones in the presence of AMD3100. Addition of AMD3100 to PBMC from individuals infected with viruses displaying the SI phenotype resulted in a complete block of the SI viruses. Recovered viruses showed reduced sensitivity to AMD3100 and could no longer induce syncytium formation in MT-2 cells. These viruses replicated in CCR5-transfected cells but not in CXCR4-transfected cells. Conversely, the NSI strains remained insensitive to AMD3100 and continued to replicate solely in CCR5-transfected cells. Phylogenetic analysis revealed a drastic change in the viral population upon AMD3100 treatment as predicted from the selection of MT R5 quasispecies. Surprisingly, the clinical isolate FCP (an SI strain) did not show significant changes in the V3 loop region after incubation with AMD3100. However, there were notable changes in the V2 loop coding region which led us to suspect that these changes are responsible for the phenotypic changes observed. The V2 loop genotype has also been associated with the SI-NSI phenotype and HIV tropism (23, 29). Nevertheless, our results clearly show genotypic and phenotypic changes in all treated clinical isolates.

The bicyclam AMD3100 is not active against MT strains of HIV-1 (28). Conversely, AMD3100 was equally active against dual-tropic viruses (which use CCR3, CCR5, CCR8, and CXCR4) (27) in PBMC. More recently, Zhang and Moore (37) have also reported that inhibition of a dual-tropic virus (R5-X4) was inhibited (although only partially) by AMD3100. These results suggest that selection with AMD3100 will favor the emergence of R5 strains over dual-tropic variants. Our results show that only those quasispecies that use CCR5 are allowed to survive while both X4 and R5X4 strains are selected out. That is, AMD3100 exerts selective pressure over both X4 (i.e., the AOM and FCP isolates) and R5-X4 (i.e., the CST isolate) strains. Furthermore, the clinical isolate CST represents a viral population comprising quasispecies that may use CCR5, CXCR4, or both. After selection with AMD3100, the CST isolate seems to replicate less efficiently in CCR5 cells. The replication of both R5 and R5X4 quasispecies may account for the relatively high p24 antigen production in U87 CD4 cells of the untreated CST isolate; however, after treatment with AMD3100, p24 antigen production reflects only the replication of the selected R5 quasispecies.

De Vreeze et al. (14) have developed a partially AMD3100-resistant HIV-1 strain that continued to replicate in MT-4 (CXCR4*) cells. This AMD3100-resistant strain was selected from a highly adapted laboratory strain (NL4-3) that deviates from the consensus sequence of primary clinical isolates. Furthermore, the AMD3100-resistant strain was selected in the lymphoid cell line (MT-4) that does not allow replication of MT strains. By slowly increasing the concentration of AMD3100 after subsequent passages, the parental NL4-3 strain accumulated an increasing number of mutations that finally rendered the virus resistant to AMD3100. Conversely, the present results indicate that in a system in which R5 strains are able to replicate at the expense of the X4 strains, the R5 strains take over the population, while the X4 strains vanish. Our results suggest that AMD3100 favors the selection of preexisting quasispecies without the need for ongoing mutations. Under the conditions used, passage of NL4-3 in PHA-stimulated PBMC in the presence of 1 μg of AMD3100/ml resulted in the “knockout” of the NL4-3 virus and proviral DNA-negative cells at 21 days after infection. We postulate that the treatment of AIDS patients with a CXCR4 antagonist may revert the SI-X4-TT phenotype to a less pathogenic phenotype. Suboptimal concentrations of AMD3100 would allow SI-X4 variants to escape the inhibitory activity of AMD3100; nevertheless, many mutations accumulating in the gp120 gene of AMD3100-resistant virus could indicate that resistance may not be easily acquired in vivo (14). Mirroring what happens during the course of infection (that is, that R5 strains evolve in some individuals into X4 or R5X4 [dual-tropic] HIV strains [36]), the phenotype of NSI slow-replicating HIV-1 converts to SI fast-replicating strains after prolonged culture in SUP-T1 cells (11, 12, 24). These viruses are able to efficiently replicate in transformed T-cell lines and to form syncytia when grown in MT-2 cells. HIV-1 isolates 168.1 (NSI) and 168.10 (SI) are sequential isolates obtained from the same asymptomatic individual by coculture of his peripheral blood lymphocytes (PBL) with healthy donor PBL (11, 24). In the T-cell line SUP-T1, the syncytium-inducing capacity of a chimeric HXB-2 virus containing only the V3 region from 168.1 or 168.10 accorded with the phenotype of HIV-1 isolates 168.1 (NSI) and 168.10 (SI) (12). Upon prolonged propagation in SUP-T1 cells, the NSI virus 168.1 tended to give rise to virus mutants with an SI and high replicative capacity. We have confirmed, by detection of mRNA by reverse transcriptase PCR (data not shown), that SUP-T1 cells express low but detectable levels of chemokine receptor CCR5 and high levels of CXCR4 (13), explaining why R5 strains, such as 168.1, can infect this cell line, albeit at a low rate of virus replication. In contrast, X4 strains easily infect and propagate in SUP-T1 cells. In this model, AMD3100 prevented the emergence of the SI phenotype and genotype that is observed in untreated infected cells despite slow but continuous viral replication. No CPE or syncytium formation was detected in the AMD3100-treated cells even after 305 days of the first detection of syncytia (405 days postinfection).

