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Chemokine Expression Patterns in the Systemic and Genital Tract Compartments are Associated with HIV-1 Infection in Women from Benin

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Abstract
Introduction Understanding the genital mucosal immunity and the factors involved in linking innate to adaptive immunity is crucial for the design of efficient preventive strategies against human immunodeficiency virus (HIV)-1.
Methods Levels of both genital mucosal and blood chemokines were compared between 58 HIV-1-uninfected and 50 HIV-1-infected female commercial sex workers (CSWs) as well as 53 HIV-1-uninfected non-CSW control women at low risk for exposure, recruited in Cotonou, Benin.
Results HIV-1-infected CSWs had significantly higher blood and genital levels of monocyte chemotactic protein (MCP-3/CCL7) and monokine induced by gamma interferon (MIG/CXCL9) compared with those in both the HIV-1-uninfected CSW and non-CSW groups. In the HIV-1-infected group, levels of MCP-3 and MIG were significantly higher in the genital mucosa than in the blood. However, the blood levels of macrophage inflammatory protein (MIP-1a/CCL3) and MIP-1b/CCL4 were higher in HIV-1-uninfected CSWs compared with those in the other groups.
Conclusion Increased production of chemokines in the genital tract may favour the recruitment of HIV-1 target cells causing a mucosal environment that promotes viral replication and dissemination, whereas higher expression of β-chemokines at the systemic level is associated with protection from HIV-1 infection.

Keywords Africa · chemokine · female genital tract · HIV · immunity mucosal and systemic

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Introduction

Human immunodeficiency virus (HIV) vaccines and microbicides hold promise for preventing the acquisition of HIV-1 infection [1, 2], but the success of designing such agents needs a clear understanding of the mechanisms of HIV-1 transmission at the initial site of infection [3]. Most HIV-1 infections occur during heterosexual intercourse, and women are more likely to become infected than men (http://www.unaids.org/en/KnowledgeCentre/HIVData/EpiUpdate/EpiUpdArchive/2007default.asp). Initial exposure to HIV-1 during sexual transmission occurs in the genital tract; however, little is known about HIV-1-specific immune responses at this site.

Chemokines are known to function as regulatory molecules in leukocyte maturation, trafficking and recruit-

ing to sites of inflammation [4]. Besides these functions in the immune system, certain chemokines and their receptors are involved in HIV-1 pathogenesis. The β-chemokines macrophage inflammatory protein (MIP-1α/CCL3), MIP-1β/CCL4 and regulated on activation normal T cell expressed and secreted (RANTES)/CCL5 are natural ligands for the HIV-1 co-receptor CCR5. The antiviral activity of these molecules is exerted by competition with HIV-1 for receptor binding capacity and the attraction of immune cells. High copy numbers of CCL3L1 and CCL4L1 genes (encoding MIP-1α and MIP-1β, respectively) are associated with higher chemokine production and lower risk of HIV-1 infection [5, 6]. Genital RANTES levels were increased in HIV-1-exposed persistently seronegative women [7, 8]. On the other hand, chemokines may have an opposite effect by promoting inflammation including up-regulating of HIV-1 receptors thereby recruiting more targets cells for HIV-1 replication. Indeed, elevated genital levels of RANTES have been correlated with increased numbers of HIV-1-susceptible cells in the cervical mucosa [9]. Elevated serum levels of monocyte chemotactic protein (MCP-1/CCL2) have been positively correlated with the plasma viral load by driving recruitment of target cells [10]. HIV-1-infected individuals had higher levels of plasma interferon (IFN)-inducible protein (IP-10/CXCL10) and monokine induced by IFN-γ (MIG/CXCL9) compared with those in HIV-1-uninfected subjects [11].

We have recently shown that HIV-1-infected female commercial sex workers (CSWs) had significantly higher levels of tumour necrosis factor (TNF)-α and IFN-γ in cervicovaginal lavage (CVL) samples compared with those in both HIV-1-unifected CSWs and HIV-1-uninfected non-CSW control women [12]. Moreover, increased levels of interleukin (IL)-1β, IL-6 and TNF-α in the female genital tract have been associated with enhanced HIV-1 shedding at this site [13]. Importantly, these observations were not reflected at the systemic level [12, 13] suggesting that the mucosal inflammation observed in HIV-1-infected women is associated with recruitment, differentiation and activation of immune cells, which act as targets favouring viral replication and viral dissemination at the initial site of exposure.

In order to characterise the factors involved in the modulation of the inflammatory response associated with HIV-1 susceptibility, we have measured and compared the expression levels of chemokines in the CVL samples and serum of HIV-1-infected CSWs, HIV-1-uninfected CSWs, and HIV-1-uninfected non-CSW control subjects at low risk for HIV exposure. We report that in HIV-1-infected CSWs, levels of MCP-3/CCL7 and MIG were significantly higher in the genital mucosa than in the blood, suggesting a chemotactic gradient favouring the recruitment of immune cells contributing to the mucosal inflammatory response observed in these women. However, the blood levels of MIP-1α and MIP-1β were higher in HIV-1-uninfected CSWs compared with those in the other groups demonstrating that the expression of these β-chemokines at the systemic level is associated with protection from HIV-1 infection.

