

Age-Related Changes in Expression of CXCR4 and CCR5 on Peripheral Blood Leukocytes from Uninfected Infants Born to Human Immunodeficiency Virus Type 1-Infected Mothers

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Cross-sectional analysis of human immunodeficiency virus-exposed, uninfected infants revealed high proportions of CXCR4-expressing cells in their cord blood, which declined at 4.5 months and increased between 9 and 15 months to levels approaching those of uninfected adults. Proportions of CCR5-expressing cells, however, were very low in cord blood and subsequently increased with age.

The immune system of the infant is functionally less mature at birth and undergoes a process of sequential maturation and development that is reflected in qualitative and quantitative changes in a number of leukocyte subsets. Infants have increased numbers of lymphocytes compared with adults, as well as different distributions of the major lymphocyte subsets (8, 15, 24, 25).

Chemokines and their receptors are involved in controlling lymphocyte trafficking, which is a critical component of systemic immunity. Human immunodeficiency virus type 1 (HIV-1) has evolved the means to utilize chemokine receptors to facilitate its entry into CD4⁺ cells, the major ones being CXCR4 and CCR5, which mediate infection with T-cell-tropic and macrophage-tropic viral strains, respectively (1, 7, 10, 12).

The purpose of this study was to assess whether age-related changes exist in the expression of CXCR4 and CCR5 in infants, as this could have relevance for infant immune responses to microbes and for differences in disease susceptibility and progression found between HIV-1-infected adults and children. Ethical clearance for this study was obtained from the University of the Witwatersrand Ethical Committee.

We monitored coreceptor expression in cord blood ($n = 27$) and in infants 4.5 months ($n = 9$), 9 months ($n = 16$), and 15 months ($n = 9$) of age who were born to HIV-1-seropositive mothers, none of whom breastfed, recruited as part of the PETRA trial (18). All 61 of the infants included had been determined to be HIV-1 seronegative by enzyme-linked immunosorbent assay (ELISA; Abbott HIV-1 IMX) at 18 months of age, and hence defined as HIV-exposed, uninfected infants. T-lymphocyte maturation abnormalities have been detected in uninfected, HIV-exposed infants (4), and so the results reported here must not be seen as necessarily representative of uninfected infants who are not exposed to HIV-1. However, a

direct comparison of cord blood subsets of infants born to non-HIV-infected mothers showed no significant differences from those of uninfected infants born to HIV-infected mothers. This suggests that at least in utero exposure is not sufficient to result in substantial alterations in the cell subsets studied (data not shown). Healthy, non-HIV-infected adults ($n = 18$; mean age, 37.8 years; range, 26 to 57 years) were included for comparison. Mononuclear cell subsets outlined in Table 1 and Fig. 1 and 2 were analyzed by flow cytometry as described by Shalekoff et al. (26).

Percentages of lymphocyte subsets among different age groups are shown in Table 1. Other studies have assessed cell subsets in healthy, non-HIV-exposed infants but looked at different age groups (5, 6, 11, 25). However, there appear to be no major differences in any subsets compared to the present study of HIV-exposed, uninfected children.

The relative expression of CXCR4 and CCR5 on various leukocyte subsets of infants of various ages is shown in Fig. 1 and 2, respectively. The proportion of cells expressing CXCR4 was highest in cord blood (Fig. 1). Thereafter, proportions of CXCR4-expressing cells declined at 4.5 months and then increased to adult levels, a pattern similar among the different cell types. CXCR4 expression was significantly higher in cord blood than in adult blood, although differences were very modest. The most dramatic modulation of CXCR4 with respect to age occurred on NK cells, CD14⁺ monocytes, and granulocytes (Fig. 1D, F, and G, respectively). In contrast to that found for CXCR4, the proportions of cells expressing CCR5 were initially very low and gradually increased with age (Fig. 2). Similar patterns were observed for all cell types except CD19⁺ lymphocytes, where there was no significant age-related increase in CCR5 expression (Fig. 2E), and CD14⁺ monocytes, where there was only a significant increase in expression in the 9-month ($P < 0.05$) and adult ($P < 0.01$) groups compared with the cord blood samples (Fig. 2F). Correlations between CXCR4 (cord blood samples excluded) and CCR5 expression and age are shown in Table 2.

