

Cellular Human Immunodeficiency Virus (HIV)–Protective Factors: A Comparison of HIV–Exposed Seronegative Female Sex Workers and Female Blood Donors in Abidjan, Côte d’Ivoire

Wim Jennes,¹ Souleymane Sawadogo,² Stéphanie Koblavi-Dème,² Bea Vuylsteke,^{1,2} Chantal Maurice,² Thierry H. Roels,^{2,3} Terence Chorba,^{2,3} John N. Nkengasong,^{2,3} and Luc Kestens¹

¹Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium; ²Projet RETRO–Côte d’Ivoire, Abidjan, Côte d’Ivoire; ³Division of HIV/AIDS Prevention, National Center for HIV, STD and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

Cellular factors that may protect against human immunodeficiency virus (HIV) infection were investigated in 27 HIV-exposed seronegative (ESN) female sex workers (FSWs) and 27 HIV-seronegative female blood donors. Compared with blood donors, ESN FSWs had significantly decreased expression levels of C-X-C chemokine receptor 4 (CXCR4), but not of C-C chemokine receptor 5, on both memory ($P < .001$) and naive ($P = .041$) CD4⁺ T cells. CXCR4 down-regulation was associated with prolonged duration of commercial sex work by ESN FSWs. CD38 expression on CD8⁺ T cells was significantly increased among ESN FSWs, compared with that among blood donors ($P = .017$). There were no differences in HLA-DR and CD62L expression between blood donors and ESN FSWs. Proportions of T cells producing the β -chemokines RANTES (regulated on activation, normally T cell-expressed and -secreted), macrophage inflammatory protein (MIP)–1 α , and MIP-1 β or the cytokines interleukin (IL)–2, IL-4, interferon- γ , and tumor necrosis factor- α , were similar in the 2 groups. These data indicate that ESN FSWs differ from HIV-seronegative female blood donors with respect to immunological factors that have no clear protective potential against HIV transmission.

In several populations, small proportions of individuals remain human immunodeficiency virus (HIV)–seronegative, despite frequent sexual exposure to HIV,

which suggests that host factors may contribute to protection against infection [1–5]. Proposed correlates of protection against HIV infection in HIV-exposed seronegative (ESN) subjects that are well documented include inherited and acquired host factors, such as a homozygous 32-bp deletion in the gene encoding the HIV-1 coreceptor CCR5 [6–11], particular genetic HLA polymorphisms [12, 13], mucosal and systemic anti-HIV IgA [14–18], and HIV-specific helper and cytotoxic T cells [19–25].

In addition to CD4, the β -chemokine receptor CCR5 is used as a coreceptor by primary macrophage (M)–tropic HIV-1 strains that are sexually transmitted [26–29], whereas the α -chemokine receptor CXCR4 is used by T cell (T)–tropic variants of HIV-1 that emerge at later disease stages [30–32]. Decreased expression levels of CCR5 on CD4⁺ T cells, in the absence of the 32-bp deletion, have been correlated to decreased suscepti-

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Reprints or correspondence: Wim Jennes, Institute of Tropical Medicine, Dept. of Microbiology, Laboratory of Immunology, Nationalestraat 155, 2000 Antwerp, Belgium (wjennes@itg.be).

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bility to M-tropic HIV-1 in vitro [33, 34], to decreased HIV-1 load levels in infected subjects [35], and to increased protection against HIV-1 infection in ESN subjects [36]. However, studies exist in which the latter could not be confirmed [37, 38]. Although susceptibility to T-tropic HIV-1 strains in vitro has been correlated directly to CXCR4 expression levels [39–41], no associations have been found between CXCR4 levels and the transmission of HIV-1 in vivo.

β -chemokines are the natural ligands for the CCR5 coreceptor, and, although their HIV-suppressive capacity has been shown in vitro [42], their role in protection against HIV infection in vivo remains controversial. Some studies have found increased β -chemokine levels in ESN subjects, compared with those in unexposed control subjects [25, 36, 43–45]. However, other studies have not found any difference [37, 38, 46]. Similarly, Th1 cytokines could play a role in protection against infection with HIV in association with cell-mediated immunity [47]. Indeed, among ESN populations, increased levels of Th1 cytokines have been shown in the presence of mucosal and systemic cellular immune activation [48] or together with a decreased Th2 cytokine profile [49, 50]. However, in other studies, decreased Th1 cytokine production was found among ESN subjects [46, 51].