Methods

Study populations Female CSWs were enrolled through a dedicated sex worker clinic in Cotonou, Benin, and were divided into two groups: HIV-1-uninfected CSWs (n=58) and HIV-1-infected CSWs (n=50). The HIV-1-uninfected non-CSW control subjects at low risk for exposure (n=53) were enrolled from a general health clinic in Cotonou. This study was approved by the Ministère de la Santé du Bénin and by the CHUM human research ethics board. Women were invited to participate in the study as they attended clinics. Women were excluded from the study if less than 18 years old, menstruating or pregnant. All subjects provided written informed consent. At enrolment, participants were asked to answer a questionnaire about demographic information, sexual behaviour, duration of prostitution, number of sex partners, condom use, vaginal douching practices and reproductive history. Each participant underwent a genital examination by a physician. Vaginal specimens were obtained for diagnosis of candidiasis, *Trichomonas vaginalis* infection and bacterial vaginosis by microscopic examination. Endocervical swabs were obtained to test for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection using BD ProbeTec ET system (Strand Displacement Assay, Becton Dickinson, Heidelberg, Germany). Peripheral blood was taken for HIV, syphilis and herpes simplex (HSV)-2 serologies and for HIV-1 viral load, CCR5 genotype and cytokine determination. Plasma and serum were kept frozen at −80°C until use. HIV-1 positivity was defined by the presence of HIV-1 antibodies tested with Vironostika HIV Uni-Form II Ag/Ab (Organon Teknika, Boxtel, The Netherlands). Non-reactive samples were considered HIV-seronegative, whereas reactive samples were tested with Genie II HIV-1/HIV-2 (Bio-Rad, Hercules, CA, USA). Genie II dually reactive samples (to HIV-1 and HIV-2) and discordant samples (Vironostika reactive/Genie II non-reactive) were further tested by INNO-LIA HIV I/II Score (Innogenetics NV, Technologiepark 6, Gent, Belgium). Plasmatic HSV-2 IgG detection was determined with the Captia anti-HSV-2 IgG specific test (Trinity Biotech, Bray, Ireland). HIV-1 viral loads were determined in the plasma of all HIV-1-infected CSWs using VERSANT HIV-1 RNA 3.0 Assay (bDNA; Siemens Medical Solutions Diagnostics, Tarrytown, NY, USA). DNA samples were genotyped for the CCR5 32-bp deletion allele [6], and all women were found to be homozygous for the wild-type allele.

Table I Distribution of Demographic, Sexual Behaviour and Genital Tract Infection Characteristics in HIV-1-uninfected and HIV-1-infected CSWs, and HIV-1-uninfected Non-CSW Control Subjects

	HIV-1-uninfected CSWs *N*=58	HIV-1-infected CSWs *N*=50	HIV-1-uninfected non-CSW controls *N*=53	*P* value[a]
Age, mean (SD), years	34.2 (11.9)	34.4 (8.9)	32.5 (9.3)	NS
Duration of sex work, mean (SD), years	4.2 (3.1)	4.0 (2.6)	NA	NS
Number of clients last week, mean (SD)	17.2 (13.6)	12.1 (11.2)	NA	NS
Days since last menses, mean (SD)	14.5 (8.2)	18.5 (15.5)	20.1 (10.3)	NS
Regular partner	32/56 (57.0%)	29/47 (61.7%)	43/53 (81.1%)	0.04
Vaginal douching	55/58 (94.8%)	42/42 (100%)	49/53 (92.5%)	NS
Condom always used with clients	47/55 (85.5%)	37/47 (78.7%)	NA	NS
Vaginosis	21/51 (43.1%)	22/43 (51.2%)	18/53 (34.0%)	NS
Candidiasis	6/51 (11.8%)	6/38 (15.8%)	12/53 (22.6%)	NS
NG and/or CT infection	6/49 (12.2%)	5/39 (12.8%)	0/53 (0%)	0.03
HSV-2-positive serology	38/47 (80.9%)	39/41 (95.1%)	25/53 (47.2%)	<0.0001

All risk factor data were collected via a questionnaire administrated before samples were collected. Gynaecological exams and biological sampling were performed by a physician without knowledge of HIV status of the women to avoid potential bias

CSW commercial sex worker, *HIV-1* human immunodeficiency virus type 1, *HSV-2* herpes simplex 2, *N* number of participants, *NA* non-applicable, *NS* nonsignificant, *NG/CT Neisseria gonorrhoeae/Chlamydia trachomatis*

[a] *P* values for the comparison across all groups were calculated with one-way analysis of variance for the age, days since last menses, Mann–Whitney *U* test for the duration of sex work and average number of clients, chi-square test for regular partner, vaginal douching, condom use, vaginosis, candidiasis, NG/CT and HSV-2

Mucosal sample collection and preparation CVL samples were obtained from all study participants by a physician using a 10-ml syringe filled with sterile phosphate-buffered solution and aimed directly into the cervical os; CVL fluids were then collected, transferred immediately into 20 ml of RPMI-1640, kept on ice and processed within 1 h. CVL samples were centrifuged at 1,500 rpm for 10 min to remove cells and debris, and supernatants were stored at −80°C until shipped on dry ice to Montreal, Canada. CVL samples were concentrated with Amicon Ultra-15 3 kDa (Millipore, Billerica, MA, USA) prior to chemokine measurement.

