UvA-DARE (Digital Academic Repository)

Decreasing sensitivity to RANTES (regulated on activation, normally T cell-expressed and -secreted) neutralization of CC chemokine receptor 5-using, non-syncytium-inducing virus variants in the course of human immunodeficiency virus type 1 infection

Koning, F.A.; Kwa, D.; Boeser-Nunnink, B.; Dekker, J.; Vingerhoed, J.; Hiemstra, H.; Schuitemaker, H.

[Link to publication](#)

Decreasing Sensitivity to RANTES (Regulated on Activation, Normally T Cell–Expressed and –Secreted) Neutralization of CC Chemokine Receptor 5–Using, Non–Syncytium-Inducing Virus Variants in the Course of Human Immunodeficiency Virus Type 1 Infection

Fransje A. Koning, David Kwa, Brigitte Boeser-Nunnink, Jos Dekker, Jose Vingerhoed, Harry Hiemstra,[a] and Hanneke Schuitemaker

Department of Clinical Viro Immunology, Sanquin Research at CLB, and Landsteiner Laboratory of the Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

In approximately half of human immunodeficiency virus (HIV) type 1–infected individuals, the development of CXC chemokine receptor 4–using, syncytium-inducing (SI) virus variants precedes a rapid progression to acquired immunodeficiency syndrome (AIDS). In other individuals, only CC chemokine receptor 5–using (R5), non-SI (NSI) virus variants are present throughout infection. These individuals may be either long-term survivors (LTSs) or rapid progressors. The basis for this variable disease progression in individuals with only R5 virus variants is not yet fully understood. In this study, the β-chemokine sensitivity of biological HIV-1 clones isolated from 13 individuals who harbored only R5, NSI virus variants (7 LTSs and 6 progressors) was investigated. We found a statistically significant decrease in sensitivity of virus variants to RANTES (regulated on activation, normally T cell–expressed and –secreted) neutralization during the course of progressive infection, but not during follow-up of LTSs. Our data suggest that a role exists for RANTES neutralization sensitivity of HIV-1 in AIDS pathogenesis.

For entry of human immunodeficiency virus (HIV) type 1 into a target cell, a coreceptor is necessary, in addition to the principal receptor CD4. The 2 major coreceptors for HIV-1 are CCR5 and CXCR4 [1, 2]. In general, HIV-1 infection is established by macrophag-

The Journal of Infectious Diseases 2003;188:864–72

etropic, CCR5-using (R5), non–syncytium-inducing (NSI) virus variants. In approximately half of HIV-1–infected individuals, progression to AIDS is preceded by the development of CXCR4-using (X4), syncytium-inducing (SI) HIV-1 variants [3]. This expanded coreceptor usage, however, is not a prerequisite for disease progression [4, 5], because many infected individuals progress to AIDS in the presence of only R5, NSI virus variants [3, 6, 7]. Although long-term, nonprogressive HIV-1 infection is associated with the presence of only R5 virus variants [8, 9], some individuals with only R5 HIV-1 variants progress to AIDS rapidly. It was found that these rapid progressors with R5, NSI virus variants had a higher virus load in vivo and that their viruses showed a higher replication rate and increased cytopathicity in vitro, compared with those of long-term survivors (LTSs) [10].

Because the β-chemokines RANTES, macrophage in-

Patient	HIV-1 status at study entry	Diagnosis[a] (months after seroconversion or entry)	Genotype CCR5	CCR2b
68	+	AS (151)	wt	wt
441	+	AS (152)	wt	wt
583	+	AS (149)	wt	wt
16	−	AS (143)	Δ32/wt	wt
78	+	AS (124)	Δ32/wt	wt
337	+	AS (142)	Δ32/wt	64l/wt
434	−	AS (140)	Δ32/wt	wt
53	+	PCP (76)	wt	wt
142	+	KS (109)	wt	wt
424	−	EC (38)	wt	wt
38	+	KS (101)	Δ32/wt	wt
172	−	KS (25)	Δ32/wt	wt
617	+	NHL (136)	Δ32/wt	wt

NOTE. 64l/wt, CCR2b 64l heterozygote; AS, asymptomatic; Δ32/wt, CCR5 Δ32 heterozygote; EC, esophageal candidiasis; HIV-1, human immunodeficiency virus type 1; KS, Kaposi sarcoma; NHL, non-Hodgkin lymphoma; PCP, *Pneumocystis carinii* pneumonia; wt, CCR5 or CCR2b homozygous wild-type genotype; +, positive; −, negative.

[a] Diagnosis by the end of follow-up. The end of follow-up is defined as April 1997 or start of therapy, for long-term survivors, and the moment of AIDS diagnosis, for progressors.

flammatory protein (MIP)–1α, and MIP-1β down-regulate CCR5 expression and reduce HIV-1 CCR5 usage [11–13], one might assume that a role exists for β-chemokines in the pathogenesis of AIDS and in vivo evolution of HIV-1. In HIV-1–infected individuals, the expression of β-chemokines is strongly enhanced in the lymph nodes [14]. Furthermore, CD4+ cells from exposed, uninfected individuals showed a high level of β-chemokine production, compared with those from control subjects [15, 16], and β-chemokine production in HIV-positive individuals without AIDS was found to be higher than that in individuals who had progressed to AIDS [17]. Moreover, in nonprogressors, both CD8+ and CD4+ cells produced β-chemokines, whereas, in patients with AIDS, only the CD8+ cells did [18]. These results suggest that a high β-chemokine production of CD4+ cells might confer protection against both HIV-1 infection and progression to AIDS.