In the present study, we determined whether African ESN female sex workers (FSWs) differed from African HIV-seronegative female blood donors in one or several immunological aspects with potential HIV-protective capacity. Freshly obtained whole-blood samples were used to measure ex vivo cell surface expression levels of immune activation markers and HIV-1 coreceptors and to detect intracellular β -chemokine and cytokine production, all at the level of a single cell, by flow cytometry. In addition, we verified whether these potential correlates of HIV protection were associated with indicative markers of HIV exposure among FSWs, such as the duration of commercial sex work, the number of clients per day, the consistency in using condoms, and the occurrence of sexually transmitted infections (STIs).

SUBJECTS AND METHODS

Study population. HIV-seronegative FSWs were enrolled consecutively as part of an ongoing surveillance study at a confidential clinic in Abidjan [52], and blood samples and standard questionnaires with information on sociodemographics and sexual behavior were collected. Voluntary HIV-seronegative female blood donors were enrolled at the national blood transfusion center in Abidjan; only blood samples were collected. The study lasted 2 months and took place in 2 distinct 1-month time periods. In November 1999, 14 FSWs and 15 blood donors were enrolled; in May 2000, 13 FSWs and 12 blood donors were enrolled.

Laboratory methods for HIV and STI diagnosis. Whole

blood was drawn from FSWs and control subjects into EDTA tubes (Becton Dickinson). Within 4 h of blood collection, plasma was separated from cells by centrifugation at 500 g, aliquoted, and stored at -70°C .

The HIV-seronegative status of all subjects was determined in plasma by use of the current HIV testing algorithm of Projet RETRO-Côte d'Ivoire, defined by a combination of ELISAs and Western blots [53, 54]. The HIV-negative status of ESN FSWs was confirmed by reverse HIV-1 reverse-transcriptase (RT) polymerase chain reaction (PCR) in plasma samples [55].

Bacterial and protozoan STIs were tested in the 27 FSWs: antitreponemal antibodies were detected using the *Treponema pallidum* hemagglutination assay (TPHA; Fujirebio) and the rapid plasma reagin (RPR) test (Macro-Vue, Becton Dickinson). A diagnosis of syphilis was made if results for both the TPHA and RPR tests were positive [56]. *Neisseria gonorrhoeae* infection was diagnosed by culture on modified Thayer-Martin medium or by PCR [56]. *Chlamydia trachomatis* infection was diagnosed by Microtrak EIA (Syva) or by PCR. *Trichomonas vaginalis* infection was diagnosed by use of wet-mount techniques.

T cell activation markers. Phenotypic analyses of peripheral blood lymphocytes were performed on fresh whole blood within 4 h of collection in EDTA. To quantify the levels of CD4⁺ and CD8⁺ T cell activation, whole blood was incubated in the presence of the following monoclonal antibody (MAb) combinations: anti-CD8, anti-CD38, and anti-HLA-DR; anti-CD3, anti-CD8, and anti-HLA-DR; and anti-CD4, anti-CD45RO, and anti-CD62L MAbs. All MAbs were fluorochrome-labeled and purchased from Becton Dickinson. The whole-blood staining procedure was done as described elsewhere [57]. Samples were analyzed using a FACScan flow cytometer and CellQuest software (both from Becton Dickinson). Calibration beads (Dako) were run on a weekly basis, to ensure the stability of the flow cytometer. Percentages of HLA-DR⁺ cells were analyzed within the CD4⁺ and CD8⁺ T cell subsets. Percentages of CD38⁺ and CD38⁺ HLA-DR⁺ double-positive cells were analyzed within the bright CD8⁺ lymphocyte subset. Percentages of CD62L⁺ cells were analyzed within CD45RO⁺ (memory) and CD45RO⁻ (naive) CD4⁺ lymphocyte subsets. For all analyses, quadrant markers were set using negative isotype controls.

HIV-1 coreceptor expression. Levels of HIV-1 coreceptor expression were analyzed on fresh whole blood within 4 h of collection in EDTA. Cells were stained with a combination of peridinin-chlorophyll-protein-labeled anti-CD4 and fluorescein isothiocyanate-labeled anti-CD45RO (both from Becton Dickinson), and phycoerythrin (PE)-labeled anti-CCR5 and anti-CXCR4 MAbs (both from Pharmingen), to assess the membrane expression of CCR5 and CXCR4 on CD45RO⁺ (memory) and CD45RO⁻ (naive) CD4⁺ lymphocytes. Whole-

blood staining was done as described above for T cell activation markers. HIV-1 coreceptor expression levels were calculated as percentages of positive cells or as median logarithmic fluorescence intensity (MFI) levels.

