Suppression of Dualtropic Human Immunodeficiency Virus Type 1 by the CXCR4 Antagonist AMD3100 Is Associated with Efficiency of CXCR4 Use and Baseline Virus Composition[▽]

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In a phase I/II evaluation of the CXCR4 antagonist AMD3100, human immunodeficiency virus RNA levels were significantly reduced in a single study subject who harbored CXCR4 (X4)-tropic virus, but not in subjects who harbored either dual/mixed (DM)-tropic or CCR5 (R5)-tropic virus (C. W. Hendrix et al., *J. Acquir. Immune Defic. Syndr.* 37:1253–1262, 2004). In this study, we analyzed the envelope clones of DM-tropic virus in baseline and treated virus populations from 14 subjects. Ten subjects exhibited significant reductions in CXCR4-mediated infectivity after 10 days of AMD3100 therapy relative to baseline (X4 suppressor group), while four subjects had no reduction of CXCR4-mediated infectivity (X4 nonsuppressor group). The baseline viruses of the X4 suppressor group infected CXCR4-expressing cells less efficiently than those of the X4 nonsuppressor group. Clonal analysis indicated that the baseline viruses from the X4 suppressor group contained a higher proportion of R5-tropic variants mixed with CXCR4-using variants, while the X4 nonsuppressor group was enriched for CXCR4-using variants. AMD3100 suppressed X4-tropic variants in all subjects studied, but not all dualtropic variants. Furthermore, dualtropic variants that used CXCR4 efficiently were suppressed by AMD3100, while dualtropic variants that used CXCR4 poorly were not. This study demonstrated that AMD3100 has the ability to suppress both X4-tropic and certain dualtropic variants *in vivo*. The suppression of CXCR4-using variants by AMD3100 is dependent on both the tropism composition of the virus population and the efficiency of CXCR4 usage of individual variants.

Human immunodeficiency virus type 1 (HIV-1) envelope (*env*)-mediated entry is a multistep process, which requires a chemokine coreceptor (predominantly CCR5 or CXCR4) in addition to CD4 (3, 6). HIV-1 gp120 binds to CD4 on the cell surface, promoting a conformational change that allows gp120 to engage the coreceptor, which in turn triggers conformational changes in gp41 that lead to fusion of the viral and host membranes (1). Some viruses utilize both coreceptors and are characterized as dualtropic, while others use either CCR5 (R5 tropic) or CXCR4 (X4 tropic) exclusively. R5-tropic viruses are predominant at transmission and at early stages of infection, while most CXCR4-using (dualtropic and X4-tropic) viruses are present at later stages of disease (18, 21).

The switch from R5-tropic to CXCR4-using virus, first characterized as a switch from a non-syncytium-inducing to a syncytium-inducing phenotype in MT2 cells, occurs in approximately 50% of untreated HIV-1-infected individuals and is associated with decreased CD4⁺ T-cell counts and rapid disease progression (12, 13, 20). A number of recent studies have consistently demonstrated that the frequency of CXCR4-using viruses is approximately 20% in early infection (17) and increases to approximately 50% in late-stage disease (16; E. Coakley et al., presented at the 2nd International Workshop on Targeting HIV Entry, Boston, MA, 20 to 21 October 2006). The prevalence of CXCR4 use in antiretroviral treatment-

experienced subjects is 50%, or slightly higher, depending on the cohort studied (17; E. Coakley et al., presented at the 2nd International Workshop on Targeting HIV Entry; J. Demarest et al., presented at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 30 October to 2 November 2004). Consequently, there is an interest in the development of drugs that block CXCR4-mediated HIV-1 infection in addition to CCR5 inhibitors.

Agents that inhibit HIV-1 entry are widely studied. Enfuvirtide targets virus-cell membrane fusion and is approved for use in North America and Europe. Compounds that block the interaction of HIV-1 gp120 with either CD4 (11, 15) or the CCR5 or CXCR4 chemokine coreceptor (5, 9, 22) are being evaluated in several clinical trials. The phase III trial of maraviroc, a CCR5 antagonist, provides compelling evidence that this class of compounds can efficiently block R5-tropic HIV-1 infection (J. Lalezari et al., presented at the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, 2007 [abstr. 104bLB]; M. Nelson et al., presented at the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, 2007 [abstr. 104aLB]).