Chemokine measurement Chemokine levels were determined in serum and CVL samples using the Bio-Plex cytokine/chemokine assay kit I and II (Bio-Rad), which

Table II Chemokine Levels in Cervicovaginal Lavage Samples from HIV-1-uninfected and HIV-1-infected CSWs, and HIV-1-uninfected non-CSW Control Subjects

	HIV-1-uninfected CSWs *N*=58	HIV-1-infected CSWs *N*=47	HIV-1-uninfected non-CSW controls *N*=52	*P* value[a]
IP-10	2,011 (4,142)	3,389 (5,289)	1,451 (3,432)	NS
MCP-1	39.3 (124)	52.7 (84.3)	19.4 (54.7)	0.013
MIP-1α	2.7 (5.2)	1.9 (7.1)	0.8 (2.5)	0.0002
MIP-1β	95.6 (117)	135 (176)	64.4 (107)	0.022
RANTES	14.0 (33.1)	26.2 (66.9)	3.4 (7.5)	0.0002
MCP-3	9.6 (23.0)	16.1 (20.9)	4.3 (9.5)	0.002
MIG	1,826 (3,083)	7,054 (9,183)	1,553 (2,373)	<0.0001

Data are mean (SD) pg ml^{-1}

The lower detection limit for each assay was 2.7 pg ml^{-1} for IP-10, 1.7 pg ml^{-1} for MCP-1, 1.5 pg ml^{-1} for MCP-3 and RANTES, 0.5 pg ml^{-1} for MIG and 1 pg ml^{-1} for MIP-1 and MIP-1β

CSW commercial sex worker, *HIV-1* human immunodeficiency virus type 1, *IP-10* interferon-inducible protein-10, *MCP* monocyte chemotactic protein, *MIG* monokine induced by gamma interferon, *MIP* macrophage inflammatory protein, *N* number of participants, *NS* nonsignificant RANTES regulated on activation normal T cell expressed and secreted

[a] *P* values for comparison across all groups were calculated with one-way analysis of variance test

allows simultaneous detection of IP-10, MCP-1, MCP-3, MIG, MIP-1α, MIP-1β and RANTES. Analysis was performed on a Luminex® 200 System (Luminex Corporation, Austin, TX, USA). The final concentration for a given chemokine in the CVL sample was determined as follows: concentration obtained with the Luminex analyser (pg ml^{-1})/(CVL concentration factor)×total CVL volume prior to concentration. The lower detection limit (LDL) for each assay was determined as the last point on the standard curve, 2.7 pg ml^{-1} for IP-10, 1.7 pg ml^{-1} for MCP-1, 1.5 pg ml^{-1} for MCP-3 and RANTES, 0.5 pg ml^{-1} for MIG and 1 pg ml^{-1} for MIP-1α and MIP-1β. Sample below the LDL was assigned a value of 0 pg ml^{-1}.

Statistical analysis Statistical analysis was performed using the GraphPad PRISM 5.0 for Windows (GraphPad Software, San Diego, CA, USA). One-way analysis of variance and chi-square tests were used to assess the significance of

