Response to Treatment and Disease Progression Linked to CD4+ T Cell Surface CC Chemokine Receptor 5 Density in Human Immunodeficiency Virus Type 1 Vertical Infection

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The factors governing interindividual variability in disease progression among children vertically infected with human immunodeficiency virus type 1 (HIV-1) remain unclear. Because it has recently been shown in infected adults that the density of CC chemokine receptor 5 (CCR5) molecules at the surface of nonactivated (human leukocyte antigen [HLA]–DR$^-$) CD4$^+$ T cells correlates with disease progression, the same correlation was sought in children. HLA-DR$^-$CD4$^+$ T cell surface CCR5 density was constant over time and correlated with the bioclinical stage and with the CD4 cell slope observed before antiretroviral treatment. In addition, CCR5 density was negatively correlated with the intensity of the decrease in viremia during antiretroviral therapy and was positively correlated with CD4 cell slope since birth. These results are compatible with the hypothesis that CCR5 density is a key factor governing disease progression in pediatric HIV-1 infection and, thereby, an indicator of prognosis. Moreover, they suggest that therapies aimed at reducing CCR5 accessibility should slow down HIV disease evolution in children.

The course of human immunodeficiency virus type 1 (HIV-1) infection is highly variable among vertically infected children. One-third of them will develop AIDS during their first year of life, whereas the others will have more slowly progressing disease, with a few even remaining asymptomatic for several years [1]. Although a high level of HIV RNA in plasma has been correlated with disease progression, its predictive value for an individual child is only moderate because of a marked overlap in levels of viremia between those with rapid and slow progression, particularly during the first year of life [2, 3]. Understanding the factors governing the evolution of HIV-1 infection in children is a major goal for defining strategies to slow down this evolution. Yet, the reasons for this interindividual variability are poorly understood.

Most HIV-1 strains transmitted from mother to infant use the CC chemokine receptor 5 (CCR5) rather than the CXC chemokine receptor 4 (CXCR4) coreceptor [4, 5]. CCR5 and CXCR4 are chemokine receptors belonging to the G protein–coupled receptor superfamily. In vitro, the mean number of CCR5 molecules on the surface of the target cell (CCR5 density) determines its infectability by CCR5-using (R5) strains [6–9]. We have recently shown among infected adults that CCR5 density is correlated with the level of R5 virus RNA in plasma [10] and with the rate of CD4$^+$ T cell loss [11]. The present study was aimed at testing the hypothesis that, similarly, CCR5 density could govern the course of HIV-1 infection in vertically infected children. To test this hypothesis, we looked for a correlation between CCR5 expression and bioclinical evolution in a group of 40 infected children.

Subjects and Methods

Study subjects. All children (20 of each sex) vertically infected with HIV-1 who were being monitored at university hospitals in Geneva, Switzerland, and Montpellier, France, were recruited for this study. They ranged in age from 10 to 201 months (arithmetic mean, 108 months; 95% confidence interval [CI], 92–123). At each visit (1 visit per child; 2 visits if CCR5 density or virus load was being monitored), blood was drawn, CD4 cell count was determined, and the plasma HIV RNA level was quantified by a commercial assay (Amplicor HIV-1 Monitor, version 1.5; Roche Diagnostic Systems) according to the manufacturer's instructions. This assay was selected for its ability to quantitate HIV RNA from various subtypes, including non-B subtypes. Children were classified in clinical classes N, A, B, and C and biologic classes 1–3 according to the 1994 recommendations of the Centers for Disease Control and Prevention for children [12]. Ten percent were in class N (age range, 5–

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Informed consent was obtained from parents or guardians of patients and volunteers. The study was approved by the local ethics committees.

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Table 1. Bioclinical characteristics of children vertically infected with human immunodeficiency virus type 1 (HIV-1) in study of CC chemokine receptor 5 (CCR5) density on $CD4^+$ T cell surface and disease progression.