One of the most-well-characterized CXCR4 antagonists is the bicyclam molecule AMD3100 (2, 4, 7, 8). Further studies have shown that AMD3100 also inhibits dualtropic virus *in vitro* (19). In a phase I/II clinical trial, AMD3100 was shown to significantly reduce HIV RNA levels (0.9-log₁₀-unit reduction) in a single study subject who harbored pure X4-tropic virus but not in the remaining subjects who harbored either dual/mixed (DM)-tropic or R5-tropic viruses (9). In addition, AMD11070, another CXCR4 antagonist, was shown to reduce the fraction

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TABLE 1. Coreceptor tropism of virus population from DM subjects at baseline and day 11

Group ^a and subject	AMD3100 dose (μg/kg/h)	Tropism, day 11	RLU produced in:			
			CCR5 ⁺ cells		CXCR4 ⁺ cells	
			Baseline	Day 11	Baseline	Day 11
X4 suppressor						
74	80	R5	52,546	87,159	308	56
31	20	R5	114,206	316,970	575	107
9	5	R5	39,361	50,543	998	112
32	40	R5	46,180	140,449	1,904	107
28	20	R5	409,212	384,574	2,046	81
35	40	R5	34,494	153,409	2,443	56
73	80	R5	143,194	134,000	3,021	97
11	5	DM	224,031	256,901	13,710	199
10	5	R5	813,948	932,650	32,597	77
33	40	DM	241,910	170,994	48,606	505
Median (range)			128,670 (34,494–813,948)	162,201 (50,543–932,650)	2,244 (308–48,606)	102 (56–505)
X4 nonsuppressor						
70	40	DM	124,598	157,147	216,896	256,772
2	2.5	DM	272,689	346,774	342,258	496,095
21	10	DM	35,731	223,413	389,548	209,882
71	40	DM	720,638	851,150	1,647,981	1,130,617
Median (range)			198,644 (35,731–720,638)	285,093 (15,714–851,149)	365,903 (216,896–1,647,980)	376,433 (209,881–1,130,616)

^a Defined by changes in viral infectivity in CXCR4⁺ cells after AMD3100 treatment.

of CXCR4-using viruses in a phase I/II study (G. Moyle et al., presented at the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, 2007 [abstr. 511]).

DM-tropic virus populations can comprise either pure dualtropic variants or mixtures of R5-tropic, X4-tropic, and/or dualtropic variants. The effect of AMD3100 on X4-tropic and dualtropic variants in a DM-tropic virus population in vivo is not known. In this study, we evaluated the targeted antiviral activity of AMD3100 by characterizing the *env* clones of the baseline and treated virus populations in subjects with DM-tropic viruses at baseline. Both the tropism composition of the virus population (the relative proportions of R5-tropic, X4-tropic, and dualtropic clones) and the efficiency of CXCR4-mediated entry of individual variants were found to be associated with the ability of AMD3100 to suppress CXCR4-using variants in vivo.

MATERIALS AND METHODS

Study cohort. Forty HIV-1-positive individuals were enrolled in a phase I/II multicenter, open-label, dose-escalating study of AMD3100 administered as a 10-day intravenous infusion (9). Subjects were randomized across a range of doses (2.5, 5, 10, 20, 40, 80, and 160 μg/kg of body weight/h). The protocol was approved by the local institutional review board, and all subjects gave written informed consent prior to their participation in the study. The coreceptor tropism of baseline plasma viruses (drug screen or treatment initiation) and all available day 11 plasma viruses (completion of 10 days of AMD3100) was determined. Of the 40 subjects, 14 subjects who harbored DM-tropic viruses at baseline, and for whom paired samples taken at day 11 were available, were selected for this follow up study. Viral loads at baseline and at day 11 of treatment were measured using the Roche HIV-1 RNA Amplicor monitor assay.

Determination of HIV-1 coreceptor phenotype. Coreceptor tropism was measured using the Trofile assay (Monogram Biosciences) (24). Specifically, a replication-defective retroviral vector containing a luciferase gene was used to co-transfect human embryonic kidney 293 cell cultures (AIDS Research and Reference Reagent Program, NIH) along with *env* expression vectors containing patient-derived viral envelope sequences (24). Pseudotyped viruses were harvested 2 days after transfection and were assessed for their ability to infect U87 cells expressing CD4 and either CCR5 or CXCR4 (provided by Nathaniel Landau) (24). Viruses were classified as R5 tropic, X4 tropic, or DM tropic based

on two criteria: (i) the production of luciferase activity (expressed in relative light units [RLU]) in U87 CD4 CCR5 and U87 CD4 CXCR4 cells and (ii) the specific inhibition of luciferase activity by a CCR5 antagonist [a member of the 4-(piperidin-1-yl)butane family, provided by Merck] or a CXCR4 antagonist (AMD3100, provided by AnorMED) (24).