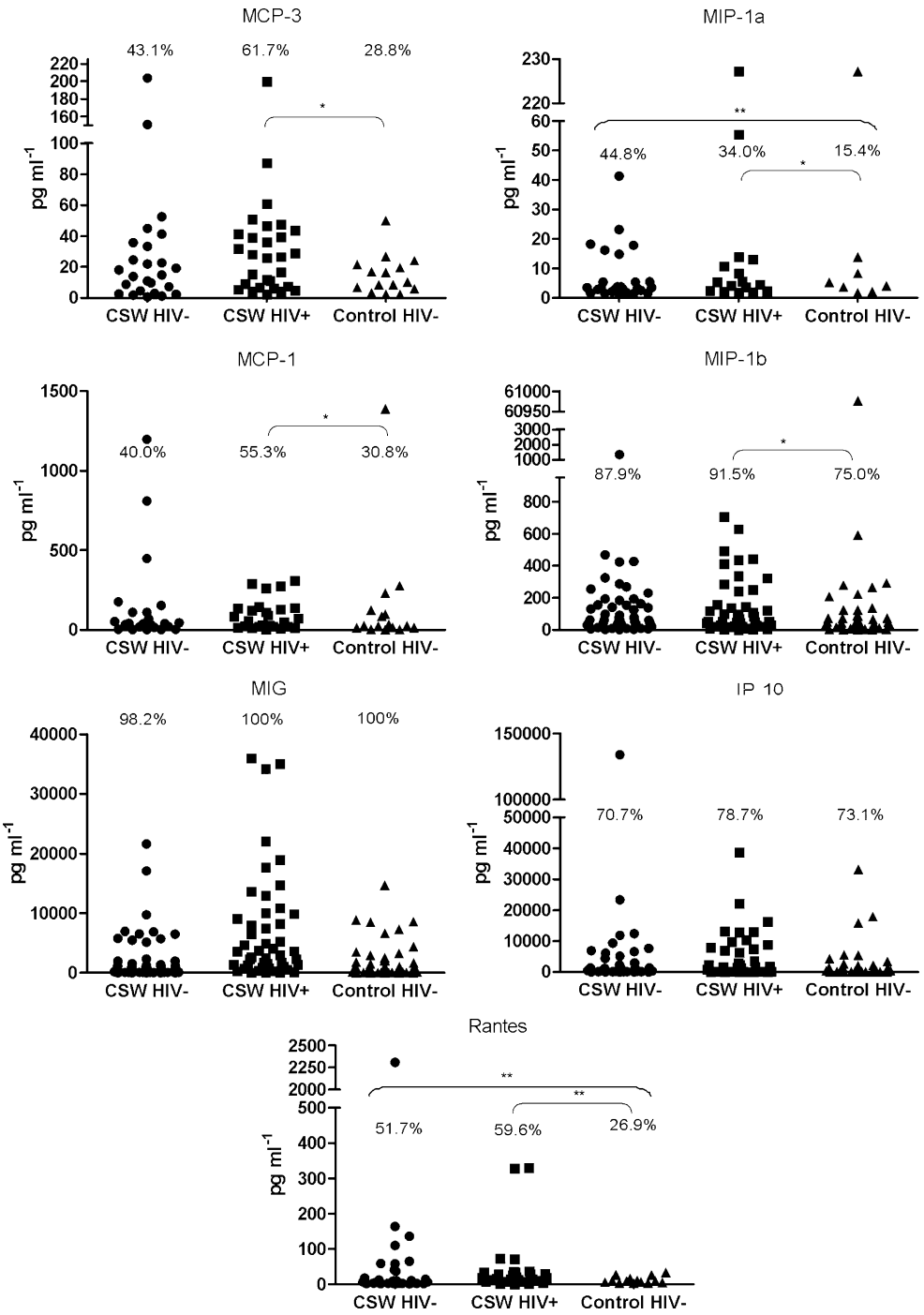

Fig. 1 Distribution of IP-10, MCP-1, MCP-3, MIG, MIP-1α, MIP-1β and RANTES CVL levels according to the study groups. CVL chemokines levels were quantified by Bio-Plex cytokine/chemokine assay (Bio-Rad) and normalised to a standard curve. The LDL for each assay was determined as the last point on the standard curve, 2.7 pg ml^{-1} for IP-10, 1.7 pg ml^{-1} for MCP-1, 1.5 pg ml^{-1} for MCP-3 and RANTES, 0.5 pg ml^{-1} for MIG and 1 pg ml^{-1} for MIP-1α and MIP-1β. Sample measurements below the LDL were assigned a value of 0. Values are expressed in pg ml^{-1}. Owing to the high number of samples below the assay LDL, chemokine levels were dichotomized as detectable and undetectable in all analyses. Comparisons of the chemokine detection rates (percent of women producing chemokine levels above the LDL) between two study groups were examined with the chi-square test. Significance levels are shown as *single asterisk*: $P<0.05$ and *double asterisk*: $P<0.001$. Differences that were not statistically significant are not illustrated. *IP* interferon-inducible protein, *LDL* lower detection limit, *MCP* monocyte chemotactic protein, *MIG* monokine induced by gamma interferon, *MIP*, macrophage inflammatory protein, *RANTES* regulated on activation normal T cell expressed and secreted

the associations between continuous and categorical variables across all study groups. Comparisons of continuous and categorical variables between two groups were assessed by the Mann–Whitney *U* and chi-square tests, respectively. Spearman's rank test was used to determine correlations between continuous variables.

Results

Demographic, Sexual Behaviour and Genital Infection Characteristics of the Study Groups

These data were collected to address the issue of confounding variables for risk of HIV-1 infection and mucosal immune responses. The three groups were similar with respect to age, days from last menses, vaginal douching and the presence of vaginosis and candidiasis as determined by microscopic examination of vaginal specimens (Table I). The HIV-1-uninfected non-CSW control subjects were more likely to have a regular partner (P=0.04) and less likely to be positive for *C. trachomatis* and/or *N. gonorrhoeae* by strand displacement assay on endocervical swabs (P=0.03) and to be HSV-2 seropositive (P<0.0001) than the HIV-1-infected and HIV-1-uninfected CSW women. Duration of sex work, average numbers of clients during the past week and condom use were equivalent between the HIV-1-infected and HIV-1-uninfected CSW groups.

Chemokine Expression Patterns in the Cervicovaginal Lavage Samples

HIV-1-infected CSWs had higher levels of MCP-3 and MIG than did the HIV-1-uninfected CSWs (P=0.023, P<0.0001, respectively) and the HIV-1-uninfected non-CSW control women (P=0.0004, P<0.0001, respectively; Table II). The level of MCP-1 was significantly different between the HIV-1-infected CSW and HIV-1-uninfected non-CSW groups (P=0.004), with higher levels observed in HIV-1-infected CSWs. The percentage of women producing significant amounts of MCP-1 and MCP-3 (concentration above the LDL) was significantly higher in the HIV-1-infected CSW group compared with that in HV1-uninfected non-CSW group (Fig. 1). Interestingly, MIP-1α expression level was higher in HIV-1-uninfected CSWs compared with that in both HIV-1-infected CSWs (P=0.016) and HIV-1-uninfected non-CSW women (P=0.001). The expression level and detection rate of MIP-1β and RANTES were significantly different between the CSW and the non-CSW groups. HIV-1-uninfected non-CSW subjects had lower levels of MIP-1β and RANTES than did the HIV-1-infected (P=0.009, P=0.004, respectively) and the HIV-1-uninfected (p=0.043, P=0.021, respectively) CWS groups. Accordingly, MIP-1β and RANTES detection rates were lower in the non-CSW group compared with that in both CSW groups (Fig. 1). There was no significant correlation between the CVL chemokine expression patterns and the presence of *N. gonorrhoeae* and/or *C. trachomatis* genital infections or HSV-2 seropositivity either within groups or among all study participants. There was no correlation between the HIV-1 viral load and the levels of chemokines in the CVL samples of the HIV-1-infected CSWs.