We hypothesized that, in addition to the level of β-chemokine production in the host, the sensitivity of the virus to β-chemokine neutralization might also influence disease progression. If β-chemokines exert a selective pressure in vivo favoring the emergence of variants that are insensitive to their control, one might consider SI variants as escape variants. A decrease in sensitivity of the virus to these β-chemokines during progression to AIDS may also occur in individuals who do not develop SI variants. Therefore, we compared primary R5 HIV-

1 variants, which were isolated at a relatively early and a late moment in infection from progressors and LTSs, for their sensitivity to β-chemokine neutralization.

SUBJECTS, MATERIALS, AND METHODS

Subjects. From the Amsterdam Cohort of Homosexual Men (ACH), 13 participants who harbored only NSI HIV-1 variants for the entire duration of follow-up were selected [5]. All patients gave written, informed consent before entering the original studies and gave consent for storing specimens for later studies of any type. This work was performed as part of the Amsterdam Cohort Studies on HIV infection and AIDS, a collaboration between the Municipal Health Service, the Academic Medical Center, and Sanquin Research at CLB (Amsterdam). By April 1997, 7 of these individuals had been asymptomatic and seropositive for at least 11 years, with relatively stable CD4+ T cell counts, in the absence of antiretroviral therapy. These individuals were classified as LTSs: ACH 16, 68, 78, 337, 434, 441, and 583 (mean duration of seropositive follow-up, 142 months; range, 136–146 months); 3 individuals were classified as rapid progressors: ACH 53, 172, and 424 (AIDS diagnosis after 25–76 months of follow-up); 2 individuals were classified as typical progressors: ACH 38 and 142 (AIDS diagnosis after 99 and 109 months of follow-up, respectively); and 1 individual was classified as a slow progressor: ACH 617 (AIDS diagnosis after 136 months of follow-up). Analysis of CD4+ T cell counts, quantification of serum HIV-1 RNA load, and CCR5 and CCR2b genotyping of the individuals were performed previously [5] (figure 1 and table 1).

Virus isolation, SI phenotyping, and characterization of coreceptor usage. Biological virus clones were isolated from patients' peripheral blood mononuclear cell (PBMC) samples obtained at ≥2 time points [5]. One time point was chosen early in the course of follow-up (mean, 22 and 17 months for LTSs and progressors, respectively), and the other was chosen as late as possible in the course of follow-up, for LTSs (mean, 113 months), or around the time of AIDS diagnosis, for progressors (mean, 77 months). At least 2 biological virus clones/time point were tested for sensitivity to neutralization by β-chemokines.

SI phenotyping was performed on the MT2 cell line. Characterization of coreceptor usage was performed on astroglioma cell lines stably transfected with CD4 and either CCR3, CXCR4, or CCR5 and specified on Δ32 homozygous PBMCs [5]. Cell-free virus stocks were grown on phytohemagglutinin (PHA)–stimulated donor PBMCs and were preserved at −70°C.