Clonal analysis of viral populations. Forty-eight *env* clones were isolated from each viral population and screened for their ability to mediate pseudoviral infection of U87 cells expressing CD4 and either CCR5 or CXCR4 (23). The coreceptor tropism of a subset of viable clones from selected subjects was confirmed using the standard Trofile assay (24). The sequences of the gp160 *env* regions of these clones were determined using standard dye-deoxy chain terminator chemistry (ABI, Foster City, CA).

Phylogenetic analysis of *env* clones. Two subjects (subjects 33 and 35) had baseline (day 0), on-treatment (day 11), and posttreatment (days 18 and 39) samples available. Ten to 15 clones from each time point were sequenced, and phylogenetic analysis of gp160 *env* nucleotide sequences was performed using neighbor-joining methods (14) and bootstrap resampling (1,000 replicates). For all phylogenetic trees shown in the study, coreceptor tropism designations of *env* clones were assigned based on the results of the Trofile assay (24). The amino acid sequences of the V3 loop regions of these clones and their correlation with coreceptor tropism before and after AMD3100 treatment were also compared.

Statistical analyses. The Wilcoxon signed-rank test was used to compare viral infectivities (measured as RLU) in the CXCR4⁺ and CCR5⁺ cells of X4 suppressor and X4 nonsuppressor groups at baseline and day 11. All analyses were performed using Prism, version 4.03 (GraphPad, San Diego CA).

Nucleotide sequence accession numbers. The gp160 *env* nucleotide sequences obtained in this study have been submitted to GenBank under accession numbers EU604549 to EU604642.

RESULTS

Reduction of viral infectivity by AMD3100 is correlated with CXCR4 usage of DM-tropic viruses. The coreceptor tropism of viruses isolated from 14 subjects at baseline and after 10 days of AMD3100 treatment (day 11) was determined using the Trofile assay (24). Under AMD3100 drug pressure, DM-tropic virus populations in some subjects converted to R5 tropism while others remained DM tropic (Table 1). The baseline viruses displayed a broader range of infectivity in CXCR4-expressing cells than in CCR5-expressing cells, as measured by

TABLE 2. *env* clonal composition of DM subjects at baseline and day 11

Group ^a and subject	Tropism (<i>env</i> pools), day 11	% of clones that were:					
		R5 tropic		X4 tropic		Dualtropic	
		Baseline	Day 11	Baseline	Day 11	Baseline	Day 11
X4 suppressor							
74	R5	95	100	0	0	5	0
31	R5	92	100	4	0	4	0
9	R5	93	98	0	0	7	2
32	R5	80	94	0	0	20	6
28	R5	72	100	14	0	14	0
35	R5	61	100	39	0	0	0
73	R5	82	100	3	0	15	0
11	DM	52	97	0	0	48	3
10	R5	65	98	15	0	20	2
33	DM	57	85	8	0	35	15
X4 nonsuppressor							
70	DM	0	0	0	0	100	100
2	DM	8	10	0	0	92	90
21	DM	0	0	58	0	42	100
71	DM	0	0	0	0	100	100

^a Defined by changes in viral infectivity in CXCR4⁺ cells after AMD3100 treatment.