Intracellular staining of β -chemokines and cytokines. Peripheral blood mononuclear cells (PBMC) were separated from fresh whole blood within 4 h of collection and stained intracellularly for β -chemokines and cytokines, as described elsewhere [57]. In brief, PBMC were incubated overnight in the presence of monensin (1.5 μ g/mL; Sigma) for the constitutive intracellular accumulation of the β -chemokine RANTES and for 5 h with PMA (0.02 μ g/mL) and ionomycin (1 μ g/mL) in the presence of brefeldin A (10 μ g/mL) (all from Sigma) for the stimulation-induced intracellular accumulation of the β -chemokines macrophage inflammatory protein (MIP)-1 α and MIP-1 β and the cytokines interleukin (IL)-2, IL-4, interferon (IFN)- γ , and tumor necrosis factor (TNF)- α . PBMC were subsequently surface stained for CD3 and CD8, fixed, permeabilized, and stained intracellularly with anti-RANTES or anti-MIP-1 α (both from Pharmingen); anti-IL-2, anti-IL-4, anti-IFN- γ , or anti-TNF- α (all from Becton Dickinson) PE-labeled MAbs; or anti-MIP-1 β carboxyfluorescein-labeled MAb (R&D Systems) for 30 min at room temperature. Samples were analyzed using a FACScan flow cytometer and CellQuest software (both from Becton Dickinson). Percentages of β -chemokine-positive and cytokine-positive cells were analyzed within the CD4⁺ and CD8⁺ T cell subsets. For all analyses, quadrant markers were set using negative isotype controls.

Statistical analysis. Because there were 2 different study periods and because we wanted to minimize bias as a result of changing laboratory conditions, ESN FSWs and blood donors with approximately the same date of laboratory analysis were used for comparisons ($n = 20$ for both groups). Median values were calculated for normal and nonnormal distributions. Differences between groups were tested for statistical significance by use of the nonparametric Mann-Whitney U test. Correlation analyses were performed for all 27 subjects in both study groups by use of the nonparametric Spearman's rank correlation test. The level of significance for all statistical tests was set at $P = .05$.

RESULTS

Characteristics of the study population. The median age of the 27 ESN FSWs was 23 years (range, 17–37 years), compared with 23 years (range, 18–52 years) for the 27 female blood donors ($P = .326$). The median duration of commercial sex work reported by 27 ESN FSWs was 6 months (range, 1–120 months). Three FSWs (11%) reported doing commercial sex work for ≥ 3 years, 11 (41%) reported doing commercial sex work for 6 months to 3 years, and 13 (48%) reported doing commercial sex work for < 6 months. The reported number of

clients on the last working day ranged from 1 to 14 (median, 3 clients). Ten FSWs (37%) reported “always” using condoms, 14 (52%) reported “often” using condoms, and 3 (11%) reported “rarely” or “never” using condoms. The reported consistency in condom use decreased with prolonged duration of commercial sex work ($r = -0.517$; $P = .006$) and increased with higher reported numbers of clients per day ($r = 0.554$; $P = .003$). The number of clients per day tended to decrease with prolonged duration of commercial sex work ($r = -0.364$; $P = .062$). Of the 27 ESN FSWs, 5 (19%) had 1 STI: 2 tested positive for *T. vaginalis*, 2 tested positive for *C. trachomatis*, 1 tested positive for *N. gonorrhoea*, and none tested positive for *T. pallidum*.

ESN FSWs had increased T cell activation, compared with blood donors. ESN FSWs had significantly higher percentages of CD38⁺ cells in CD8⁺ lymphocytes than did blood donors (table 1). Moreover, ESN FSWs tended to have lower percentages of true naive (CD62L⁺ CD45RO⁻) CD4⁺ T lymphocytes, compared with blood donors. No differences were found in percentages of HLA-DR⁺ cells in CD4⁺ and CD8⁺ T cell subsets. Since multiple statistical comparisons were performed (table 1), there is an increased probability that the statistically significant result for CD38 expression on CD8⁺ T cells has occurred by chance. Among the ESN FSWs, no associations were found between CD38, CD62L, or HLA-DR expression and the duration of commercial sex work, number of clients per day, condom use, or the occurrence of an STI.

ESN FSWs had similar expression levels of the HIV-1 coreceptor CCR5 but significantly decreased levels of CXCR4, compared with blood donors. Figure 1 shows representative histograms for the analysis of percentages of positive cells and MFI levels of CCR5 and CXCR4 in one blood donor and one ESN FSW for CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocytes. For CCR5 (figure 1A), the markers M1 and M2 clearly discriminate CCR5⁻ and CCR5⁺ cell distributions in the

Table 1. Expression of T cell activation markers on CD4⁺ and CD8⁺ T cell subsets from human immunodeficiency virus (HIV)-seronegative female blood donors and HIV-exposed seronegative (ESN) female sex workers (FSWs).