β-chemokine neutralization assay. PBMCs from 10 uninfected, CCR5 Δ32 wild-type, healthy blood donors were isolated, pooled, and cryopreserved. All experiments, including titration of virus stocks, were performed on this stock of cryo-
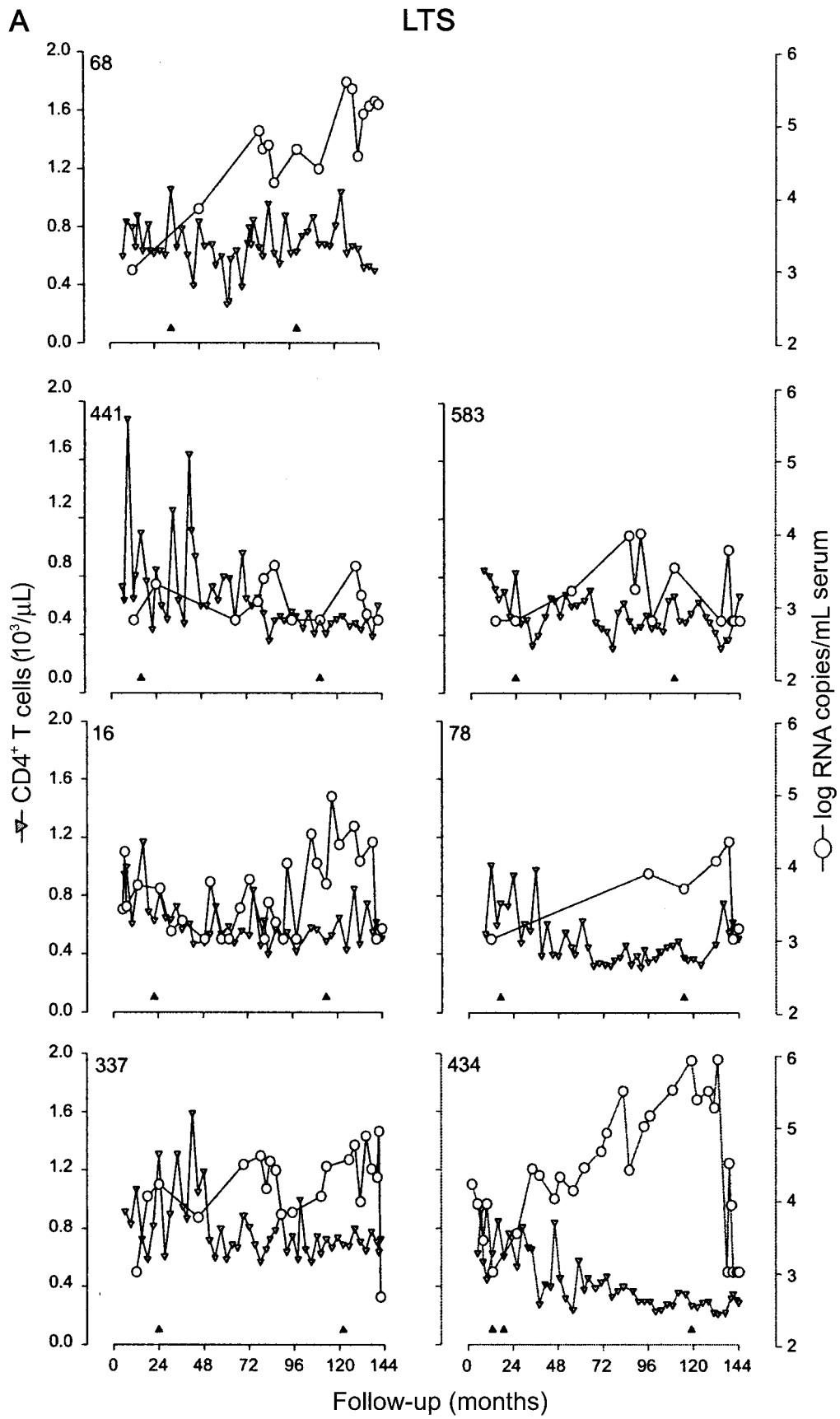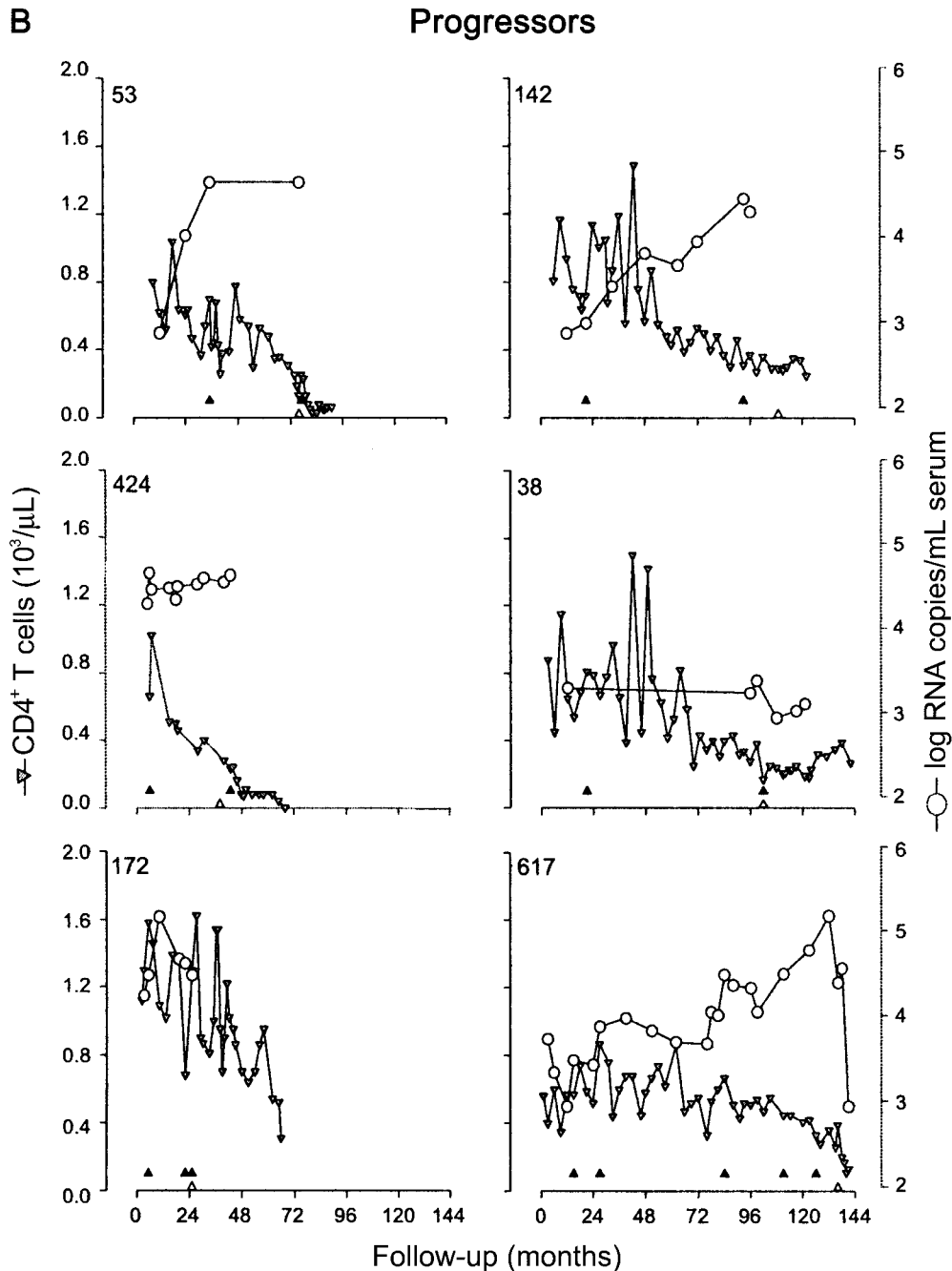
A

LTS

Figure 1.