Results presented here confirm those from other studies (2,

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TABLE 1. Percentages of lymphocyte subsets for study subjects

Cell type or ratio	Median % total lymphocytes (range) for group				
	Cord Blood (n = 27)	4.5 mo (n = 9)	9 mo (n = 16)	15 mo (n = 9)	Adult (n = 18)
CD3 ⁺ T cells	62 (48–73)	59 (49–66)	57 (39–71)	61 (59–68)	71 ^a (57–77)
CD4 ⁺ cells	41 (24–59)	41 (28–51)	35 (20–54)	41 (30–48)	40 (33–54)
CD8 ⁺ cells	27 (11–37)	20 ^a (11–29)	20 (9–38)	18 (9–35)	28 ^a (18–39)
CD4/CD8 ratio	1.6 (0.7–5.7)	2.1 (1.1–4.4)	1.9 (0.8–5.6)	2.2 (1.0–5.0)	1.5 ^a (0.9–3.0)
CD3 ⁻ CD16 ⁺ /CD56 ⁺ NK cells	14 (5–36)	8 ^a (3–13)	8 (2–25)	7 (6–15)	15 ^a (4–22)
CD19 ⁺ B cells	15 (6–24)	28 ^a (20–39)	30 (14–48)	25 (17–84)	11 ^a (5–17)
CD45RA ⁺ lymphocytes	89 (80–99)	90 (84–92)	86 ^a (65–93)	84 (83–87)	65 ^a (43–94)
CD45RA ⁺ CD4 cells	89 (55–98)	85 (80–89)	80 ^a (57–89)	79 (72–87)	51 ^a (27–93)
CD45RA ⁺ CD8 cells	98 (92–100)	93 ^a (70–96)	85 (62–98)	83 (66–94)	75 (44–99)
CD45RO ⁺ lymphocytes	8 (4–25)	12 ^a (7–31)	16 ^a (6–32)	16 (11–19)	38 ^a (27–62)
CD45RO ⁺ CD4 cells	9 (4–23)	15 ^a (9–34)	23 ^a (13–45)	21 (12–25)	55 ^a (37–78)
CD45RO ⁺ CD8 cells	4 (2–9)	8 ^a (3–45)	20 ^a (5–55)	21 (11–34)	34 ^a (10–61)
CD38 ⁺ lymphocytes	95 (85–97)	91 ^a (82–95)	91 (73–95)	86 ^a (77–92)	59 ^a (42–77)
CD38 ⁺ CD8 cells	97 (87–100)	95 (86–98)	95 (83–98)	93 (73–97)	48 ^a (22–75)

^a Differences compared to the previous age group were determined by the Mann-Whitney U test ($P < 0.05$).

16) in that the percentage of CD4 cells that expressed CCR5 was lower in cord blood than in adult blood (1.4 versus 23%, respectively). However, Mo et al. (16) found that cord blood monocytes expressed only slightly less CCR5 than did adult cells (62 versus 78%), and although in our study a higher proportion of cord blood monocytes (11%) expressed CCR5 than did cord blood CD4 cells, this was still significantly less than that of adult monocytes (22%). The reason for these differences may be that isolated peripheral blood monocytes were used for their experiments (16), whereas whole blood was used in our study. Possible effects of HIV exposure on our study also cannot be excluded.