T cell activation marker (T cell subset)	Blood donors ($n = 20$)	ESN FSWs ($n = 20$)	P^a
HLA-DR (CD4 ⁺)	12.9 (9.4–18.2)	12.7 (9.7–16.4)	.766
HLA-DR (CD8 ⁺)	22.0 (14.5–32.0)	23.0 (18.6–33.3)	.589
CD38 (CD8 ⁺)	70.1 (62.1–78.1)	76.6 (72.3–84.6)	.017
HLA-DR/CD38 (CD8 ⁺)	56.6 (48.7–67.2)	63.3 (52.7–71.8)	.144
CD62L (CD45RO ⁺ /CD4 ⁺)	56.4 (51.4–66.4)	60.1 (48.4–67.4)	.607
CD62L (CD45RO ⁻ /CD4 ⁺)	96.1 (93.7–97.9)	94.0 (86.8–96.3)	.062

NOTE. Data are median (interquartile range) ex vivo percentage of T cell activation marker expression.

^a Two-tailed nonparametric Mann-Whitney U test. P values $< .05$ are in bold.

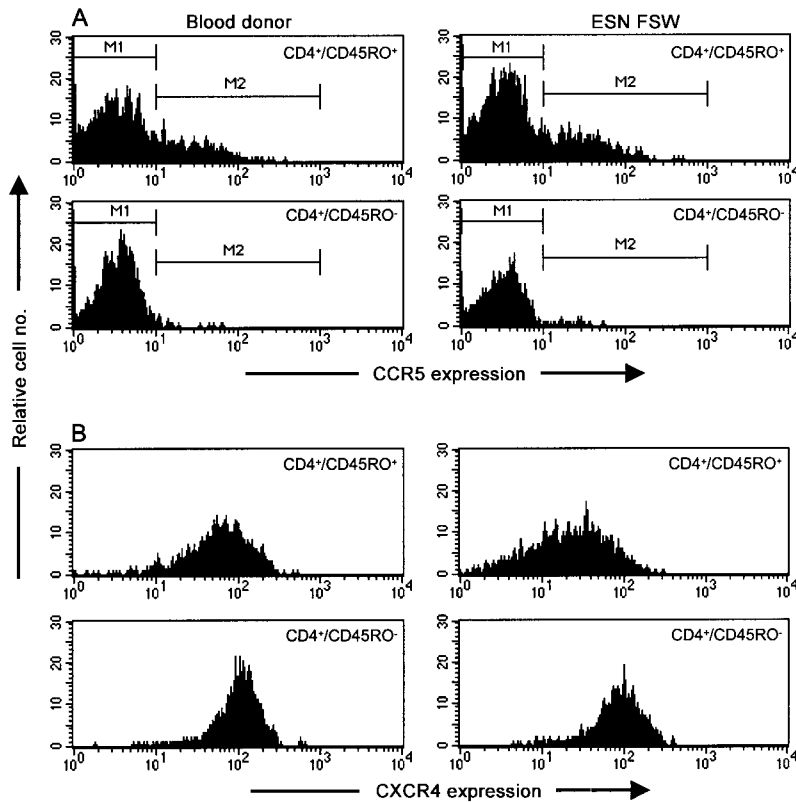


Figure 1. Representative flow cytometry analysis of ex vivo CCR5 and CXCR4 expression on CD4⁺ lymphocytes from one female blood donor and one human immunodeficiency virus–exposed seronegative (ESN) female sex worker (FSW). *A*, For CCR5, the markers M1 and M2 discriminate CCR5⁻ and CCR5⁺ cell distributions, respectively, in the CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets. Relative cell nos. of CCR5⁺ cells (marker M2) were recalculated in percentages of CCR5⁺ cells and in median logarithmic fluorescence intensity (MFI) levels of the CCR5⁺ distributions in both the CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets. *B*, For CXCR4, MFI levels of the whole CXCR4 distributions in the CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets were calculated.

CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets. Therefore, relative cell numbers of CCR5⁺ cells (marker M2) were recalculated in percentages of CCR5⁺ cells and in MFI levels of the CCR5⁺ distributions within the CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets. MFI levels of the CCR5⁺ clusters were calculated as a measurement of CCR5 expression density on CCR5⁺ cells. CXCR4 expression on lymphocytes was not characterized by discrete distributions of positive and negative cells (figure 1*B*). Therefore, MFI levels of the whole CXCR4 distributions in the CD45RO⁺ CD4⁺ and CD45RO⁻ CD4⁺ lymphocyte subsets were calculated. In both ESN FSWs and blood donors, CCR5 was almost exclusively expressed on CD45RO⁺ (memory) CD4⁺ T cells, whereas the expression of CXCR4 was higher on CD45RO⁻ (naive) CD4⁺ cells than on CD45RO⁺ (memory) CD4⁺ T cells (table 2), which confirms previous observations [58, 59].