Figure 1 *(continued).* Longitudinal analysis of CD4+ T cell counts and virus load of long term survivors (LTSs) *(A)* and progressors *(B)*. Patient nos. are indicated in the upper left corner of each graph. CD4+ T cell counts (cells ×10³/µL) are indicated by triangles (left *Y*-axis), and human immunodeficiency virus (HIV) RNA loads are indicated by open circles (right *Y*-axis). Filled arrowheads on the *X*-axis indicate time points of clonal isolation of HIV-1 variants that were analyzed for β-chemokine neutralization sensitivity. Open arrowheads indicate the time point of AIDS diagnosis. Follow-up is indicated in months after HIV-1 seroconversion or seropositive entry in cohort studies. Figure is adapted from De Roda Husman et al. [5].

preserved, healthy-donor PBMC mixture, to eliminate possible variations caused by differences in infectability of PBMCs.

Neutralization assays were performed as follows. Pooled donor PBMCs, stimulated for 3 days with PHA, were depleted of CD8+ cells by use of magnetic beads (MACS; Miltenyi Biotec), according to the manufacturer's protocol, and then were preincubated for 3 h at 37°C with 2-fold serial dilutions of either RANTES, MIP1-α, or MIP1-β (PeproTech) or a 1:1 :1 mixture of these 3 β-chemokines with the same total concentration (hereafter referred to as "Mix"; concentrations used
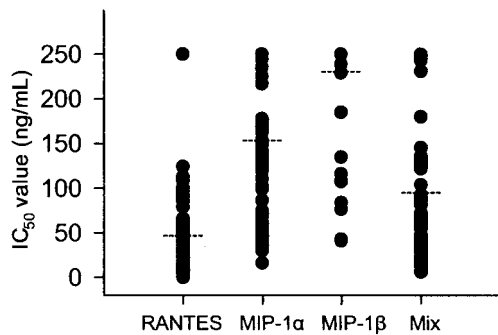
Figure 2. Potency of inhibition of R5 human immunodeficiency virus type 1 replication by β-chemokines. Each dot represents the IC_{50} value of an individual biological human immunodeficiency virus clone of the given β-chemokine (X-axis). The mean IC_{50} value of each β-chemokine is depicted by a horizontal line. MIP, macrophage inflammatory protein; Mix, 1:1:1 mixture of the 3 β-chemokines.

were 8, 16, 32, 63, 125, and 250 ng/mL). Cells were then inoculated in 96-well plates (10^5 cells/well) with 20 $TCID_{50}$ of a virus isolate (final volume, 200 μL/well) and were incubated overnight at 37°C. Supernatant was removed, and cells were resuspended in fresh medium with appropriate β-chemokine concentrations. Production of p24 was measured in supernatant samples taken at days 7, 10, and 14 after infection, using an in-house p24-antigen capture ELISA [19]. p24 antigen levels from cultures inoculated in the absence of β-chemokines were designated as maximum virus production, and the ratios of p24 production in β-chemokine–containing cultures were calculated relative to these maximum values. Supernatant from cells that were not incubated with either β-chemokines or virus was used for background values of the p24 ELISA. All measurements were performed in triplicate.

Determination of IC_{50} and IC_{90}. The β-chemokine concentrations causing 50% and 90% reduction in p24 antigen production (IC_{50} and IC_{90}) 14 days after infection were determined by a 4-parametric logistic analysis [20]. If the appropriate degree of inhibition was not achieved at the highest β-chemokine concentration, a value >250 ng/mL was recorded, and, in figures and statistical tests, we assumed these to be equal to 250 ng/mL.

Statistical analysis. The Mann-Whitney U test was used to compare unpaired groups. Wilcoxon signed rank test was used to compare IC_{50} and IC_{90} values per individual over time. Statistical analyses were performed using SPSS software (version 10.0; SPSS).

RESULTS

Biological HIV-1 clones from 13 individuals with only R5, NSI virus variants (7 LTSs and 6 progressors) [5] were tested for their sensitivity to β-chemokine neutralization. Biological virus

clones from each individual, obtained at $\geqslant 2$ time points during the course of infection, the first relatively early and the other relatively late during follow-up for LTSs or around the time of AIDS diagnosis for the progressors, were analyzed. Figure 1 shows the moments of clonal virus isolation and AIDS diagnosis (if applicable) and longitudinal data on CD4+ T cell counts and HIV RNA load, for the individuals studied. Table 1 shows the CCR5 and CCR2b genotype of the individuals and their diagnosis at the end of follow-up.

For each biological virus clone tested, the IC_{50} and IC_{90} values of the β-chemokines at day 14 after inoculation were determined using a 4-parametric logistic model. In table 2 (LTS) and table 3 (progressors), the mean IC_{50} values of the biological virus clones, at each time point and for each individual, are given for RANTES, MIP-1α, MIP-1β, and Mix. In addition, the minimum and maximum IC_{50} values found at each time point are given. For 2 individuals, 434 and 172, virus isolation was performed at time points <6 months apart. In these cases, the mean IC_{50} values were calculated from IC_{50} values of the isolates of those time points together (tables 2 and 3). For individual 617, virus isolation was performed at 5 different time points during follow-up. From these data, the first time point was used as "early" and the last as "late," in figures and calculations. If an IC_{50} value was >250 ng/mL (the highest concentration used), we used IC_{50} values of 250 ng/mL in figures and statistical tests.

RANTES was the most potent inhibitor of replication of primary R5, NSI HIV-1 isolates. Mix and MIP-1α were less potent inhibitors, whereas MIP-1β hardly inhibited the replication of any of the viruses, not even at the highest concentration used (250 ng/mL) (figure 2 and tables 2 and 3).

