3. RESULTS

3.1 HIV-1 and herpesvirus loads in blood and semen

HIV-1 RNA load was quantified in semen and blood plasmas from therapy-naïve HIV-1-infected individuals. The median $CD4^+$ T cell count in this cohort was 281 cells/mm^3 (interquartile range [IQR]:185–430). HIV-1 RNA was detected in blood plasma from all the enrolled patients (100%, 74/74) with a median load of 4.58 log_{10} copies/mL (IQR: 3.6–5.2). A significant negative correlation was found between the $CD4^+$ T cell count and the HIV-1 plasma load (ρ = –0.49, p<0.0001) (Fig. 3.1A). In seminal plasma, HIV-1 RNA was detected in 94% of the patients (47/50) with a median load of 3.89 log_{10} copies/mL (IQR: 3–5). There was a significant positive correlation between the HIV-1 loads in blood plasma and semen plasma (ρ=0.64, p<0.0001) (Fig. 3.1B).

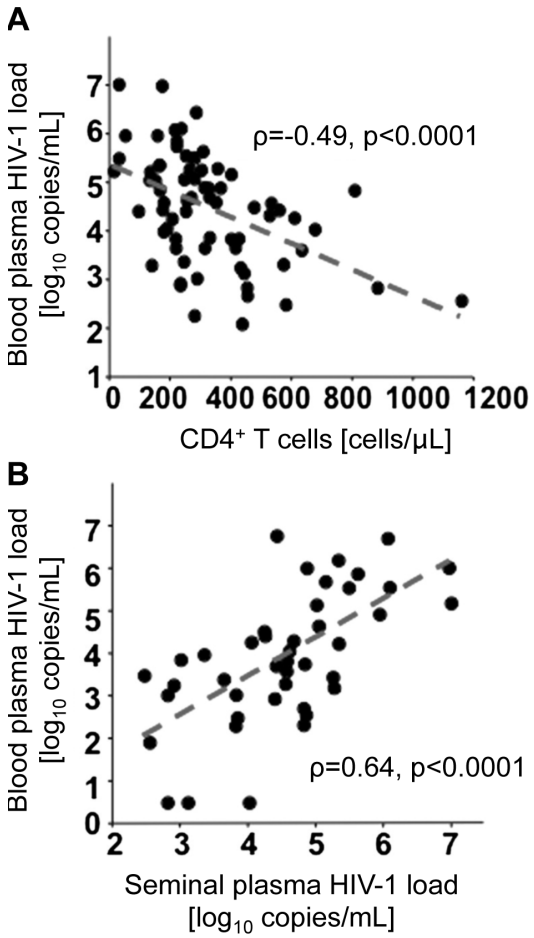

Figure 3.1 HIV-1 loads in blood and seminal plasma.
*Correlation between (**A**) HIV-1 load and $CD4^+$ T cell count in blood plasma;*
*(**B**) HIV-1 load in blood and seminal plasma.*

Seminal and blood plasmas were tested for six herpesviruses known to be associated to HIV-1 infection (HSV-2, EBV, CMV, HHV-6, HHV-7 and HHV-8), and the results are summarized in Table 3.1.

	HIV-1-uninfected		HIV-1-infected	
	Blood plasma	**Seminal plasma**	**Blood plasma**	**Seminal plasma**
HSV-2	0	0	0	4/50 (8%) 2.1 [1.9–2.3]
EBV	0	1/28 (3.5%) 5.5	21/74 (28%)* 3.2 [2.7–3.6]	28/50 (56%)* 3.5 [3.1–4.7]
CMV	0	1/28 (3.5%) 3.2	12/74 (16%)* 3.1 [2.9–3.4]	35/50 (70%)* 5.3 [4.4–6.1]
HHV-6	0	3/28 (6%) 3.7 [3.7–4.5]	0	1/50 (2%) 3.3
HHV-7	0	3/28 (6%) 3.0 [2.2–3.1]	0	6/50 (12%) 2.7 [2.3–3.1]
HHV-8	0	0	0	3/50 (6%) 3.3 [3.1–3.5]

Table 3.1 Frequency of detection and median load (with interquartile range in square parenthesis) of herpesvirus DNA in blood and seminal plasmas from HIV-1-infected and HIV-uninfected men. Viral load are expressed as log_{10} copies/mL. Asterisks denote a significant difference in the frequency of detection between HIV-1-infected and HIV-uninfected individuals ($p<0.01$).

HSV-2 DNA was found in seminal plasma of 8% the HIV-1-infected individuals (median load 2.1 log_{10} copies/mL [IQR: 1.9–2.3]) but in none of their blood plasma samples. No HSV-2 DNA was detected in blood or semen samples from HIV-1-uninfected individuals.

Among the HIV-1-infected individuals, HHV-6, HHV-7, and HHV-8 DNA were detected in 2%, 12%, and 6% of the seminal plasma samples, respectively (median loads, 3.3 [1 patient], 2.7 [IQR, 2.3–3.1], and 3.3 [IQR, 3.1–3.5] log_{10} copies/mL, respectively). Among HIV-uninfected individuals, HHV-6, HHV-7, and HHV-8 DNA were detected in 6%, 6%, and 0% of the seminal plasma samples, respectively (median loads, 3.7 [IQR, 3.7–4.5] and 3.0 [IQR, 2.2–3.1] log_{10} copies/mL, respectively). There was no significant difference in seminal loads of these viruses between HIV-1-infected and HIV-uninfected individuals. None of these herpesviruses was detected in blood plasma from either HIV-1-infected or HIV-uninfected men.