luciferase activity (RLU). Based on infectivity in CXCR4-expressing cells before and after AMD3100 treatment, we were able to classify the 14 subjects into two groups: X4 suppressors (10 subjects) and X4 nonsuppressors (4 subjects). These two groups differed in the ability of viruses to infect CXCR4-expressing cells at baseline. The infectivity of baseline viruses in CXCR4-expressing cells ranged from 306 to 48,606 RLU for X4 suppressors, compared to 216,896 to 1,647,981 RLU for X4 nonsuppressors. In contrast, the infectivity of baseline viruses in CCR5-expressing cells was similar for X4 suppressors (range, 34,494 to 813,948 RLU) and X4 nonsuppressors (range, 35,731 to 720,638 RLU). Following 10 days of AMD3100 therapy, 8 of 10 subjects classified as X4 suppressors had virus populations that became R5 tropic, and the other 2 remained DM tropic but displayed a >50-fold decrease in infectivity in CXCR4-expressing cells. In contrast, none of the four subjects classified as X4 nonsuppressors had virus populations showing reductions in infectivity in CXCR4-expressing cells after 10 days of AMD3100 therapy. Comparison of luciferase activities generated from baseline and day 11 virus samples showed reductions in viral infectivity on CXCR4 target cells for the 10 subjects classified as X4 suppressors ($P = 0.002$ by the Wilcoxon signed-rank test) but not for X4 nonsuppressors ($P = 0.625$). Viral infectivity in CCR5 target cells did not differ between the two groups ($P > 0.08$). No dose-related difference between the two groups was observed, since subjects on low and high doses were found in both groups (Table 1).

The composition of the DM-tropic virus population affects the antiviral activity of AMD3100 in vivo. To further explore whether the difference in the responses of DM-tropic viruses to AMD3100 treatment was due to different types of CXCR4-using variants (for example, X4 tropic or dualtropic) in the DM-tropic virus populations, individual *env* clones were isolated from baseline and treated samples of all 14 DM subjects, and the ability of individual clones to infect CCR5 or CXCR4 target cells was assessed. For each subject, approximately 20 to

38 functional clones were analyzed, and the percentage of *env* clones exhibiting R5, X4, or dual tropism in the virus population was determined (Table 2). All baseline samples from the 10 subjects in the X4 suppressor group contained R5-tropic clones mixed with X4-tropic and/or dualtropic clones. In contrast, only one of four baseline samples from the X4 nonsuppressor group had a mixture of R5-tropic and dualtropic clones. The remaining baseline X4 nonsuppressor samples contained only dualtropic clones ($n = 2$) or a mixture of dualtropic and X4-tropic clones ($n = 1$). Baseline virus populations in the X4 suppressor group tended to have more R5-tropic clones (range, 52 to 95%) and fewer CXCR4-using clones (range, 5 to 48%). In contrast, those in the X4 nonsuppressor group had markedly fewer R5-tropic clones (range, 0 to 8%) and more CXCR4-using clones (range, 92 to 100%). Thus, the proportion of CXCR4-using clones isolated from the virus populations at baseline differed greatly between these two groups, with predominantly dualtropic variants in X4 nonsuppressors.

Following 10 days of therapy with AMD3100, CXCR4-using clones in the virus populations from the X4 suppressor group at baseline were either undetectable or their numbers were greatly reduced, and there was a concomitant increase in the proportion of R5-tropic clones. In contrast, the relative proportions of CXCR4-using clones (predominantly dualtropic clones) and R5-tropic clones in X4 nonsuppressors showed no change from those at baseline. Interestingly, the X4-tropic clones identified in the baseline samples of seven subjects were suppressed by AMD3100, regardless of whether they were present in X4 suppressors or X4 nonsuppressors. However, the ability of AMD3100 to suppress dualtropic clones differed between the two groups. For all 10 subjects in the X4 suppressor group, disappearance of dualtropic clones or a reduction in their proportion was observed at day 11. Conversely, for all four subjects in the X4 nonsuppressor group, no reduction in the relative proportion of dualtropic clones at day 11 was found. Furthermore, only X4-tropic clones, not dualtropic

FIG. 1. Analysis of tropism of viruses from subjects 35 and 33 before, during, and after treatment with AMD3100. (A and C) The coreceptor tropism of the virus population at baseline and on days 11, 18, and 39 was determined using Trofile. Infectivity in CD4⁺ CCR5⁺ (open bars) and CD4⁺ CXCR4⁺ (solid bars) U87 cells, as measured by luciferase activity (RLU) in a single measurement, is shown. (B and D) Proportions of clones with the indicated tropisms (R5, X4, dual-X, and dual-R), represented as percentages of the total number of functional clones for each time point, are shown. Open bars, R5-tropic clones; solid bars, X4-tropic clones; shaded bars, dual-X-tropic clones; hatched bars, dual-R-tropic clones.

clones, were suppressed at day 11 in a single subject, subject 21, who had a mixture of X4-tropic and dualtropic clones at baseline.

Taken together, these data show that AMD3100 can suppress X4-tropic variants in all subjects tested here and that the ability to suppress dualtropic variants in virus populations was associated with the relative proportions of R5-tropic and dualtropic variants present in baseline virus populations.