Patient	Age at recruitment, years	CDC stage before treatment	$CD4^+$ T cell slope before treatment, % of cells lost/year	Current $CD4^+$ T cell slope, % of cells lost/year	CCR5 genotype	$CD4^+$ T cells expressing HLA-DR, %	DR^-CD4^+ T cells expressing CCR5, %	Total $CD4^+$ T cells expressing CCR5, %	CCR5 density on DR^-CD4^+ T cells[a]	CCR5 density on total $CD4^+$ T cells[a]	DR^-CD4^+ T cells expressing CXCR4, %	Total $CD4^+$ T cells expressing CXCR4, %	CXCR4 density on DR^-CD4^+ T cells[a]	CXCR4 density on total $CD4^+$ T cells[a]	Viral phenotype
1	15	B2	1.2	1.2	WT/WT	29	6	14	8432	8669	10	10	2562	3128	NSI
2	7	B1	7.2	0.7	WT/WT	5	5	8	5939	6468	9	1	2219	1991	SI
3	15	A2	3.4	1.6	WT/WT	7	11	13	8575	6684	1	1	2346	1811	NSI
4	9	B1	1.5	1.1	WT/WT	6	9	12	9880	12,198	20	19	2527	1615	NSI
5	5	B3	66.0	4.5	WT/WT	9	28	35	18,556	14,907	1	1	2548	2170	N
6	5	N2	50.0	2.6	WT/WT	11	34	40	13,433	11,216	11	11	2579	2394	NSI
7	4	A1	7.2	2.9	WT/WT	10	16	21	7244	5316	5	5	2045	1823	ND
8	11	B3	4.9	2.4	WT/WT	9	13	16	12,572	13,194	8	8	4817	2464	N
9	12	A2	1.9	1.8	WT/WT	7	19	22	13,582	11,602	11	10	2334	2830	NSI
10	12	A2	ND	1.3	Δ32/WT	2	5	6	8230	7088	1	1	4303	3127	SI
11	9	A3	6.3	1.4	WT/WT	4	9	12	12,714	9632	2	1	3375	3262	SI
12	14	B2	4.9	1.5	WT/WT	39	9	19	6921	5868	7	5	5644	4076	ND
13	7	N1	2.2	1.3	WT/WT	13	19	26	6316	5066	5	5	3281	3262	NSI
14	5	N1	2.7	1.3	WT/WT	24	50	62	5308	4852	5	5	5374	3452	N
15	<1	C1	ND	ND	WT/WT	8	12	18	6716	6058	8	10	3745	2912	NSI
16	11	A2	3.6	1.8	WT/WT	28	18	25	7826	6272	10	9	5226	3626	NSI
17	12	B1	8.8	8.8	WT/WT	24	52	56	8443	7012	8	7	3254	2517	NSI
18	14	A3	2.9	1.2	WT/WT	17	27	34	9910	11,508	2	2	4251	3516	NSI
19	7	A1	13.3	2.0	WT/WT	32	26	41	5355	4907	12	9	4365	2978	NSI
20	8	A3	ND	0.6	WT/WT	4	9	12	8198	8990	35	35	2725	2782	N
21	9	A2	13.8	2.4	WT/WT	22	24	38	13,672	15,322	34	29	3103	3781	ND
22	6	B3	48.0	4.5	WT/WT	12	11	17	13,357	14,567	40	40	7848	8025	NSI
23	3	A1	33.0	1.1	WT/WT	6	11	15	10,465	10,838	43	42	1799	1786	ND
24	8	N1	4.9	0.0	WT/WT	4	8	10	5865	7192	38	36	1509	1502	N
25	13	A2	2.2	1.2	WT/WT	8	17	20	8999	9476	12	12	1723	1725	N
26	5	B3	12.9	2.1	WT/WT	10	10	16	9340	10,162	25	45	1682	1675	N
27	7	C3	26.4	5.0	WT/WT	59	29	59	5869	10,435	34	31	3678	3807	SI
28	9	B3	96.0	2.7	WT/WT	28	26	37	13,197	14,546	21	21	3197	3499	NSI
29	7	C3	7.8	2.7	WT/WT	9	12	16	10,976	11,394	33	32	1965	2083	N
30	7	B1	ND	1.5	WT/WT	10	5	8	7950	8738	32	30	1996	2003	ND
31	7	B1	0.0	0.0	WT/WT	4	8	10	8601	8856	ND	25	ND	2595	ND
32	3	A1	96.0	3.0	WT/WT	6	3	6	10,405	12,317	3	3	1760	1738	ND
33	10	A2	ND	0.3	WT/WT	6	10	14	11,368	12,605	35	33	2251	2264	N
34	13	A3	ND	1.4	WT/WT	15	9	18	12,030	15,312	19	26	2205	2228	NSI
35	12	B3	4.5	1.1	Δ32/WT	11	15	19	9465	10,279	29	30	2444	2464	SI
36	4	A1	ND	0.0	WT/WT	2	4	5	7339	7446	23	23	3099	3138	N
37	14	C3	ND	0.3	WT/WT	10	22	29	6115	8237	78	77	3891	3981	N
38	13	C3	5.0	0.9	WT/WT	11	23	33	12,756	13,744	27	24	2919	2928	N
39	3	B1	ND	1.6	WT/WT	8	5	9	6223	6903	32	30	1849	1878	ND
40	16	A3	ND	1.4	WT/WT	17	12	21	10,810	10,389	45	38	4863	4889	NSI