Chemokine Expression Patterns in Serum

Based on our previous findings showing important differences in immunoregulatory cytokine expression patterns in the systemic and genital tract compartments of these

Table III Chemokine Levels in Serum from HIV-1-uninfected and HIV-1-infected CSWs, and HIV-1-uninfected non-CSW Control Subjects

	HIV-1-uninfected CSWs N=50	HIV-1-infected CSWs N=50	HIV-1-uninfected non-CSW controls N=53	P value[a]
IP-10	290 (301)	776 (569)	259 (190)	<0.0001
MCP-1	8.9 (6.9)	6.0 (5.3)	6.9 (5.2)	0.04
MIP-1α	29.0 (68.0)	16.2 (43.8)	12.3 (23.9)	0.05
MIP-1β	215 (109)	143 (86.8)	196 (98.9)	0.0005
RANTES	94,126 (117,263)	141,069 (134,907)	156,774 (158,527)	0.04
MCP-3	2.0 (6.8)	4.1 (7.8)	0.9 (4.3)	0.002
MIG	549 (460)	1,798 (1,283)	260 (163)	<0.0001

Data are mean (SD) pg ml^{-1}

The lower detection limit for each assay was 2.7 pg ml^{-1} for IP-10, 1.7 pg ml^{-1} for MCP-1, 1.5 pg ml^{-1} for MCP-3 and RANTES, 0.5 pg ml^{-1} for MIG and 1 pg ml^{-1} for MIP-1α and MIP-1β

CSW commercial sex worker, *HIV-1* human immunodeficiency virus type 1, *IP-10* interferon-inducible protein-10, *MCP* monocyte chemotactic protein, *MIG* monokine induced by gamma interferon, *MIP* macrophage inflammatory protein, *N*,number of participants, *NS* nonsignificant RANTES regulated on activation normal T cell expressed and secreted

[a] P values for comparison across all groups were calculated with one-way analysis of variance test

women [12], we also examined the pattern of chemokine expression in the serum of all participants. HIV-1-infected CSWs had higher levels of IP-10, MCP-3 and MIG than did the HIV-1-uninfected CSWs ($P<0.0001$, $P=0.02$, $P<0.0001$, respectively) and the HIV-1-uninfected non-CSW control women ($P<0.0001$, $P=0.002$, $P<0.0001$, respectively; Table III). In addition, the percentage of women with detectable level of MCP-3 was significantly higher in the HIV-1-infected CSW group compared with that in both the HV1-uninfected CSW and non-CSW groups (Fig. 2). Interestingly, MIP-1α and MIP-1β levels were higher in HIV-1-uninfected CSWs compared with those in both HIV-1-uninfected non-CSW controls ($P=0.03$, $P=0.002$, respectively) and HIV-1 infected CSWs ($P=0.0002$ for MIP-1β). HIV-1-uninfected CSWs had higher level of MCP-1 than did HIV-1-infected CSWs