Percentages and MFI levels of CCR5⁺ memory, naive and total CD4⁺ T cells were similar between ESN FSWs and blood donors (table 2). Percentages of CCR5⁺ memory CD4⁺ cells among ESN FSWs correlated significantly with percentages of

HLA-DR⁺ CD4⁺ T cells ($r = 0.621$; $P = .003$), HLA-DR⁺ CD8⁺ T cells ($r = 0.502$; $P = .024$), and HLA-DR⁺ CD38⁺ CD8⁺ T cells ($r = 0.582$; $P = .007$). None of these correlations was found among blood donors. Among ESN FSWs, no associations were found between CCR5 expression levels and the duration of commercial sex work, number of clients per day, condom use, or the occurrence of an STI.

MFI of CXCR4 expression on memory, naive, and total CD4⁺ T cells was significantly lower in ESN FSWs than in blood donors (table 2). No associations were found between CXCR4 expression and T cell activation among either ESN FSWs or blood donors. CXCR4 expression levels tended to correlate inversely with the duration of commercial sex work for ESN FSWs, within both naive ($r = -0.279$; $P = .176$) and memory ($r = -0.276$; $P = .182$) CD4⁺ T cells. To explore further the relationship between CXCR4 expression and the duration of commercial sex work, 10 ESN FSWs who had done sex work for <6 months were compared with 10 ESN FSWs who had done sex work for ≥6 months, with approximately the same date of laboratory analysis, to eliminate bias as a result of chang-

Table 2. Expression of human immunodeficiency virus (HIV) type 1 coreceptors CCR5 and CXCR4 in CD4⁺ lymphocyte subsets from HIV-seronegative female blood donors and HIV-exposed seronegative (ESN) female sex workers (FSWs).

HIV-1 coreceptor, lymphocyte subset	Blood donors (n = 20)	ESN FSWs (n = 20)	P ^a
CCR5^b			
All CD4 ⁺ cells, %	19.6 (15.2–24.1)	17.8 (15–21.2)	.534
All CD4 ⁺ cells, MFI	20.6 (17.9–25.2)	24.6 (20.2–30.3)	.144
CD45RO ⁻ CD4 ⁺ cells, %	1.5 (0.6–3)	1.4 (0.7–3.5)	.946
CD45RO ⁻ CD4 ⁺ cells, MFI	14.6 (12.1–17.6)	13.6 (12.9–19.3)	.808
CD45RO ⁺ CD4 ⁺ cells, %	29.4 (25.6–32)	27.7 (23.5–36.4)	.978
CD45RO ⁺ CD4 ⁺ cells, MFI	20.6 (18.4–25)	24.7 (20.2–31.2)	.168
CXCR4^c			
CD4 ⁺ cells, MFI	55.3 (45.3–75.3)	47.4 (37.3–56.5)	.045
CD45RO ⁻ CD4 ⁺ cells, MFI	93.5 (79.5–109.2)	81.7 (65.1–87.6)	.041
CD45RO ⁺ CD4 ⁺ cells, MFI	37.1 (29.6–49.9)	25.7 (22.2–29.6)	<.001

NOTE. Data are median (interquartile range) ex vivo percentage or median logarithmic fluorescence intensity (MFI).

^a Two-tailed nonparametric Mann-Whitney *U* test. *P* values <.05 are in bold.

^b CCR5 MFI levels were calculated as MFI levels of CCR5⁺ cells.

^c CXCR4 MFI levels were calculated as MFI levels of all cells.

ing laboratory conditions over time (figure 2). ESN FSWs who had done sex work for ≥6 months had lower CXCR4 MFI levels than did ESN FSWs who had done sex work for <6 months, reaching significant levels in the naive (CD45RO⁻) CD4⁺ T cell subset but not in the memory (CD45RO⁺) CD4⁺ T cell subset. Although CXCR4 MFI levels on naive CD4⁺ cells tended to correlate inversely with age among ESN FSWs ($r = -0.331$; $P = .106$), this was not found to be the case among blood donors. In addition, duration of sex work did not correlate with age ($r = 0.183$; $P = .360$) among ESN FSWs, and median age in the groups of ESN FSWs who had done sex work for ≥6 months or <6 months was not different ($P = .239$). Therefore, age could be excluded as a significant confounding factor in the analysis. Among ESN FSWs, no associations were found between CXCR4 expression and the number of clients per day, consistency of condom use, or the occurrence of an STI.