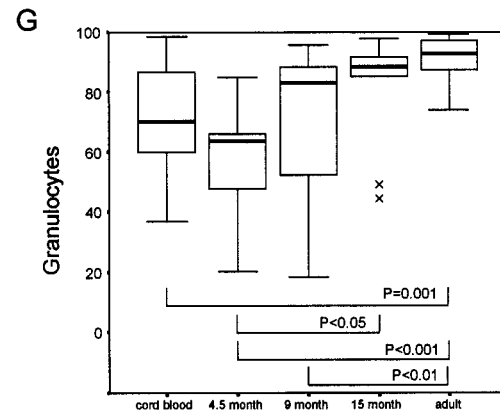
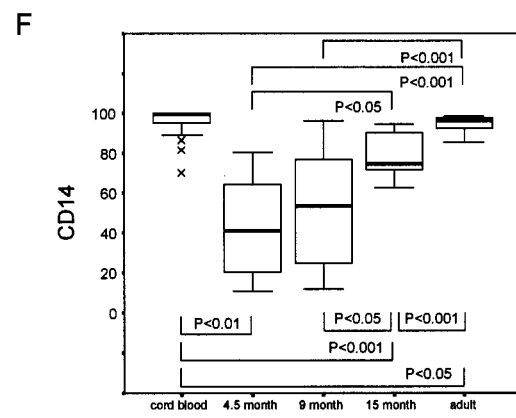
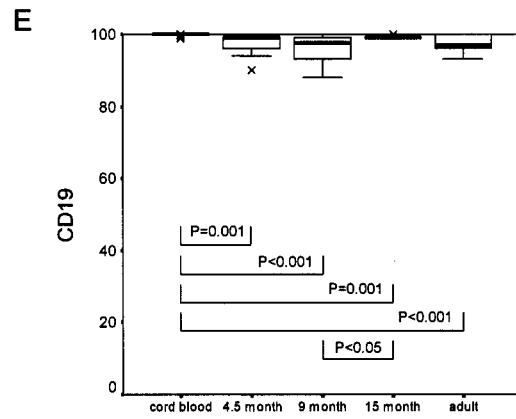
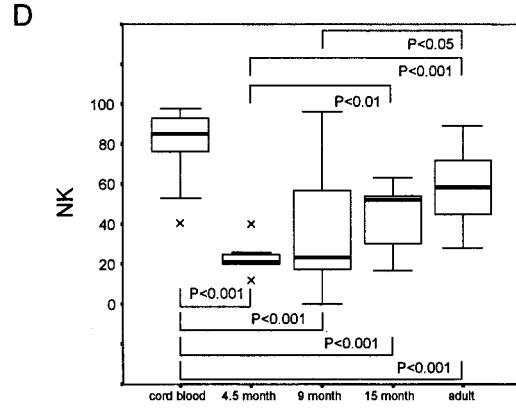
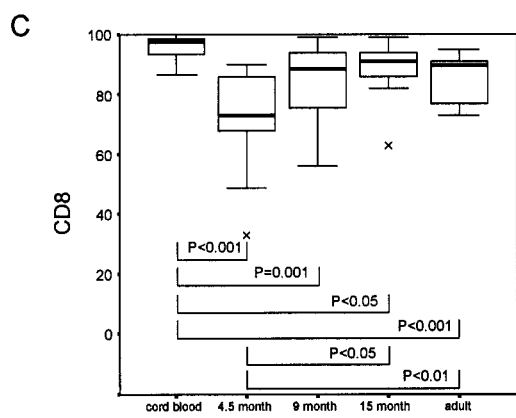
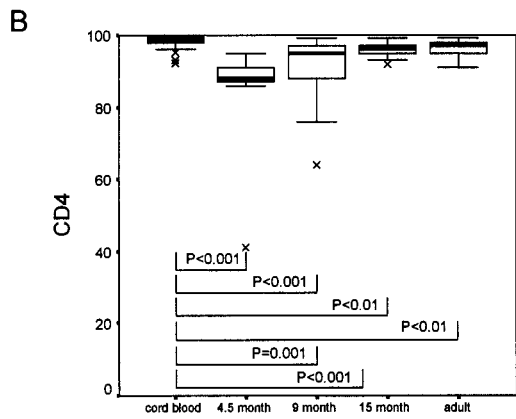
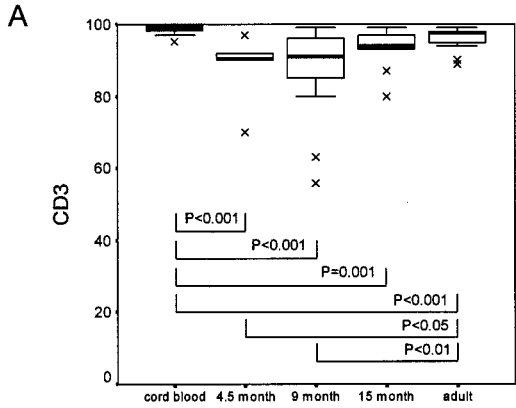
Virus populations in infected infants have been shown to be more homogeneous than those of their mothers (14, 21, 29), which suggests that a single genotype is either transmitted to or initially replicating in the child. Whether such a restriction in the virus transmitted is in any way related to the coreceptor expression on permissive cells remains largely unknown. However, HIV-1 has been shown to primarily infect CXCR4-expressing cells in placentae from nontransmitting, HIV-1-infected mothers, whereas infection of predominantly CCR5-expressing cells was demonstrated in placentae from transmitting women (3). Macrophage tropism of a mother's HIV-1 isolates has been shown to be associated with an in-

creased risk of transmission of HIV-1 to the infant (9). Taken together, these data are consistent with transmission of a population of non-syncytium-inducing (NSI) isolates that use CCR5 as a coreceptor.