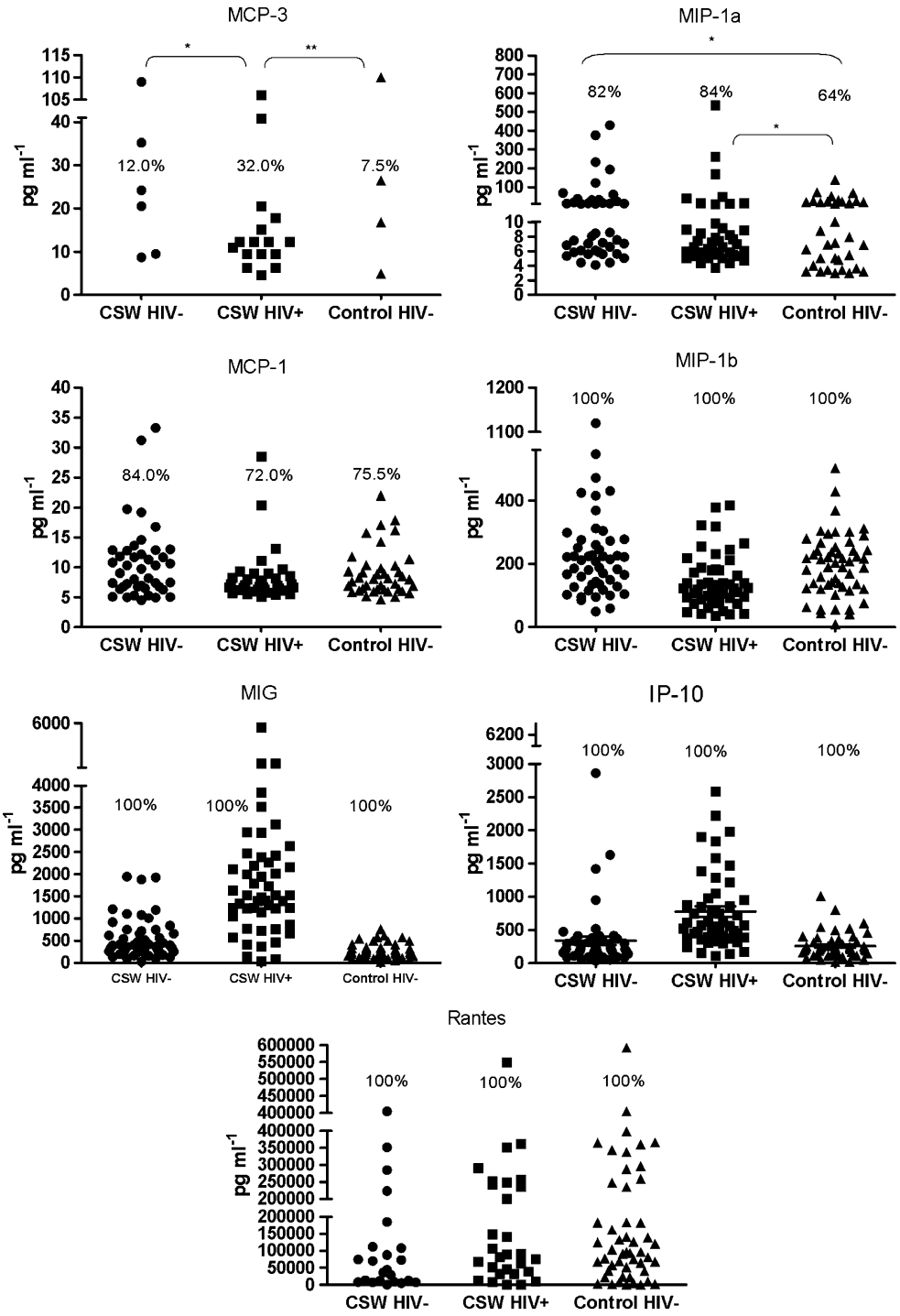

Fig. 2 Distribution of IP-10, MCP-1, MCP-3, MIG, MIP-1α, MIP-1β and RANTES serum levels according to the study groups. Serum chemokines levels were quantified by Bio-Plex cytokine/chemokine assay (Bio-Rad) and normalised to a standard curve. The LDL for each assay was determined as the last point on the standard curve, 2.7 pg ml^{-1} for IP-10, 1.7 pg ml^{-1} for MCP-1, 1.5 pg ml^{-1} for MCP-3 and RANTES, 0.5 pg ml^{-1} for MIG and 1 pg ml^{-1} for MIP-1α and MIP-1β. Sample measurements below the LDL were assigned a value of 0. Values are expressed in pg ml^{-1}. Owing to the high number of samples below the assay LDL, chemokine levels were dichotomized as detectable and undetectable in all analyses. Comparisons of the chemokine detection rates (percent of women producing chemokine levels above the LDL) between two study groups were examined with the chi-square test. Significance levels are shown as *single asterisk*: $P<0.05$ and *double asterisk*: $P<0.001$. Differences that were not statistically significant are not illustrated.
IP interferon-inducible protein, *LDL* lower detection limit, *MCP* monocyte chemotactic protein, *MIG* monokine induced by gamma interferon, *MIP* macrophage inflammatory protein, *RANTES* regulated on activation normal T cell expressed and secreted

(P=0.01). The detection rate of MIP-1α was significantly higher in CSWs compared to controls, whereas those of MCP-1 and MIP-1β were similar among all groups. Although RANTES level was lower in HIV-1-uninfected CSWs compared with those in the other groups, the detection rate was similar among all groups. There was no correlation between the HIV-1 viral load and the levels of chemokines in the serum of the HIV-1-infected CSWs.

The chemokine expression levels differed considerably between the blood and genital tract compartments of all participants. Levels of MCP-3 and MIG were significantly higher in the mucosal than in the blood samples (P=0.002, P=0.0004, respectively). Inversely, levels of MIP-1α and MIP-1β were significantly higher in the blood than in the genital mucosa (P<0.0001, P<0.0001, respectively). The differences in the level of chemokine expression between the blood and the female genital tract compartments allowed us to identify a chemotactic gradient associated with HIV-1 infection (Fig. 3).

Discussion

Understanding the mucosal immunology and its link to the systemic immune system is pivotal for the design of strategies for blocking HIV-1 at ports of entry and preventing its dissemination. The present study shows that the pattern of chemokine expression in the genital mucosa displays characteristic features that are distinct from those of the systemic immune compartment and is associated with HIV-1 infection.

HIV-1-infected CSWs had significantly higher blood and genital levels of MCP-3 and MIG compared with those in both the HIV-uninfected CSW and non-CSW groups. Importantly, the levels of these chemokines were significantly higher in the genital mucosa than in the blood. These results are consistent with our previous findings showing that the production of pro-inflammatory cytokines such as TNF-α and IFN-γ is increased in the genital but not in the systemic compartment of HIV-1-infected CSWs [12] and suggest a chemotactic gradient favouring the recruitment of immune cells in the genital mucosa contributing to the local inflammation observed in these women. The mucosal inflammation may favour disease progression/perpetuation by recruiting more HIV-1 target cells and causing a mucosal environment that promotes viral replication and viral dissemination beyond the initial site of infection [14, 15]. Accordingly, a recent study has shown that increased genital level of RANTES correlates with an increase in the number of HIV-1 susceptible target cells such as $CD4^+$ T and immature dendritic cells in the cervical mucosa [9]. Previous studies demonstrating the association between elevated genital RANTES level and HIV-1 resistance [7, 8] did not control for potential confounding factors that may result from high-risk sexual practices themselves, rather than representing a mechanism of immune protection from HIV-1 acquisition. Indeed, we found that both the HIV-1-uninfected and HIV-1-infected CSWs had higher genital levels of MIP-1β and RANTES than did the non-CSW controls, suggesting that their production might be related to the practice of sex work. The CSWs were more likely to have multiple partners, genital co-infections such as *C. trachomatis* and *N. gonorrhoeae* and to be HSV-2 seropositive than did the non-CSW controls (Table I). Exposure to semen can elicit the expression of cytokines and chemokines in the female genital tract [16]. Given that CSWs have multiple partners and are presumably exposed to a broader spectrum of semen than are non-CSW women, this could potentially contribute to the relatively higher level of chemokines observed in the genital mucosa of CSWs. Genital co-infections can also induce the production of a wide range of pro-inflammatory cytokines and chemokines in the genital tract and have been shown to increase the risk of HIV-1 acquisition [17–21]. However, we found no correlation between the presence of genital co-infections and the risk of HIV-1 infection or the chemokine and cytokine [12] expression patterns. We cannot exclude the possibility that other genital infections such as chancroid, donovanosis and human papillomavirus could have influenced the risk of infection or the production of chemokines and cytokines. Although we did not test specifically for these infections, none of the participants had evidence of characteristic genital chancres, ulcers and/or condylomas associated with these illnesses (data not shown).