NOTE. CDC, Centers for Disease Control and Prevention; CXCR4, CXC chemokine receptor 4; N, negative culture; ND, not determined; NSI, non–syncytium inducing; SI, syncytium inducing; WT, wild type.

[a] Cell surface coreceptor density is expressed as mean no. of coreceptor molecules/cell.

8 years; mean, 6.2 years), 42.5% in class A (age range, 3–16 years; mean, 9.6 years), 35% in class B (age range, 3–15 years; mean, 8.7 years), and 12.5% in class C (age range, 1–14 years; mean, 8.4 years). CD4 cell loss per year was evaluated for each child by calculating the difference between the percentage of $CD4^+$ T cells *(Y)* and the normal percentage of $CD4^+$ T cells at the age of the child [13] *(X)* and by dividing this difference by the age of the child in months *(Z),* as follows: $[(X - Y)/Z] \times 12$. This formula is an indicator of the progressive CD4 cell loss during the whole life of the child, resulting in the deficit in $CD4^+$ T cells observed at the moment of the study. The bioclinical characteristics of the 40 children are shown in table 1. All of them were treated. We monitored plasma virus load after the onset of the treatment for 21 children who received a triple therapy consisting of 2 nucleoside agents and 1 protease inhibitor. Nineteen age-matched healthy children (negative control group) were recruited at the university hospital of Montpellier, France.

CCR5 phenotyping. $CD4^+$ T cell surface densities of CCR5 and CXCR4 were determined by quantitative flow cytometry, as described elsewhere [10]. For this purpose, blood cells were directly labeled with phycoerythrin-conjugated anti-CD4 monoclonal antibody (MAb) and phycoerythrin–cyanin-5 anti–HLA-DR MAb and were indirectly labeled with anti-CCR5 (2D7) or anti-CXCR4 (12G5) MAb (Pharmingen) and a fluorescein isothiocyanate–conjugated anti-immunoglobulin probe (H+L; Jackson ImmunoResearch Laboratories). After gating on $CD4^+$, $CD4^+DR^+$, or $CD4^+DR^-$ T cells, the intensity of CCR5 or CXCR4 expression on $CCR5^+$ or $CXCR4^+$ cells was analyzed by conversion of fluorescein isothiocyanate fluorescence into mean number of surface–bound MAb molecules per cell, using populations of standard microbeads precoated with different well-defined quantities of MAb (QIFIKIT; Dako) and concurrently labeled with the same fluorescein isothiocyanate–conjugated anti-immunoglobulin probe.