Reemergence of CXCR4-using viruses after AMD3100 treatment. Two subjects (subjects 33 and 35) in the X4 suppressor group who received 40 $\mu\text{g/kg/h}$ of AMD3100 had additional posttreatment plasma samples available (days 18 and 39) for coreceptor tropism analysis of *env* populations and clones. The virus population of subject 35 comprised R5-tropic (39%) and X4-tropic (61%) clones at baseline, R5-tropic clones only at days 11 and 18, and a mixture of R5-tropic (63%) and X4-tropic (37%) clones at day 39 (Fig. 1A and B). The virus population of subject 33 comprised R5-tropic (57%), X4-tropic (8%), and dualtropic (35%) clones at baseline. On days 11 and 18, the infectivity of the virus population in CXCR4-expressing cells was reduced more than 50-fold from that at baseline, and no X4-tropic clones were detected. The percentages of R5-

tropic clones increased to 85% and 92%, and those of dualtropic clones decreased to 15% and 8%, respectively, at days 11 and 18. On day 39, the infectivity of the virus population in CXCR4-expressing cells was comparable to that of the baseline sample. The proportion of dualtropic clones increased to 55%, and that of R5-tropic clones decreased to 45% (Fig. 1C and D).

The suppression of dualtropic variants in a population is associated with the infectivity of individual variants in CXCR4 target cells. It should be noted that in subject 33, all dualtropic clones detected at day 11 were phenotypically distinct from the majority of dualtropic clones detected in the paired baseline sample. We have previously described the existence of dualtropic viruses that have differing abilities to use CXCR4 and CCR5. Dual-X-tropic viruses use CXCR4 efficiently and have V3 sequences distinct from those of R5-tropic viruses from the same patient (X4-like), while dual-R-tropic viruses use CXCR4 poorly and have V3 sequences that are similar or identical to those of R5-tropic viruses from the same patient (R5-like) (10). In addition to the presence of R5-tropic clones at all time points, subject 33 had a baseline virus population composed of X4-tropic (8%), dual-X-tropic (30%), and dual-

TABLE 3. Coreceptor usage and V3 sequence analysis of *env* clones from subjects 33 and 35 at baseline and days 11, 18, and 39

Subject and time point	No. of clones	Tropism	Avg RLU		V3 loop amino acid sequence
			R5	X4	
Subject 35	Baseline	R5	469,080	84	CTRPSNNTRKSINMGPGRAFYTGTGEIIGDIRQAH
		R5	366,742	113	N.....
		X4	82	355,190	H...RVTL...S.VY.....T...R...
		X4	71	388,674	N.H...RVTL...S.VY.....T...R...
	Day 11	R5	201,554	72	
	Day 18	R5	179,136	79	
	Day 39	R5	248,231	85	
		X4	75	252,333	H...RVTL...S.VY.....T...R...
	Subject 33	R5	1,016,370	104	CTRPNNNTRKSINIGPGRAWYATGQIIGDIRQAYC
		Dual-R	1,858,036	307	
		Dual-X	305,294	1,730,180	RVTM...KV...T.....K...
		X4	111	1,679,250	RVTM...KV.....G.....
	Day 11	R5	1,097,747	110	
		Dual-R	1,310,893	1,368	
	Day 18	R5	1,856,059	172	
		Dual-R	2,278,396	258	
		Dual-X	97,013	1,345,964	RVTM...KV...T.....K...
	Day 39	R5	1,487,627	114	
		Dual-X	302,686	1,899,393	RVTM...KV...T.....K...
		Dual-X	739,163	170,982	GVTM...KV...T.....K...

R-tropic (5%) clones. Under drug pressure, only dual-R-tropic clones (15%), not X4-tropic or dual-X-tropic clones, were detected at day 11. The dual-X-tropic clones reemerged following cessation of AMD3100 treatment: 4% dual-X-tropic clones were detected at day 18, and this proportion increased to 55% by day 39 (Fig. 1D). Consistent with our previous observations, the average luciferase readout in CXCR4-expressing cells was 10⁶ RLU for dual-X-tropic clones and 10³ RLU for dual-R-tropic clones (Table 3). Dual-R-tropic clones were also observed in one other X4 suppressor, subject 10 (Fig. 2). In this

subject, the baseline virus population comprised R5-tropic (65%), X4-tropic (15%), and dual-X-tropic (20%) clones. The virus population at day 11 was essentially R5 tropic, with 2% dual-R-tropic clones, but no dual-X-tropic or X4-tropic clones were identified. Although limited and preliminary, the data from these two cases suggest that AMD3100 can suppress X4-tropic and dual-X-tropic variants but not dual-R-tropic variants.