Decreasing RANTES neutralization sensitivity of HIV-1 isolates over the course of infection. In figure 3, the mean RANTES IC_{90} values for the different biological virus clones of the early and late time points for each individual are depicted. A significant increase of IC_{50} and IC_{90} values for RANTES and
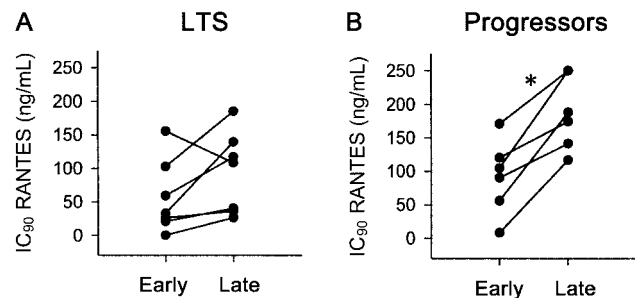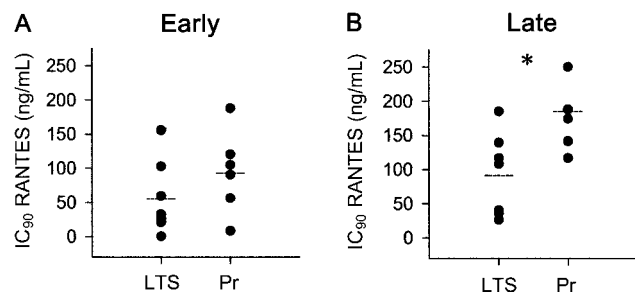

Figure 3. RANTES neutralization sensitivity over time for long-term survivors (LTSs) and progressors. Mean IC_{90} RANTES values per time point are depicted, separated for LTSs *(A)* and progressors *(B)*. The early and late time points for each patient were linked. *$P < .05$, Wilcoxon signed rank test.

Mix over time for all patients together ($n = 13$) was observed, indicating a significant decrease in sensitivity of HIV-1 to β-chemokine neutralization ($P < .05$ for RANTES IC$_{50}$, RANTES IC$_{90}$, and Mix IC$_{90}$, Wilcoxon signed rank test) during the course of HIV-1 infection.

Of interest, the decrease in sensitivity to RANTES neutralization over time was only statistically significant for the HIV-1 biological clones isolated from the progressors ($P < .05$ for RANTES IC$_{50}$, RANTES IC$_{90}$, and Mix IC$_{90}$; figure 3B) and not for the HIV-1 biological clones isolated from the LTSs (figure 3A). Indeed, from 3 of the 6 progressors, at least 1 biological HIV-1 clone from the late time point was very insensitive to β-chemokine neutralization (RANTES IC$_{90}$, >250 ng/mL), whereas only 1 late biological virus clone from the 7 LTSs was found to be very insensitive to β-chemokine neutralization (patient 441; data not shown).

The time lapse between the early and late time points was longer for the LTSs than for the progressors (tables 2 and 3). This indicates that the larger decrease in sensitivity to RANTES neutralization over time, of biological virus clones isolated from the progressors, is not explained by a larger time span between the early and late time points or by isolation on a moment later in follow-up.

Figure 4 shows a comparison of the mean IC$_{90}$ values of the early time point and those of the late time point, between LTSs and progressors. Although, at the early time point, the biological HIV-1 clones of the progressors tended to be more resistant

Figure 4. Difference in sensitivity to RANTES neutralization between long-term survivors (LTSs) and progressors (Pr). Mean IC$_{90}$ RANTES values for early *(A)* and late *(B)* time points for each patient are depicted. *$P < .05$, Mann-Whitney U test.

Table 2. β-chemokine neutralization sensitivity of biological virus isolates from 7 long-term survivors.

Patient, months after SC that virus was isolated	No. of biological virus clones analyzed	IC$_{50}$, ng/mL[a]			
		RANTES	Mix	MIP-1α	MIP-1β
68					
33	3	8.4 (4.7–12.9)	34.8 (23.9–46.2)	75.4 (16.2–173.7)	244.4 (238.8 –>250)
100	3	10.8 (9.8–11.6)	32.8 (31.6–35.2)	151.6 (98.9–225.4)	>250 (>250)
441					
16	3	18.1 (10.1–33.2)	40.1 (23.1–65.7)	181.1 (124.0– >250)	>250 (>250)
111	3	22.8 (15.6–31.6)	29.5 (23.9–34.4)	210.3 (131.0– >250)	>250 (>250)
583					
24	3	0.1 (0.01–0.1)	49.0 (28.2–63.4)	78.0 (31.5–127.9)	>250 (>250)
109	3	7.3 (0.01–14.6)	26.0 (21.2–30.7)	45.4 (43.0–47.7)	>250 (>250)
16					
22	3	7.7 (6.8–8.5)	14.2 (12.2–18.1)	46.9 (30.9–61.8)	243.1 (229.2– >250)
114	3	10.0 (5.1–16.0)	23.2 (6.9–40.9)	>250 (>250)	194.7 (84.1– >250)
78					
17	3	9.2 (3.5–14.7)	24.3 (14.3–39.6)	68.1 (68.1)	202.9 (108.8– >250)
115	3	37.1 (15.4–59.9)	65.6 (31.5–104.0)	188.6 (71.7–>250)	123.1 (42.9–>250)
337					
24	3	35.2 (22.1–51.1)	30.6 (22.4–35.3)	>250 (>250)	211.6 (134.9–>250)
122	3	41.0 (32.7–49.3)	61.4 (40.3–88.7)	131.8 (102.6–163.0)	>250 (>250)
434					
16[b]	4	62.1 (46.0–86.2)	83.9 (60.1–122.1)	181.3 (68.4–>250)	>250 (>250)
119	3	29.1 (23.0–40.0)	80.6 (68.6–86.6)	176.1 (129.2–>250)	>250 (>250)