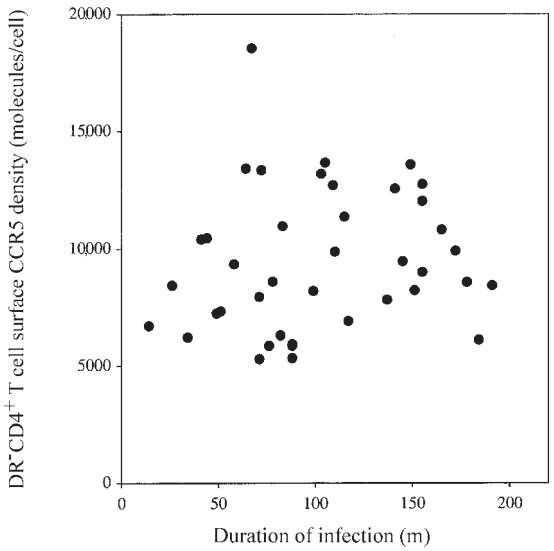

Figure 1. Absence of correlation in vertically infected children between duration of human immunodeficiency virus type 1 infection and DR^-CD4^+ T cell surface CC chemokine receptor 5 (CCR5) density. m, months.

CCR5 *genotyping.* Presence of the 32-bp deletion in the *CCR5* gene *(CCR5Δ32)* was detected by polymerase chain reaction and was confirmed by sequencing [10].

Viral phenotyping. Syncytium-inducing or non–syncytium-inducing phenotype was determined by coculturing 1×10^6 peripheral blood mononuclear cells from the patient with donor peripheral blood mononuclear cells and 5×10^6 MT2 cells in 5 mL of RPMI 1640 supplemented with 10% fetal calf serum, glutamine, and antibiotics. Coculture was done for 4 weeks. Virus production was monitored by measuring the HIV-1 p24 antigen concentration in the culture supernatant, and the presence of the syncytium-inducing strain was determined by looking for syncytia under an inverted optical microscope.

Statistical analysis. Time-course variation in CCR5 density was analyzed on 2 measures per child with a paired version of the Wilcoxon signed-rank test. Spearman rank correlations were used to evaluate the link between CCR5 density and duration of infection, bioclinical stage, CD4 cell loss, and decline in viremia during therapy. $P < .05$ was considered to be significant. The statistical program InStat 2.01 (GraphPad Software) was used in analysis.

Results

DR^-CD4^+ T cell surface CCR5 density is constant in the course of pediatric HIV-1 infection. Before examining the role of DR^-CD4^+ T cell surface CCR5 density in disease progression, we first determined whether this parameter was constant over time in infected children, as it is in adults [10, 11]. For this purpose, we monitored the DR^-CD4^+ T cell surface CCR5 density of 22 HIV-1–infected children (age range, 1–16 years), randomly chosen over a period of 12 months. DR^-CD4^+ T cell surface CCR5 density appeared to be unchanged during this period, despite some individual variations (arithmetic mean at the beginning of the study, 8718 molecules/cell [95% CI, 7298–10,140]; arithmetic mean at the end of the study, 8421 molecules/cell [95% CI, 6754–10,088]; $P = .603$). We also measured DR^-CD4^+ T cell surface CCR5 density in 19 healthy children. In this control group, CCR5 density was not correlated with age ($r = 0.164$ [95% CI, -0.327 to 0.585]; $P = .503$). In the 40 vertically infected children we studied, DR^-CD4^+ T cell surface CCR5 density was also not correlated with age ($r = 0.178$ [95% CI, -0.150 to 0.472]; $P = .271$; figure 1). Thus, DR^-CD4^+ T cell surface CCR5 density appeared to be steady over time in infected children.