ESN FSWs and blood donors had similar percentages of β -chemokine-positive T cells. RANTES was the most abundant β -chemokine detected in both CD4⁺ and CD8⁺ T cells, being constitutively expressed at higher percentages than mitogen-stimulated MIP-1 α or MIP-1 β (table 3). Percentages of RANTES-, MIP-1 α - and MIP-1 β -positive cells in CD4⁺ and CD8⁺ T cell subsets were comparable between ESN FSWs and

blood donors (table 3). Among ESN FSWs, percentages of HLA-DR-positive cells correlated with percentages of RANTES-positive cells in the CD8⁺ T cell subset ($r = 0.459$; $P = .032$) and with percentages of MIP-1 β -positive cells in both CD4⁺ ($r = 0.478$; $P = .033$) and CD8⁺ ($r = 0.549$; $P = .012$) T cell subsets. Among blood donors, percentages of HLA-DR-positive cells correlated with MIP-1 β -positive cells in the CD8⁺ T cell subset ($r = 0.522$; $P = .018$). Among ESN FSWs, no correlations were found between proportions of β -chemokine-positive T cells and duration of commercial sex work, number of clients per day, the occurrence of an STI, or condom use.

ESN FSWs and blood donors had similar percentages of cytokine-positive T cells. Percentages of CD4⁺ and CD8⁺ T cells that were positive for the Th1 cytokines IL-2, IFN- γ , and TNF- α and the Th2 cytokine IL-4 were comparable among ESN FSWs and blood donors (table 3). Percentages of CD38⁺ HLA-DR⁺ CD8⁺ T cells correlated with percentages of IL-2-positive ($r = 0.538$; $P = .01$), IFN- γ -positive ($r = 0.621$; $P = .002$), and TNF- α -positive ($r = 0.525$; $P = .012$) CD8⁺ T cells among ESN FSWs and with IFN- γ -positive CD8⁺ T cells ($r = 0.407$; $P = .06$) among blood donors. Among ESN FSWs, no correlations were found between proportions of cytokine-

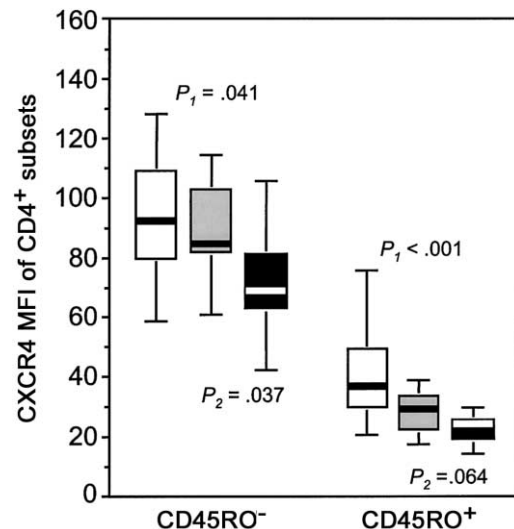


Figure 2. CXCR4 median logarithmic fluorescence intensity (MFI) levels of CD45RO⁻ (naive) and CD45RO⁺ (memory) CD4⁺ cell subsets for 20 female blood donors (white boxes), 10 human immunodeficiency virus-exposed seronegative (ESN) female sex workers (FSWs) who had done sex work for <6 months (gray boxes), and 10 ESN FSWs who had done sex work for ≥6 months (black boxes). Box plots represent the lowest value, the 25th percentile, the 50th percentile (median), the 75th percentile, and the highest value, respectively, indicated by horizontal lines in ascending order. Differences between groups were tested for statistical significance by use of the nonparametric Mann-Whitney *U* test. *P*₁, female blood donors vs. all ESN FSWs; *P*₂, ESN FSWs who had done sex work for <6 months vs. ESN FSWs who had done sex work for ≥6 months.

Table 3. Percentages of β -chemokine-positive cells and Th1 and Th2 cytokine-positive cells in CD4⁺ and CD8⁺ T cell subsets from human immunodeficiency virus (HIV)-seronegative female blood donors and HIV-exposed seronegative (ESN) female sex workers (FSWs).