Persistent use of the CCR5 receptor has been associated with slow progression of HIV-1 disease (30), while use of the CXCR4 receptor is associated with rapid progression (22). Perinatally infected children experience more rapid progression to disease than do HIV-1-infected adults (20, 23), and thus, the question of an association between CXCR4 and CCR5 receptor expression and clinical disease is important. However, infants are more likely to be infected with NSI strains of HIV-1 and progress to symptomatic disease rapidly with no evidence of syncytium-inducing (SI) isolates (27). SI isolates are, however, found in older HIV-1-infected children (27).

It is unlikely that peripheral blood receptor expression is a restricting factor for susceptibility to infection by CCR5-utilizing strains of HIV-1, as these strains are very readily transmitted from mother to child (9). An explanation for this may be that CCR5 can be upregulated by cellular activation, thereby allowing increased viral entry, or that entry of even small amounts of virus can lead to active virus turnover through some postentry enhancement of replication promoted through

FIG. 1. Expression of CXCR4 on whole-blood leukocytes from subjects in five different age groups. Lymphocytes were identified by using a forward scatter-side scatter gating strategy, and then the percentages of CXCR4-expressing CD3, CD4, CD8, NK, and CD19 cells were determined. CXCR4-expressing monocytes were analyzed by gating on CD14-positive cells, and CXCR4-expressing granulocytes were analyzed on the basis of their forward scatter and side scatter properties and absence of CD14 staining. Results are shown as percentages of positive cells. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated. Comparison of CXCR4 expression on different leukocyte subsets between the different study groups was done by use of the Mann-Whitney U test with SPSS software (SPSS, Chicago, Ill.).