NOTE. MIP, macrophage inflammatory protein; Mix, 1:1:1 mixture of the 3 β-chemokines; SC, seroconversion.
[a] Data are mean (range) IC$_{50}$ of the biological virus clones.
[b] Clones from time points <6 months apart are taken together.

to RANTES neutralization than did those of LTSs, only at the late time point was the resistance to RANTES neutralization in progressors significantly higher than the resistance to RANTES neutralization in LTSs ($P < .05$ for RANTES IC_{90}, Mann-Whitney U test; figure 4B).

Sensitivity to β-chemokine neutralization, $CD4^+$ T cell counts, and plasma virus load. To determine whether sensitivity of biological virus clones to β-chemokine neutralization is related to the individual's $CD4^+$ T cell count or plasma virus load, we determined the median $CD4^+$ cell count (660 cells/μL) and virus load (3.9 log HIV RNA copies/mL) at the early and late time points for all patients. $CD4^+$ cell counts and virus loads were classified as "high" or "low" relative to the median. For the HIV-1 biological clones obtained at time points when $CD4^+$ T cell counts were relatively high ($n = 13$), we found significantly lower β-chemokine IC_{50} and IC_{90} values than for the HIV-1 clones obtained at time points when $CD4^+$ T cell counts were low ($n = 12$) ($P < .05$ for RANTES IC_{90} [figure 5A] and for Mix IC_{90}, MIP-1α IC_{50}, and MIP-1α IC_{90} [data not shown]). In other words, low $CD4^+$ T cell counts were associated with a low sensitivity to HIV-1 β-chemokine neutralization.

HIV-1 biological clones obtained at time points when plasma virus loads were relatively high ($n = 13$) had significantly higher β-chemokine IC_{50} and IC_{90} values than virus clones obtained at time points when plasma virus loads were relatively low ($n = 13$) ($P < .05$ for RANTES IC_{90} [figure 5B] and for RANTES IC_{50}, MIP-1α IC_{90} [data not shown]). High plasma virus load was thus associated with low sensitivity to β-chemokine neutralization.

DISCUSSION

In the present study, we have demonstrated a decreasing sensitivity to RANTES neutralization of primary HIV-1 variants during the course of infection. Our hypothesis was that β-chemokines exert selective pressure in vivo, favoring the emergence of variants that are insensitive to their control. Other researchers have shown decreasing sensitivity to β-chemokine neutralization over time, due to the appearance of X4 HIV-1 variants [21, 22]. Here, we have shown that a decrease in sensitivity to RANTES neutralization during the course of infection is not necessarily correlated with the presence of X4 variants or variants with coreceptor usage other than CCR5. The in-

Table 3. β-chemokine neutralization sensitivity of biological virus isolates from 6 progressors.

Patient, months after SC that virus was isolated	No. of biological virus clones analyzed	IC_{50}, ng/mL[a]			
		RANTES	Mix	MIP-1α	MIP-1β
53					
35	3	0.8 (0.6–1.0)	18.9 (14.2–23.6)	64.5 (58.0–71.0)	217.5 (185.0–>250)
77	3	11.1 (0.1–21.3)	23.2 (7.8–47.9)	68.3 (30.1–135.4)	>250 (>250)
142					
21	2	10.9 (3.6–18.2)	27.1 (23.4–30.7)	77.7 (44.5–110.9)	>250 (>250)
93	3	112.4 (100.4–124.3)	241.3 (230.8–>250)	>250 (>250)	>250 (>250)
424					
6	2	30.9 (22.8–39.0)	36.8 (28.0–45.5)	122.9 (119.6–126.1)	78.7 (41.1–116.3)
43	3	37.2 (27.2–55.1)	55.4 (42.6–69.0)	>250 (>250)	133.3 (42.6–>250)
38					
21	3	13.2 (9.6–18.6)	29.9 (15.6–43.5)	126.3 (42.9–235.4)	>250 (>250)
102	3	16.7 (6.0–27.3)	22.0 (6.2–37.8)	176.2 (102.4–>250)	>250 (>250)
172					
5	3	53.5 (41.0–62.5)	89.1 (82.2–92.8)	172.9 (165.7–177.9)	>250 (>250)
24[b]	6	86.2 (55.0–108.7)	126.0 (44.2–180.0)	169.4 (42.7–>250)	>250 (>250)
617					
15	2	82.2 (79.0–85.3)	58.8 (58.1–59.5)	134.5 (100.0–169.0)	>250 (>250)
27	2	98.5 (61.4–144.8)	67.5 (43.2–91.8)	163.4	>250 (>250)
84	3	121.5 (68.9–150.8)	180.3 (119.9–225.9)	>250 (>250)	>250 (>250)
111	3	179.2 (160.1–194.3)	195.1 (173.1–226.5)	>250 (>250)	>250 (>250)
126	2	>250 (>250)	191.8 (135.8–247.8)	>250 (>250)	>250 (>250)

NOTE. MIP, macrophage inflammatory protein; Mix, 1:1:1 mixture of the 3 β-chemokines; SC, seroconversion.
[a] Data are mean (range) IC_{50} of the biological virus clones.
[b] Clones from time points <6 months apart are taken together.

dividuals we have described here harbored only R5, NSI virus variants; therefore, the decrease in sensitivity to RANTES observed here does not reflect a change in coreceptor usage. This result is in agreement with those of Jansson et al. [4, 22], who showed that R5 virus isolates from patients with AIDS may exhibit decreased sensitivity to RANTES inhibition.