The fact that some individuals remain persistently seronegative despite high exposure to the virus, such as the HIV-1-uninfected CSWs presented herein, implies that there are host factors that are highly protective. Until now,

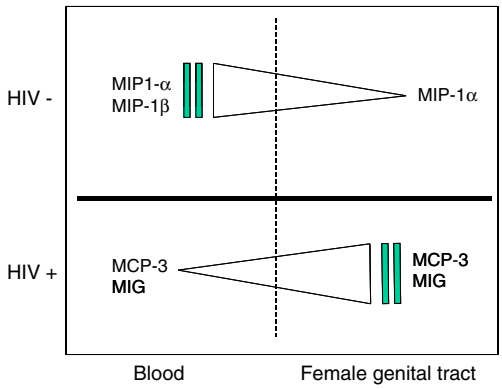

Fig. 3 Chemotactic gradient between the blood and female genital tract compartments according to the HIV status. *IP* interferon-inducible protein, *MCP* monocyte chemotactic protein, *MIG* monokine induced by gamma interferon, *MIP* macrophage inflammatory protein

HIV-1 resistance has been attributed mainly to the production of genital HIV-1-specific cytotoxic T cells and neutralising IgA [22–24]. Here, we showed that HIV-1-uninfected CSWs had significantly higher levels of MIP-1α in the genital mucosa than did both the HIV-1-infected CSWs and HIV-1-uninfected non-CSW women. Moreover, the serum levels of MIP-1α and MIP-1β were higher in HIV-1-uninfected CSWs compared with those observed in the other groups. These results are in agreement with recent reports demonstrating high copy numbers of CCL3L1 and CCL4L1 genes (encoding MIP-1α and MIP-1β, respectively) were associated with higher serum level of β-chemokines and lower risk of HIV-1 infection [5, 6]. Moreover, it has been shown that in vitro-stimulated peripheral blood mononuclear cells from HIV-1-exposed uninfected individuals produced higher level of β-chemokines compared with those from HIV-1-infected individuals [25].Taken together, these results suggest that natural resistance to HIV-1 could be partly due to a greater capacity to develop a stronger immune response, involving the early release of β-chemokines. Indeed, the production of MIP-1α and MIP-1β affects the outcome of Th1- and Th2-mediated responses and enhances the development of both humoral as well as cellular mucosal and systemic immunity [26, 27].

The levels of MIP-1α and MIP-1β were significantly lower in the genital mucosa than in the blood suggesting that the main site of production of the β-chemokines associated with HIV-1 resistance is not in the cervix or the vagina. Although the mechanism of protection remains unclear, the relatively low level of MIP-1α and MIP-1β in the genital mucosa might reduced the number of $CCR5^{+}$ target cells available for HIV-1 at the initial site exposure. On the other hand, the elevated level of MIP-1α and MIP-1β observed in the serum of the HIV-1-uninfected CSWs might reflect immune activity upstream the female lower genital tract to contain viral dissemination beyond the cervicovaginal barrier. This highlights the importance of enhancing our knowledge of the mucosal immunity at the portal of entry of the virus and its link to systemic immunity in order to develop preventive strategies such as microbicides and vaccines.

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Authors' contribution M. Roger is the lead investigator of this study and with J. Lajoie and J. Poudrier, designed the experiments, performed the analysis and wrote the manuscript. J. Lajoie performed the experiments. M. Massinga Loembe, A-C Labbé, F. Guédou and M. Alary were responsible for the participants' recruitment and provided clinical and laboratory data. M. Massinga Loembe and F Leblond were responsible for the sample processing and data collection. All authors edited and approved the final version of the manuscript.

Conflicts of interest The authors declare no conflict of interest.