DR^-CD4^+ T cell surface CCR5 density is correlated with disease progression before the onset of antiretroviral therapy. To test the hypothesis that CCR5 expression could determine the natural course of the disease in HIV-1–infected children, we compared their DR^-CD4^+ T cell surface CCR5 density with their bioclinical stage before any specific treatment had been prescribed (table 1). We excluded from our study children from whom syncytium-inducing strains were isolated, because these strains are CCR5 independent. We found a correlation between CCR5 density and severity of bioclinical disease stage before therapy.

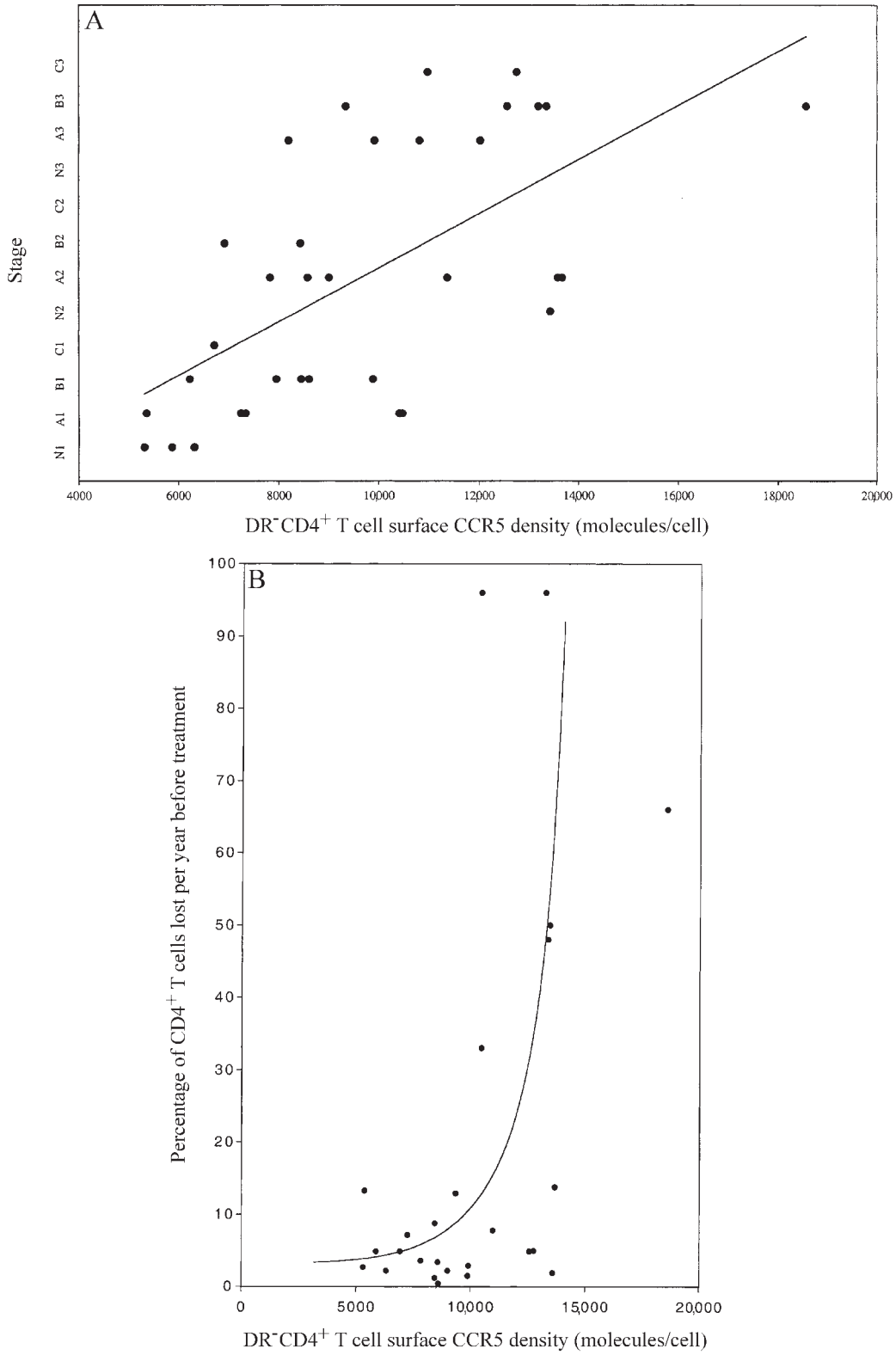

Figure 2. Correlation of $DR^{-}CD4^{+}$ T cell surface CC chemokine receptor 5 (CCR5) density with bioclinical stage *(A)* and with $CD4^{+}$ T cell slope *(B)* in nontreated human immunodeficiency virus type 1–infected children.

This correlation was the strongest when clinical stage and biologic stage were combined (figure 2*A*; $r = 0.638$ [95% CI, 0.373 to 0.807]; $P < .001$). Likewise, we calculated the annual percentage of $CD4^+$ T cell loss in each child before the onset of treatment and found a correlation between this CD4 cell loss and DR^-CD4^+ T cell surface CCR5 density ($r = 0.417$ [95% CI, 0.023 to 0.698]; $P = .034$; figure 2*B*). This correlation was logarithmic. Under a threshold of ~10,000 CCR5 molecules per cell, CD4 cell loss was small, and above this threshold it was large. This correlation was independent of the presence of the *CCR5Δ32* deletion, which has been involved in delayed disease progression [14–18]; the 2 children who were heterozygous for this deletion were not included in the calculation, because they harbored syncytium-inducing strains (table 1). On the other hand, CD4 cell loss was linked to neither the percentage of DR^-CD4^+ T cells expressing CCR5 (CCR5 frequency, $r = 0.222$ [95% CI, −0.184 to 0.564]; $P = .265$) nor the DR^-CD4^+ T cell surface CXCR4 density ($r = 0.013$ [95% CI, −0.394 to 0.417]; $P = .949$).