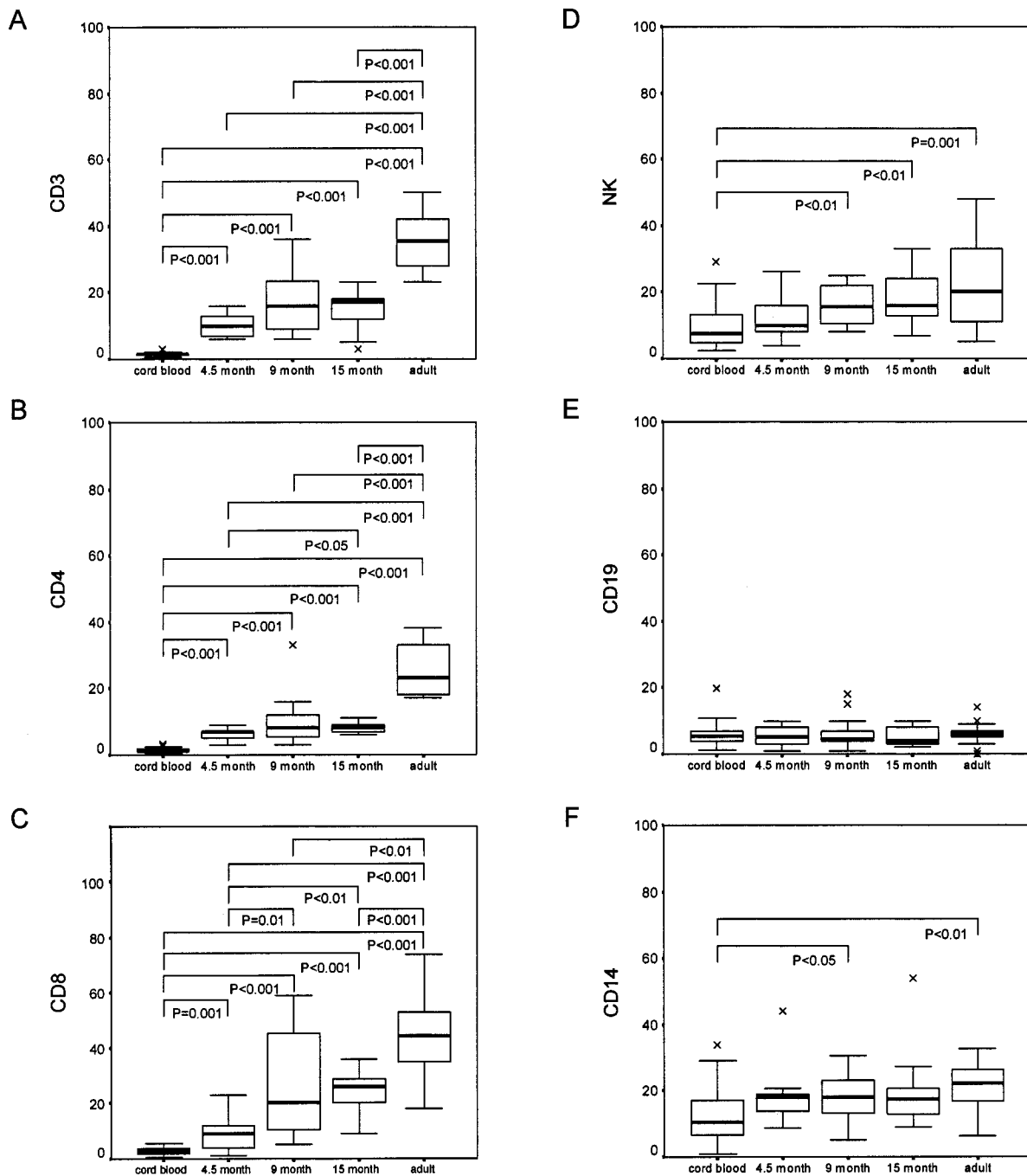


FIG. 2. Expression of CCR5 on whole-blood leukocytes from subjects in five different age groups. Lymphocytes were identified by using a forward scatter-side scatter gating strategy, and then the percentages of CCR5-expressing CD3, CD4, CD8, NK, and CD19 cells were determined. CCR5-expressing monocytes were analyzed by gating on CD14-positive cells. Results are shown as percentages of positive cells. Data are presented as medians (horizontal bar), 25th and 75th percentiles (boxes), and 10th and 90th percentiles (bars). Significant differences between groups are indicated. Comparison of CCR5 expression on different leukocyte subsets between the different study groups was done by use of the Mann-Whitney U test.

cellular activation. In addition, *in vitro* experiments have shown that the threshold of CCR5 expression required for HIV-1 replication in thymocytes is very low (17). Further support favoring the lack of restriction afforded by low CCR5 expression in cord blood cells comes from a study showing that unstimulated cord blood mononuclear cells are preferentially

infected by macrophage-tropic NSI HIV-1 isolates, whereas adult peripheral blood mononuclear cells are more susceptible to T-cell-tropic SI viral strains (19). Two other studies show an increased susceptibility of neonatal monocyte-derived macrophages to infection with NSI HIV-1 strains (13, 28).

It is clear that differences in cell maturity are reflected in the

TABLE 2. Correlations between CXCR4 and CCR5 expression and age^b

Cell type	CXCR4 expression		CCR5 expression	
	r value	P value ^a	r value	P value ^a
CD3 ⁺	0.457	0.001	0.896	<0.001
CD4 ⁺	0.519	<0.001	0.908	<0.001
CD8 ⁺	0.242	0.084	0.862	<0.001
NK	0.570	<0.001	0.433	<0.001
CD19 ⁺	0.074	0.601	-0.46	0.689
CD14 ⁺	0.760	<0.001	0.402	<0.001
Granulocytes	0.621	<0.001		

^a Significant P values are indicated in bold.

^b The Spearman correlation coefficient was calculated to analyze correlations between age and CXCR4 and CCR5 expression.

differential expression of CXCR4 and CCR5 on infant and adult peripheral leukocytes that are also mirrored by alterations in the naive and memory T-cell phenotype with age (Table 1). These findings have implications for cellular trafficking in response to the specific ligands of CXCR4 and CCR5, in that these capabilities would be expected to improve as the infant ages. Such differences may in part underlie differences in HIV disease progression between adults and children and have implications for age-dependent susceptibility to other microbial infections in early infancy. In the latter regard, it is particularly noteworthy that CXCR4-expressing NK, CD14, and granulocyte populations increase remarkably with age, whereas CCR5-expressing NK and CD14 cells show a more gradual increase. These findings suggest that CXCR4 may play a more prominent role in the innate arm of the immune system, as opposed to CCR5, which appears to be more strongly associated with cells of the acquired arm of the immune system. How persistent exposure to HIV (intrapartum and postpartum exposure through breastfeeding) might alter these immune parameters and so alter predisposition to infection, or alter the disease profiles of other infecting organisms, remains an important future question for study.

Taken together, the data show that studies of children need to take into account the differences in expression of these two important receptors in relation to age. These data add to our knowledge of the developing immune system of the infant that will provide valuable understanding with respect to the pathogenesis of neonatal disease, especially with respect to perinatal HIV-1 infection.

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