Chemokine or cytokine (T cell subset)	Blood donors (n = 20)	ESN FSWs (n = 20)	P ^a
β-Chemokines^b			
RANTES (CD4 ⁺)	15.5 (12.0–19.4)	13.1 (8.9–21.8)	.387
RANTES (CD8 ⁺)	59.8 (40.2–67.3)	56.9 (42.3–68.1)	.829
MIP-1 α (CD4 ⁺)	4.1 (2.9–7.5)	5.0 (2.9–7.1)	.626
MIP-1 α (CD8 ⁺)	9.4 (5.5–18.3)	11.1 (5.2–22.2)	.626
MIP-1 β (CD4 ⁺)	4.9 (3.2–8.3)	5.1 (2.4–7.6)	.892
MIP-1 β (CD8 ⁺)	25.5 (13.5–33.7)	20.4 (14.7–33.8)	.808
Cytokines^c			
IL-2 (CD4 ⁺)	22.2 (18.4–30.0)	25.2 (19.2–33.4)	.402
IL-2 (CD8 ⁺)	6.0 (4.3–8.9)	6.6 (5.2–11.4)	.245
IFN- γ (CD4 ⁺)	17.6 (11.8–21.9)	17.9 (12.4–23.3)	.665
IFN- γ (CD8 ⁺)	18.7 (13.2–26.1)	21.8 (14.3–32.1)	.499
TNF- α (CD4 ⁺)	27.2 (16.2–40.3)	37.7 (22.9–46.6)	.245
TNF- α (CD8 ⁺)	9.6 (5.9–17.4)	12.2 (6.4–21.4)	.499
IL-4 (CD4 ⁺)	5.2 (3.9–6.1)	6.4 (4.5–7.7)	.105
IL-4 (CD8 ⁺)	3.5 (2.7–4.6)	3.6 (2.9–5.8)	.516

NOTE. Data are median (interquartile range) in vitro percentages of β -chemokine-positive cells and Th1 and Th2 cytokine-positive cells. IFN, interferon; IL, interleukin; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor.

^a Two-tailed nonparametric Mann-Whitney *U* test.

^b Intracellular accumulation was stimulation induced, except for RANTES.

^c IL-2, IFN- γ , and TNF- α are Th1 cytokines; IL-4 is a Th2 cytokine.

positive T cells and duration of commercial sex work or use of condoms, but the number of clients per day correlated positively with proportions of IL-2-positive CD4⁺ T cells ($r = 0.512$; $P = .015$) and IL-4-positive CD4⁺ T cells ($r = 0.479$; $P = .024$). No statistically significant associations were found between proportions of cytokine-positive T cells and the occurrence of an STI among ESN FSWs.

DISCUSSION

In Abidjan, Côte d'Ivoire, HIV prevalence among FSWs has decreased extensively, from 89% in 1992 to 32% in 1998 [52] and 28% in 2000 (authors' unpublished data), thanks to intensive prevention efforts and increasing condom use. However, although reported condom use is high with clients (79% consistent condom use with clients during the last working day in 1998 [52, 60]), only 20% of the FSWs use condoms consistently with their stable partners (authors' unpublished data). The HIV incidence among FSWs participating in a trial of microbicides during 1998–2000 was 4% [61], which indicates that seronegative FSWs in Abidjan are still frequently exposed to HIV and are at risk of acquiring infection. In 2000, HIV seroprevalence

among first-time blood donors (men and women) in Abidjan was estimated at 6% [62]. Ten percent of pregnant women attending an antenatal clinic in Abidjan during 1997–2000 were HIV seropositive [63]. These data indicate that, despite targeting a population that is at lower risk for HIV infection than HIV-seronegative FSWs, blood donors, especially young female blood donors, cannot be considered to be HIV unexposed.

In this study, a group of ESN FSWs was compared with a group of HIV-seronegative female blood donors for the presence of immunological factors that could contribute to decreased susceptibility to HIV infection. Our findings indicate that ESN FSWs and HIV-seronegative female blood donors have comparable expression levels of the HIV-1 coreceptor CCR5, β -chemokines, and Th1 or Th2 cytokines, but that ESN FSWs have significantly decreased levels of CXCR4 expression and significantly increased levels of CD8⁺ T cell activation.

Increased CD8⁺ T cell activation could be a sign of anti-HIV activity by HIV-specific CD8⁺ cytotoxic T cells [48]. Indeed, several studies have described HIV-specific CTL responses in HIV-seronegative subjects in association with frequent sexual exposure to HIV [20, 21, 24, 25]. However, in the present study, CD8⁺ T cell activation among ESN FSWs did not correlate with any marker of sexual HIV exposure. It is possible that CD8⁺ T cell activation is associated with other infections and STIs in ESN FSWs, rather than being related to HIV. Similar to results of a previous study among HIV-infected FSWs in Abidjan [64], we did not observe any statistically significant association between T cell activation markers and the occurrence of protozoan or bacterial STIs. It is more likely that viral non-HIV concurrent infections and STIs (which we have not analyzed in this study), rather than protozoan or bacterial STIs, play a role in T cell activation among ESN FSW.