Phylogenetic analysis of gp160 and V3 sequences. Ten to 15 gp160 *env* clones obtained from subjects 33 and 35 at each time

FIG. 2. Analysis of tropism of viruses from subject 10 before and after treatment with AMD3100. (A) The coreceptor tropism of the virus population at baseline and day 11 was determined using Trofile. Infectivity in CD4⁺ CCR5⁺ (open bars) and CD4⁺ CXCR4⁺ (solid bars) U87 cells, as measured by luciferase activity (RLU) in a single measurement, is shown. (B) Proportions of clones with the indicated tropisms (R5, X4, dual-X, and dual-R), represented as percentages of the total number of functional clones isolated at each time point, are shown. Open bars, R5-tropic clones; solid bars, X4-tropic clones; shaded bars, dual-X-tropic clones; hatched bars, dual-R-tropic clones.

FIG. 3. Phylogenetic analysis of gp160 *env* clones isolated at baseline and on days 11, 18, and 39 for subjects 35 and 33. Phylogenetic trees show the association of the time point with the coreceptor tropism at baseline and on days 11, 18, and 39 for subjects 35 (A) and 33 (B). Colored lines represent *env* clones isolated at baseline (pink), day 11 (gold), day 18 (green), and day 39 (blue). Clones with X4 or dual-X tropism are shown within dashed circles. Asterisks indicate clones with dual-R tropism.

point were sequenced, and phylogenetic analysis was performed (Fig. 3). X4-tropic and dual-X-tropic clones clustered together and were distinct from R5-tropic clones from the same subject, regardless of drug exposure. This suggests that X4-tropic and dual-X-tropic clones are genetically distinct from R5-tropic clones and that reemerging CXCR4-using clones, following treatment with AMD3100, were similar to CXCR4-using clones present at baseline. The dual-R-tropic clones that were not suppressed by AMD3100 in subject 33 clustered more closely with R5-tropic clones than with dual-X-tropic or X4-tropic clones. Furthermore, the V3 sequences of the dual-R-tropic clones were indistinguishable from those of R5-tropic clones (Table 3). In addition, the V3 loop amino acid sequences of X4-tropic or dual-X-tropic clones that re-emerged posttreatment were the same as those isolated at baseline in both subjects, as were the V3 loop amino acid sequences of R5-tropic clones isolated pre- and post-drug exposure (Table 3).

DISCUSSION

In this study, we retrospectively analyzed the coreceptor tropism of 14 DM-tropic subjects enrolled in a phase I/II study of the CXCR4 antagonist AMD3100. Other subjects enrolled in this phase I/II study harbored X4- or R5-tropic viruses at baseline and did not demonstrate tropism changes following AMD3100 treatment. Following 10 days of AMD3100 therapy, DM-tropic viruses from 10 subjects either became R5-tropic or exhibited a reduction of infectivity (>50-fold) in CXCR4-expressing cells (X4 suppressors), while the viruses from 4 subjects remained DM-tropic and showed no reduction of infectivity in CXCR4-expressing cells (X4 nonsuppressors). The X4 suppressors also displayed lower levels of infectivity in CXCR4-expressing cells at baseline than the X4 nonsuppressors. *env* clonal analysis indicated that X4 suppressors had a much higher proportion of R5-tropic clones and a lower proportion of dualtropic clones than X4 nonsuppressors.

Following 10 days of AMD3100 treatment, all X4-tropic clones were suppressed in the virus populations studied, regardless of X4 suppressor or nonsuppressor classification. However, differences in the extent of suppression of dualtropic clones were observed. We found that AMD3100 suppresses dualtropic viruses when the virus population is predominantly R5-tropic. We hypothesize that in the absence of an R5-tropic virus population, dualtropic viruses escape AMD3100 suppression by using the CCR5 coreceptor (X4 nonsuppressors). Conversely, in mixed populations where R5-tropic viruses predominate, dualtropic viruses may compete less well than R5-tropic viruses for the CCR5 coreceptor and consequently are effectively suppressed by AMD3100 (X4 suppressors).