One might argue that our results point toward more-efficient CCR5 usage of late isolates, compared with that of early isolates, from 1 donor. Indeed, other researchers have found decreasing sensitivity to neutralization of cloned envelope sequences from virus variants from individual ACH 142, by use of anti-CCR5 monoclonal antibody 2D7 [23]. Furthermore, in vitro passage of an R5 primary isolate in the presence of AD101, a small-molecule CCR5 antagonist, resulted in selection of an R5 virus variant that was resistant to the antagonist and partially resistant to RANTES [24]. This resistance was not mediated by a change of coreceptor usage. Recently, a correlation between CCR5 affinity and sensitivity to neutralization of the coreceptor antagonist TAK-779 of Env virus variants was found [25], suggesting that increased CCR5 affinity could also explain the decreased sensitivity to β-chemokines of late primary isolates. Whether changes in CCR5 affinity indeed account for the decreasing RANTES sensitivity remains to be established.

Our results confirm the finding that RANTES is the most potent β-chemokine for inhibition of the replication of R5 HIV-1 variants [21, 26, 27]. Furthermore, we found that MIP-1α was a more-efficient inhibitor of replication of primary R5 virus isolates than was MIP-1β (figure 2), a finding that is in accordance with those of some [1, 27, 28] but not all [11, 21, 26] previous studies. Mix showed more-potent inhibition than did MIP-1α or MIP-1β but less-potent inhibition than equal total concentrations of RANTES alone. This implicates the absence of a synergistic action of the 3 chemokines.

We also found that the virus isolates from individuals with progressive disease were less sensitive to RANTES neutralization than were virus isolates from LTSs (figure 4). This difference was statistically significant for the mean values at the late time point, but not at the early time point, indicating that the virus variants from the progressors have a stronger decrease in sensitivity over time than do those from LTSs (figure 3). Furthermore, we found that both a low CD4+ cell count and a high plasma HIV-1 RNA load were associated with a low sensitivity to β-chemokine neutralization, a finding that suggests that an association exists between sensitivity to β-chemokine neutralization of the HIV-1 variants present in the individual and the rate of disease progression.

These phenomena might reflect mutual signs of disease progression or might influence each other. A high level of immune activation might give rise to high β-chemokine production, which would exert great selective pressure on the evolution of HIV-1 variants that are insensitive to β-chemokine neutraliza-
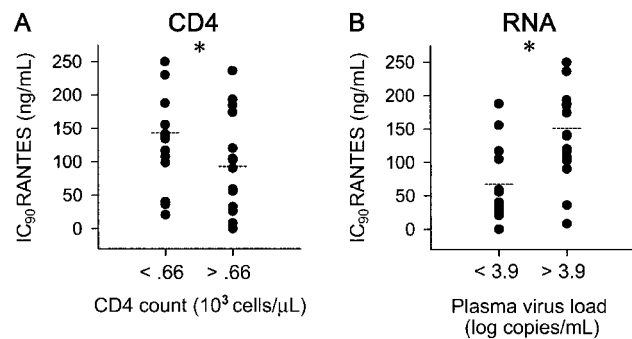
Figure 5. Association between RANTES neutralization sensitivity of biological virus clones and CD4+ T cell numbers *(A)* and plasma virus RNA load *(B)*, at the moment of virus isolation. *$P < .05$, Mann-Whitney *U* test.

tion. Our observation that HIV-1 variants isolated from CCR5 Δ32 heterozygous individuals, overall, show more resistance to RANTES neutralization than do isolates from individuals without this deletion (data not shown) might be interesting in this respect, since heterozygosity for the CCR5 Δ32 genotype has been reported to be associated with higher RANTES production levels [29]. On the other hand, high RANTES production levels also have been associated with slow disease progression [29, 30]. Therefore, the insensitivity to RANTES found in progressors might be just a consequence of more-rapid evolution due to high replication rate in vivo as reflected by high plasma virus levels, instead of a difference in selective pressure between individuals due to RANTES production levels.

Small-molecule CCR5 antagonists are being considered for therapeutic application. At present, it is unclear how HIV sensitivity profiles for these antagonists compare to those obtained with the natural ligands. Therefore, our present observation of a decreasing sensitivity of HIV variants to RANTES during the natural course of infection does not necessarily predict a limited effect of CCR5 antagonist in antiretroviral therapy during late-stage infection.

Acknowledgments

References

1. Alkhatib G, Combadiere C, Broder CC, et al. CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science **1996**; 272:1955–8.
2. Doranz BJ, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate

that uses fusin and the β-chemokine receptors CKR-5, CKR-3 and CKR-2b as fusion cofactors. Cell **1996**; 85:1149–58.

3. Koot M, Keet IPM, Vos AHV, et al. Prognostic value of human immunodeficiency virus type 1 biological phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med **1993**; 118:681–8.

4. Jansson M, Backström E, Björndal A, et al. Coreceptor usage and RANTES sensitivity of non–syncytium-inducing HIV-1 isolates obtained from patients with AIDS. J Hum Virol **1999**; 2:325–38.

5. De Roda Husman AM, van Rij RP, Blaak H, Broersen S, Schuitemaker H. Adaptation to promiscuous usage of chemokine receptors is not a prerequisite for HIV-1 disease progression. J Infect Dis **1999**; 180: 1106–15.