$CD4^+$ T cell surface CCR5 density is correlated with the response to antiretroviral therapy. The correlation we have established between CCR5 density and the evolution of the infection before treatment may be the consequence of the effect of CCR5 expression on the capacity of the target cell to sustain a productive infection. Moreover, it is logical to assume that CCR5 density could also influence the response to antiretroviral therapy, for at least 2 reasons: First, viral replication in cells expressing high densities of CCR5 molecules should be more difficult to block than viral replication in cells expressing low CCR5 densities, and, second, in high CCR5 expressers, low residual viremia will result in the productive infection of cells with a high density of membrane CCR5, and replication will be sustained. To test this hypothesis, we determined the efficiency of triple antiretroviral therapy, including a protease inhibitor, among the 21 children who received such treatment. Figure 3 shows that the intensity of the decrease in HIV-1 RNA plasma level after 3 months of treatment was correlated with DR^-CD4^+ T cell surface CCR5 density ($r = -0.484$ [95% CI, −0.763 to −0.053]; $P = .026$). Here again, neither CCR5 frequency ($r = -0.187$ [95% CI, −0.582 to 0.279]; $P = .417$) nor CXCR4 density ($r = -0.133$ [95% CI, −0.543 to 0.330]; $P = .567$) was linked to the response to treatment.

DR^-CD4^+ T cell surface CCR5 density is correlated with disease progression beyond the onset of antiretroviral therapy. If DR^-CD4^+ T cell surface CCR5 density is correlated with disease progression before the onset of antiretroviral therapy and with the quality of the response to this treatment, it is logical to assume that CCR5 density could determine the global course of the disease in treated children. Consistent with this hypothesis, we found a correlation between DR^-CD4^+ T cell surface CCR5 density and $CD4^+$ T cell slope since birth for all children ($r = 0.415$ [95% CI, 0.073 to 0.670]; $P = .016$; figure 4). $CD4^+$ T cell slope since birth was not linked to CCR5 frequency ($r = 0.292$ [95% CI, −0.061 to 0.581]; $P = .093$) nor to CXCR4 density ($r = 0.074$ [95% CI, −0.292 to 0.421]; $P = .688$).