Previous studies in Kenya and Ethiopia reported similar expression levels of CCR5 in ESN FSWs, compared with those in unexposed control subjects, as in our study, but found increased rather than decreased levels of CXCR4 [37, 38]. In these studies, however, HIV-1 coreceptor expression was measured on fresh or cryopreserved PBMC, whereas, in the present study, fresh whole blood was used. Variation in technical procedures can highly influence the analysis of HIV-1 coreceptor expression levels. This has been shown for prolonged sample storage before analysis, which causes increased CXCR4 and decreased CCR5 levels [65] and for density gradient isolated PBMC with significantly lower levels of CCR5 [66] and CXCR4 (authors' unpublished data), compared with whole blood. The use of different anticoagulants can also influence laboratory results [65, 66].

Decreased levels of CXCR4 among ESN FSWs in our study were found to be associated with prolonged duration of sex work. This association was found to be independent from T cell activation, since T cell activation did not correlate with either CXCR4 expression or the duration of sex work. In vi-

tro, HIV-1 envelope gp120 has been shown to down-regulate CXCR4 [67, 68]. Similarly, in vivo, chronic HIV infection results in down-regulation of CXCR4 expression [59, 69–71]. Therefore, in the same way as in HIV-infected subjects, chronic exposure to HIV among ESN FSWs could have caused the down-regulation of CXCR4. Alternatively, it is also possible that factors other than HIV are responsible for the down-regulation of CXCR4 in ESN FSWs, as has been reported for infection with human herpesvirus (HHV)-6 and HHV-7 [72, 73] or through allogeneic activation [74], both of which could have been acquired gradually by FSWs who had done sex work for a prolonged duration.

The relevance of CXCR4 down-regulation in ESN FSWs as a correlate of protection against HIV infection remains unclear and can only be addressed in a longitudinal study. Theoretically, it is unlikely that CXCR4 plays a role, since primary HIV-1 strains that are sexually-transmitted, M-tropic strains predominantly use CCR5 as a coreceptor [27, 29, 75], an observation that has been confirmed in Abidjan (authors' unpublished data). Moreover, the few reported cases of HIV-1 transmission via CXCR4 have all occurred in subjects homozygous for the 32-bp deletion in the CCR5 gene [76, 77]. As shown for other African populations [37, 78], this 32-bp deletion has not been detected among ESN FSWs in Abidjan [79]. Still, it would be interesting to investigate whether the down-regulated CXCR4 expression among ESN FSWs could provide a protective mechanism against T-tropic HIV-1 viruses that selectively use CXCR4 as a coreceptor. If this were proven to be the case, as a mechanism, CXCR4 down-regulation could have contributed to the selection of CCR5-using M-tropic HIV-1 strains in sexual transmission.

In most studies, β -chemokine levels in ESN subjects have been measured in plasma samples or in the supernatant of mitogen-stimulated lymphocyte cultures, without providing information on the identity of the cells that produced the β -chemokines [36–38, 43–46]. Therefore, we measured proportions of β -chemokine-producing CD4⁺ and CD8⁺ T cells in ESN FSWs by means of flow cytometry after spontaneous (for RANTES) and mitogen-stimulated (for MIP-1 α and MIP-1 β) intracellular accumulation in vitro. Our results show that proportions of β -chemokine-producing CD4⁺ and CD8⁺ T cells were comparable between ESN FSWs and blood donors and did not correlate with any indicative marker of HIV exposure of the FSWs. These data are in agreement with findings from other studies of ESN FSW populations [37, 38]. In a previous study, we found a positive correlation between proportions of β -chemokine-producing T cells and HIV-1 plasma virus load in HIV-1-infected FSWs, which indicates that intracellular β -chemokine expression is a correlate of disease progression rather than of protection [57].

Proportions of T cells producing the Th1 cytokines IL-2, TNF- α and IFN- γ were similar in ESN FSWs and blood do-

nors. Nevertheless, production of Th1 cytokines correlated more clearly with in vivo T cell activation among the ESN FSWs than among blood donors. Therefore, our data are more in line with augmented T cell activation and Th1 cytokine production in ESN subjects, as reported elsewhere [48] and do not confirm a down-regulated Th1 cytokine profile in ESN subjects in association with a postulated HIV protective low-grade activation state of CD4⁺ T cells [51]. Our data also show comparable percentages of IL-4-producing T cells among ESN FSWs and female blood donors and, therefore, do not support the recent observation of a down-regulated Th2 cytokine profile in ESN FSWs [50].

In summary, we found that ESN FSWs in Abidjan have similar expression levels of the HIV-1 coreceptor CCR5, β -chemokines, and Th1 and Th2 cytokines, compared with HIV-seronegative female blood donors, but have significantly decreased levels of CXCR4 expression, together with significantly increased levels of T cell activation. These data indicate that ESN FSWs differ from female blood donors based on immunological factors that have no clear protective potential against HIV transmission.

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