References

1. Haynes BF, Shattock RJ. Critical issues in mucosal immunity for HIV-1 vaccine development. J Allergy Clin Immunol. 2008;122:3–9.
2. Padian NS, van der Straten A, Ramjee G, Chipato T, de Bruyn G, Blanchard K, et al. Diaphragm and lubricant gel for prevention of HIV acquisition in Southern African women: a randomised controlled trial. Lancet. 2007;370:251–61.
3. Hel Z, McGhee JR, Mestecky J. HIV infection: first battle decides the war. Trends Immunol. 2006;27:274–81.
4. Glass WG, Rosenberg HF, Murphy PM. Chemokine regulation of inflammation during acute viral infection. Curr Opin Allergy Clin Immunol. 2003;3:467–73.
5. Colobran R, Adreani P, Ashhab Y, Llano A, Esté JA, Dominguez O, et al. Multiple products derived from two CCL4 loci: high incidence of a new polymorphism in HIV positive patients. J Immunol. 2005;174:5655–64.
6. Gonzalez E, Kulkarni H, Bolivar H, Mangano A, Sanchez R, Catano G, et al. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. Science. 2005;307:1434–40.
7. Iqbal SM, Ball TB, Kimani J, Thottingal P, Embree JE, Fowke KR, et al. Elevated T cell counts and RANTES expression in the genital mucosa of HIV-1-resistant Kenyan commercial sex workers. J Infect Dis. 2005;192:728–38.
8. Hirbod T, Nilsson J, Andersson S, Uberti-Foppa C, Ferrari D, Maghi M, et al. Upregulation of interferon-α and RANTES in the cervix of HIV-1 seronegative women with high-risk behavior. J Acquir Immune Defic Syndr. 2006;43:137–43.
9. Kaul R, Rebbapragada A, Hirbod T, Wachihi C, Ball TB, Plummer FA, et al. Genital levels of soluble immune factors with anti-HIV activity may correlate with increased HIV susceptibility. AIDS. 2008;22:2049–51.
10. Campbell GR, Spector SA. CCL2 increases X4-tropic HIV-1 entry into resting CD4+ T Cells. J Biol Chem. 2008;283:30745–53.
11. Brainard DM, Tager AM, Misdraji J, Frahm N, Lichterfeld M, Draeneret R, et al. Decreased CXCR3+ CD8 T cells in advanced human immunodeficiency virus infection suggest that a homing defect contributes to cytotoxic T-lymphocyte dysfunction. J Virol. 2007;81:8439–50.
12. Lajoie J, Poudrier J, Massinga-Loembe M, Guédou F, Agossa-Gbenafa C, Labbé AC, et al. Differences in immunoregulatory cytokine expression patterns in the systemic and genital tract compartments of HIV-1 infected commercial sex workers in Benin. Mucosal Immunol. 2008;1:309–16.
13. Gumbi PP, Nkwanyana NN, Bere A, Burgers WA, Gray CM, Williamson AC, et al. Impact of inflammation on cervical human immunodeficiency virus (HIV-1)-specific DC8 T-cell responses in the female genital tract during chronic HIV infection. J Virol. 2008;82:8529–36.

14. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev. 2005;206:306–35.
15. Sugaya M, Loré K, Koup RA, Douek DC, Blauvelt A. HIV-infected Langerhans cells preferentially transmit virus to proliferating autologous CD4+ memory t cells located within Langerhans Cell-T cell clusters. J Immunol. 2004;172:2219–24.
16. Sharkey DJ, Macpherson AM, Tremellen KP, Robertson SA. Seminal plasma differentially regulates inflammatory cytokine gene expression in human cervical and vaginal epithelial cells. Mol Hum Reprod. 2007;13:491–501.
17. Cohen CR, Plummer FA, Mugo N, Maclean L, Shen C, Bukosi EA, et al. Increased interleukin-10 in the endocervical secretions of women with non-ulcerative sexually transmitted diseases: a mechanism for enhanced HIV-1 transmission? AIDS. 1999;13: 327–32.
18. Boulton IC, Gray-Owen SD. Neisserial binding to CEACAM-1 arrests the activation and proliferation of CD4+ T lymphocytes. Nat Immunol. 2002;3:229–36.
19. Fichorova RN, Desai PJ, Gibson FC, Genco CA. Distinct proinflammatory host responses to *Neisseria Gonorrhoeae* infection in immortalized human cervical and vaginal epithelial cells. Infect Immun. 2001;69:5840–8.
20. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. AIDS. 2006;20:73–83.
21. Rebbapragada A, Wachihi C, Pettengell C, Sunderji S, Huibner S, Jaoko W, et al. Negative mucosal synergy between herpes simplex type 2 and HIV in the female genital tract. AIDS. 2007;21:589–98.
22. Kaul R, Plummer FA, Kimani J, Dong T, Kiama P, Rostron T, et al. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. J Immunol. 2000;164:1602–11.
23. Kaul R, Trabattoni D, Bwayo JJ, Arienti D, Zagliani A, Mwanji FM, et al. HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. AIDS. 1999;13:23–9.
24. Devito C, Hinkula J, Kaul R, Kimani J, Kiama P, Lopalco L, et al. Cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed, persistently seronegative subjects. J Acquir Immune Defic Syndr. 2002;30:413–20.
25. Suresh P, Wanchu A, Bhatnagar A, Sachdeva RK, Sharma M. Spontaneous and antigen-induced chemokine production in exposed but uninfected partners of HIV type 1-infected individuals in North India. AIDS Res Hum Retroviruses. 2007;23:261–8.
26. Schall TJ, Bacon K, Camp RD, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1 alpha) and MIP-1 beta chemokines attract distinct populations of lymphocytes. J Exp Med. 1993;177:1821–6.
27. Lillard JW, Singh UP, Boyaka PN, Singh S, Taub DD, McGhee JR. MIP-1 alpha and MIP-1 beta differentially mediate mucosal and systemic adaptive immunity. Blood. 2003;101:807–14.