In addition to the composition of the virus population, the suppression of dualtropic variants is also dependent on the type of dualtropic variant present. We have previously described the existence of two distinct types of dualtropic viruses that differ in their ability to use the CXCR4 and CCR5 coreceptors in vitro (10). The preferential use of CCR5 (dual-R) or the ability to utilize both coreceptors efficiently (dual-X) could affect the efficacy of CCR5 and CXCR4 coreceptor inhibitors. The present study demonstrates that dual-X-tropic variants, which efficiently mediated infection in both CXCR4- and CCR5-expressing cells in vitro, could be suppressed by AMD3100 in X4 suppressors for whom R5-tropic variants predominated, while dual-R-tropic variants, which use CCR5 efficiently and CXCR4 inefficiently, could not. We hypothesize that dual-R-tropic viruses preferentially use CCR5, while dual-X-tropic viruses use both CXCR4 and CCR5 efficiently but are unable to compete effectively with R5-tropic viruses in the presence of CXCR4 drug pressure. Further studies are under way to investigate the relationship between coreceptor usage by different dualtropic variants and inhibition of these variants by CXCR4 and CCR5 inhibitors.

Using currently available technologies, it is impossible to define the composition of a virus population without perform-

ing clonal analysis. DM-tropic virus populations that exhibit low levels of infectivity in CXCR4 target cells may contain either a minor virus population that uses CXCR4 efficiently (X4 or dual-X tropic) or many dualtropic variants that use CXCR4 poorly (dual-R tropic). Thus, it cannot be assumed that virus populations with low levels of infectivity in CXCR4-expressing cells harbor CXCR4-using viruses that will respond to a CXCR4 antagonist.

From this study it appears that suppression of CXCR4-using variants may or may not be associated with an overall reduction in viral load. In this trial, a single subject with an X4-tropic virus did exhibit a 0.9-log₁₀-unit reduction in viral load after 10 days of treatment with AMD3100 (9). However, no significant reductions in viral load were observed in any of the 14 subjects with DM-tropic virus, including subjects with undetectable levels of CXCR4-using variants at day 11. In X4 nonsuppressors, the majority of the virus populations comprised dualtropic variants that most likely were able to use CCR5 when under CXCR4 antagonist pressure, and thus, viral loads were not suppressed by AMD3100. In X4 suppressors, the suppression of CXCR4-using variants (dualtropic and/or X4 tropic) may have been accompanied by an increase in the proportion of R5-tropic variants, which could explain the lack of a measurable viral load reduction. However, if the population of R5-tropic variants remains unchanged, the lack of a measurable reduction in viral load may simply reflect the small proportion of CXCR4-using viruses in the population. For example, in order to realize a 0.3-log₁₀-unit reduction in viral load, 50% of the virus population would need to be suppressed.

Regardless of whether or not viral load reductions are observed, successful suppression of CXCR4-using variants by a CXCR4 inhibitor may be an important determinant of clinical outcome. In addition, CXCR4 utilization may serve as a useful screening criterion for enrollment in clinical studies of CXCR4 inhibitors. One could envision enrolling subjects with baseline virus populations comprising CXCR4-using variants (X4 tropic or dualtropic) into trials involving an inhibitor of CXCR4, and using the reduction of CXCR4-using variants as a measure of response to inhibitor treatment (G. Moyle et al., presented at the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles, CA, 2007 [abstr. 511]). This approach could be used to determine whether CXCR4-using variants are effectively suppressed by treatment, independent of a reduction in viral load or the complete suppression of infectivity in CXCR4-expressing cells.

In this study, dual-R-tropic variants that use CCR5 efficiently and CXCR4 inefficiently were not suppressed by AMD3100. It is possible that subjects with this type of dual-tropic variant may benefit from treatment with a CCR5 inhibitor. In this regard, a similar analysis of subjects with dualtropic viruses receiving a CCR5 antagonist would be highly informative (such as the phase 2b study of maraviroc for patients infected with non-CCR5-tropic HIV-1 [H. Mayer et al., presented at the XVI International AIDS Conference, Toronto, Ontario, Canada, 13 to 18 August 2006 {abstr. THLB0215}]). Further analyses of the coreceptor tropism of HIV-1 variants before and after treatment with coreceptor inhibitors are likely to provide valuable information that will advance our understanding of this important drug class and its clinical utility.

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