These results further support the notion that CXCR4 antagonism maintains the replication of NSI slowly replicating R5 strains while suppressing the replication of SI rapidly replicating X4 strains. We postulate that treatment of an HIV-positive asymptomatic individual with a CXCR4 antagonist would prolong the asymptomatic phase of its viral infection.

Recent studies by Tachibana et al. (31) and Zou et al. (38) have revealed that mice lacking CXCR4 or SDF-1 expression exhibit hematopoietic and cardiac defects, suggesting that CXCR4 and SDF-1 may play an important role in embryonic development and could have nonredundant functions in adults, thus raising some concerns about the use of CXCR4 antagonists as therapeutic agents against HIV. Furthermore, CXCR4-dependent migration to SDF-1 appears to be essential for human stem cell function in NOD-SCID mice (26). No toxicity was observed after administration of AMD3100 (10 mg/kg of body weight/day b.i.d.) to SCID-hu Thy/Liv mice for 28 days in spite of a significant decrease in HIV viral load in the infected mice (10). Low concentrations of a CXCR4 antagonist could be sufficient to prevent or delay X4 strain emergence without inducing an unwanted effect. Alternatively, other strategies, such as intrakine blockade of CXCR4 on targeted cells (7) or CD4-chemokine receptor pseudotypes...
(17), could selectively block the use of CXCR4 in T lymphocytes. Nevertheless, ongoing clinical trials with AMD3100 will have to demonstrate both its safety and efficacy as a chemotherapeutic agent against HIV and AIDS. CXCR4 antagonists could be intended as deterrents for the emergence of X4 strains, more than to decrease viral load levels, which can be effectively achieved by triple drug combinations of reverse transcriptase inhibitors and protease inhibitors (4). The concurrent observations that we have made with both laboratory HIV strains and clinical HIV isolates point to the potential usefulness of CXCR4 antagonists in preventing the switch from R5 to X4 that is generally considered a hallmark of the onset of AIDS and/or the progression of the disease. Our findings also suggest that CCR5-blocking agents might speed the evolution and outgrowth of more pathogenic HIV-1 variants that use CXCR4, thereby accelerating the course of disease. The ability of different HIV-1 strains to use coreceptors in addition to CCR5 or CXCR4 in vitro appears to be irrelevant to their drug sensitivity in primary cells (37). Combinations of both CCR5- and CXCR4-blocking agents could effectively inhibit HIV replication and prevent selection of X4 variants.

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