Discussion

Herein we have shown that DR^-CD4^+ T cell surface CCR5 density is correlated with disease progression, as shown by

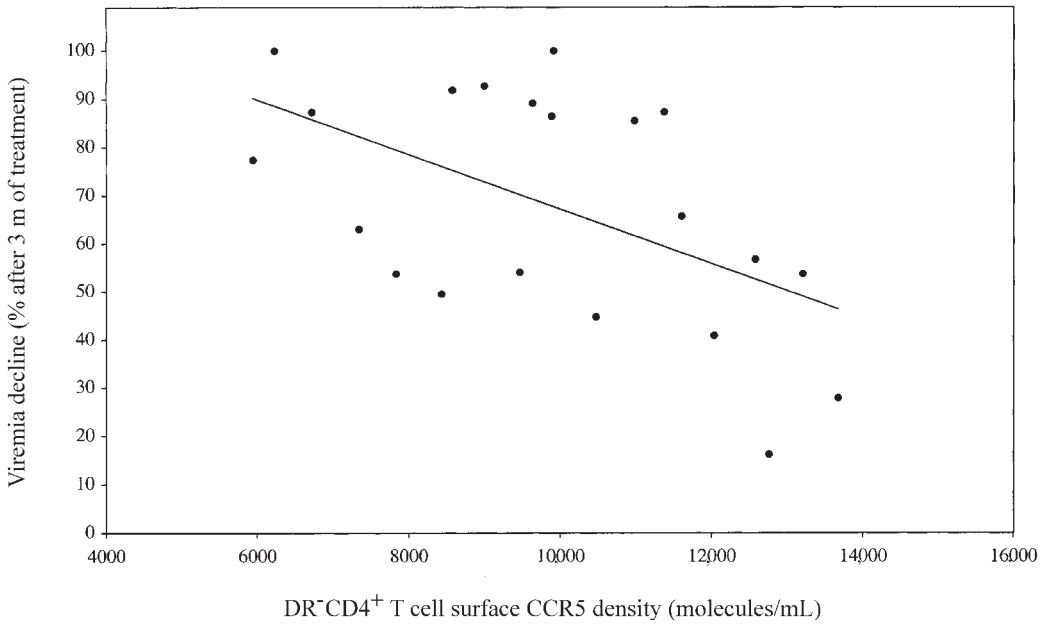

Figure 3. Correlation between DR^-CD4^+ T cell surface CC chemokine receptor 5 (CCR5) density and response to treatment (decline in viremia) among human immunodeficiency virus type 1–infected children. m, months.