6. Tersmette M, Gruters RA, De Wolf F, et al. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: studies on sequential HIV isolates. J Virol **1989**; 63:2118–25.

7. Karlsson A, Parsmyr K, Sandstrom E, Fenyö EM, Albert J. MT-2 cell tropism as prognostic marker for disease progression in human immunodeficiency virus type 1 infection. J Clin Microbiol **1994**; 32: 364–70.

8. Keet IPM, Krol A, Klein MR, et al. Characteristics of long-term asymptomatic infection with the human immunodeficiency virus type 1 with normal and low CD4+ cell counts. J Infect Dis **1994**; 169:1236–43.

9. Cao Y, Qin L, Zhang L, Safrit JT, Ho DD. Virologic and immunologic characterization of long-term survivors of human immunodeficiency virus type 1 infection. N Engl J Med **1995**; 332:201–8.

10. Blaak H, Brouwer M, Ran LJ, De Wolf F, Schuitemaker H. In vitro replication kinetics of HIV-1 variants in relation to viral load in long-term survivors of HIV-1 infection. J Infect Dis **1998**; 177:600–10.

11. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. Science **1995**; 270: 1811–5.

12. Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1β and MIP-1α. J Biol Chem **1996**; 271:17161–6.

13. Mackewicz CE, Barker E, Levy JA. Role of β-chemokines in suppressing HIV replication. Science **1996**; 274:1393–4.

14. Tedla N, Palladinetti P, Kelly M, et al. Chemokines and T lymphocyte recruitment to lymph nodes in HIV infection. Am J Pathol **1996**; 148: 1367–73.

15. Paxton WA, Martin SR, Tse D, et al. Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures. Nat Med **1996**; 2:412–7.

16. Paxton WA, Liu R, Kang S, et al. Reduced HIV-1 infectability of CD4+ lymphocytes from exposed-uninfected individuals: association with low expression of CCR5 and high production of β-chemokines. Virology **1998**; 244:66–73.

17. Garzino-Demo A, Moss RB, Margolick JB, et al. Spontaneous and antigen-induced production of HIV-inhibitory β-chemokines are associated with AIDS-free status. Proc Natl Acad Sci USA **1999**; 96: 11986–91.

18. Saha K, Bentsman G, Chess L, Volsky DJ. Endogenous production of β-chemokines by CD4+, but not CD8+ T-cell clones correlates with the clinical state of human immunodeficiency virus type 1 (HIV-1)–infected individuals and may be responsible for blocking infection with non–syncytium-inducing HIV-1 in vitro. J Virol **1998**; 72:876–81.

19. Tersmette M, Winkel IN, Groenink M, et al. Detection and subtyping of HIV-1 isolates with a panel of characterized monoclonal antibodies to HIV-p24 gag. Virology **1989**; 171:149–55.

20. O'Connell MA, Belanger MA, Haaland PD. Calibration and assay development using four-parameter logistic model. Chemometrics and Intelligent Laboratory Systems **1993**; 20:97–114.

21. Scarlatti G, Tresoldi E, Björndal Å, et al. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine mediated suppression. Nat Med **1997**; 3:1259–65.

22. Jansson M, Popovic M, Karlsson A, et al. Sensitivity to inhibition by β-chemokines correlates with biological phenotypes of primary HIV-1 isolates. Proc Natl Acad Sci USA **1996**; 93:15382–7.

23. Matthews A, Scoggins R, Olivieri K, Taylor J Jr, Chernauskas D, Camerini D. R5-AIDS HIV-1 clone exhibits higher CCR5 binding affinity than R5-pre-AIDS clones from the same patient [abstract 192-M]. In: Program and abstracts of the 9th Conference on Retroviruses and Opportunistic Infections (Seattle). Alexandria, VA: Foundation for Retrovirology and Human Health, **2002**:135.

24. Trkola A, Kuhmann SE, Strizki JM, et al. HIV-1 escape from a small molecule, CCR5-specific entry inhibitor does not involve CXCR4 use. Proc Natl Acad Sci USA **2002**; 99:395–400.

25. Reeves JD, Gallo SA, Ahmad N, et al. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density and fusion kinetics. Proc Natl Acad Sci USA **2002**; 99:16249–54.

26. Trkola A, Paxton WA, Monard SP, et al. Genetic subtype-independent inhibition of human immunodeficiency virus type 1 replication by CC and CXC chemokines. J Virol **1998**; 72:396–404.

27. Kinter AL, Ostrowski M, Goletti D, et al. HIV replication in CD4+ T cells of HIV-infected individuals is regulated by a balance between the viral suppressive effects of endogenous β-chemokines and the viral inductive effects of other inductive cytokines. Proc Natl Acad Sci USA **1996**; 93:14076–81.

28. Messele T, Rinke de Wit TF, Brouwer M, et al. No difference in in vitro susceptibility to HIV type 1 between high-risk HIV-negative Ethiopian commercial sex workers and low-risk control subjects. AIDS Res Hum Retroviruses **2001**; 17:433–41.

29. Paxton WA, Neumann AU, Kang S, et al. RANTES production from CD4+ lymphocytes correlates with host genotype and rates of human immunodeficiency virus type 1 disease progression. J Infect Dis **2001**; 183:1678–81.

30. Cocchi F, DeVico AL, Yarchoan R, et al. Higher macrophage inflammatory protein (MIP)–1α and MIP-1β levels from CD8+ T cells are associated with asymptomatic HIV-1 infection. Proc Natl Acad Sci USA **2000**; 97:13812–7.