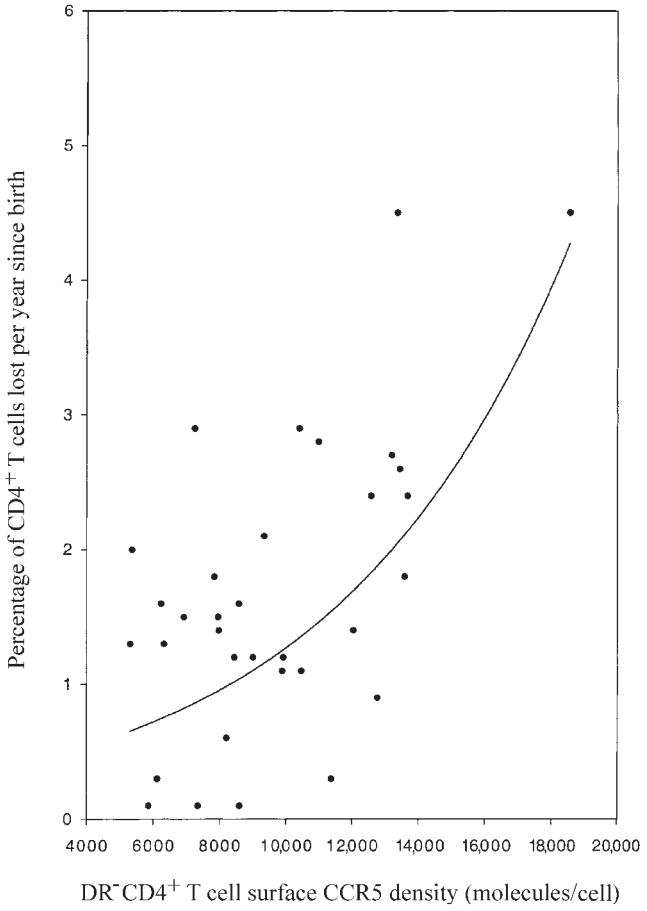

Figure 4. Correlation between $DR^{-}CD4^{+}$ T cell surface CC chemokine receptor 5 (CCR5) density and $CD4^{+}$ T cell slope since birth among human immunodeficiency virus type 1–infected children.

bioclinical stage, in children vertically infected with HIV-1. Our hypothesis is that this link is due to the effect of CCR5 density on HIV production in vitro and in vivo and thereby on CD4 cell loss. An alternative hypothesis could be that disease progression influences CCR5 expression. Our observation that CCR5 expression is globally stable over a period of 1 year in a group of infected children argues against this second hypothesis. Moreover, the individual variations in CCR5 density that we observed over time within this group were not correlated with individual progression (data not shown). Therefore, we propose that individual CCR5 expression is one factor, among others, that influences disease progression. Of interest, herein we found the correlation between CCR5 density and CD4 cell slope to be logarithmic, as was found for the correlation between CCR5 density and infectability [6] or virus load [10]. A role for CCR5 in the course of pediatric HIV infection has been reported in previous studies; in particular, heterozygosity for *CCR5Δ32* has been found to be associated with slow disease progression [14–18]. Of note, *CCR5Δ32* deletion results in the synthesis of a truncated CCR5 molecule that is unable to reach the cell surface [19]. It is possible that low CCR5 expression is the only reason that the *CCR5Δ32* deletion confers limited protection from disease progression in persons who are heterozygous for this deletion. Other factors may induce a low CCR5 expression resulting in slow disease progression, which would explain why we found that CCR5 density in children devoid of the *CCR5Δ32* deletion was correlated with CD4 cell loss. These factors might outweigh the effect of *CCR5Δ32* heterozygosity, so that the protective effect of this deletion has not been found in other studies [20–23]. Particularly, polymorphisms in the regulatory region of the *CCR5* gene, such as homozygosity for *CCR5-59356-T,* which has been associated with an increased rate of perinatal HIV-1 transmission [24], might influence disease progression.

We have recently explored the mechanism by which CCR5 density determines HIV production (authors' unpublished data). We have observed that CCR5 overexpression results in a drastic postentry boost of the virus's replicative cycle. We hypothesize that HIV production is high in children presenting with high CCR5 expression because of a high level of cell activation mediated by viral envelope–CCR5 interaction.

In addition to being predictive of the natural course of infection, CCR5 density might also be predictive of the response to treatment and thus of the course of the infection during treatment. This observation may partially explain why some persons (low CCR5 expressers) show a good response to treatment, whereas others (high CCR5 expressers) show a poor response. It also elucidates the reports on the effects of the *CCR5Δ32* deletion on the response to antiretroviral therapy [25, 26]. Quantification of $DR^{-}CD4^{+}$ T cell surface CCR5 density could thus be informative not only in regard to the natural prognosis, helping in the decision of whom to treat, but also in regard to the future response to this treatment, helping in the decision of how to treat. This information may be particularly valuable for HIV-infected children, because of the potential toxicity of antiretroviral therapies.

Reducing CCR5 density should have a doubly beneficial effect in HIV-infected patients. It should slow down disease progression and potentiate classical antiretroviral treatment. This means that anti-CCR5 therapies could have a direct protective effect, as well as an indirect protective effect, in synergy with current anti-HIV drugs.

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