On the contrary, the presence of EBV DNA was strongly associated with HIV-1 infection ($p<0.001$): it was found in 28% (median load, 3.2 log_{10} copies/mL [IQR,

2.7–3.6]) and 56% (median load, 3.5 $\log_{10}$ copies/mL [IQR, 3.1–4.7]) of blood and seminal plasma samples from HIV-1-infected individuals, respectively. Among HIV-uninfected individuals, EBV DNA was found in none of the blood plasma samples and in only 1 (3.5%; 5.52 $\log_{10}$ copies/mL) of the semen samples. Moreover, the frequency of EBV DNA in HIV-1-infected individuals was significantly higher in seminal than in blood plasma (p<0.001). Among the HIV-1-infected individuals with detectable EBV DNA, 54% had EBV DNA in seminal plasma but not in blood plasma, 27% had EBV DNA in both, and the remaining 19% had EBV DNA in blood but not seminal plasma (p<0.01) (Fig 3.2A).

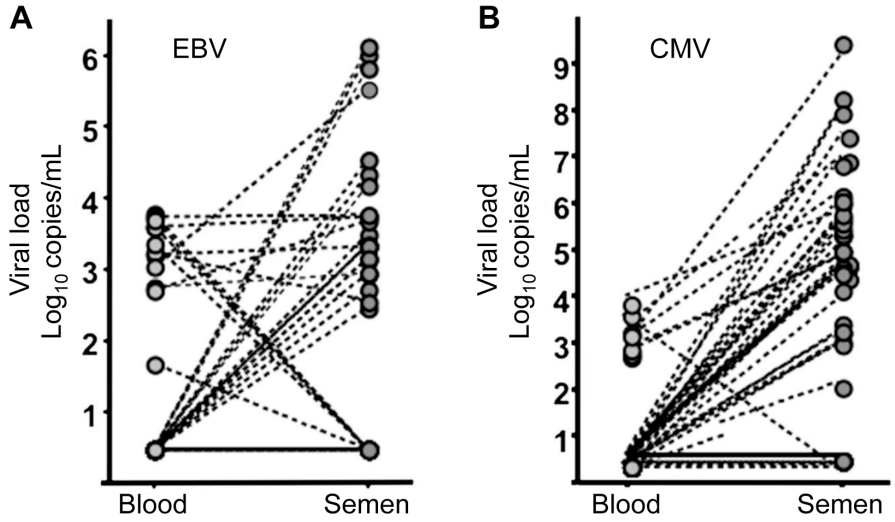

*Figure 3.2 CMV and EBV loads in blood and seminal plasma from HIV-1-infected individuals. For each individual, a dotted line connects the EBV (**A**) and CMV (**B**) loads in blood and seminal plasma. Note that high CMV or EBV seminal plasma loads are often found in patients in whom blood plasma CMV or EBV DNA, respectively, is undetectable.*

As with EBV DNA, the presence of CMV DNA was strongly associated with HIV-1 infection: CMV DNA was found in blood plasma of 16% (median load, 3.12 $\log_{10}$ copies/mL [IQR, 2.9–3.4]) and seminal plasma of 70% (median load, 5.3 $\log_{10}$ copies/mL [IQR, 4.4–6.1]) (p<0.01) of HIV-1-infected patients. In contrast, in HIV-uninfected individuals CMV DNA was not detected in any of the blood plasma samples and in only 1 (3.5%; 3.27 $\log_{10}$ copies/mL) of the seminal plasma samples. Moreover, among the HIV-1-infected patients with detectable CMV DNA, 77.4% had CMV DNA in seminal plasma but not in blood plasma, 19.4% had CMV DNA in both semen and blood plasma, and the remaining 3.2% had CMV DNA in blood plasma but not in seminal plasma (p<0.01) (Fig. 3.2B).

In summary, none of the tested herpesviruses was detected in the blood plasma of the 27 HIV-uninfected individuals. In contrast, either EBV or CMV was found in the blood plasma of 37% of the HIV-1-infected individuals (p<0.001). In 18% of these, both EBV and CMV DNA were detected, in 57% only EBV DNA was detected, and in the remaining 25% only CMV DNA was detected. No significant difference was

found in the blood plasma levels of EBV or CMV DNA between the patients with only one of the viruses and those with both of these viruses. In the seminal plasma of HIV-uninfected individuals, neither HSV-2 nor HHV-8 DNA was found; EBV or CMV DNA was found in 3.5% and HHV-6 or HHV-7 DNA in 6% of the semen samples from these individuals. On the contrary at least one among HSV-2, EBV, CMV, HHV-6, HHV-7, or HHV-8 DNA was found in seminal plasma samples from 92% of the HIV-1-infected individuals. However, of all these herpesviruses, only the frequency of EBV and CMV was significantly higher than in HIV-uninfected individuals ($p<0.01$) (Table 3.1). No significant difference was found in the semen loads of EBV or CMV between the patients with detectable DNA for only one of these viruses and patients with both.

In conclusion, HIV-1 infection was significantly associated with increased frequency of CMV and EBV DNA in both blood and seminal plasma samples. However, in the majority of the patients CMV and EBV were shed in semen even in the absence of viremia, indicating that HIV-1 infection is often associated with a compartmentalized reactivation of CMV and EBV in the male genital tract.

3.2 Relations between herpesviruses reactivation, HIV-1 load and CD4$^+$ T cell count

Because HIV-1 infection was associated with increased frequency and load of CMV and EBV DNA but not of other herpesviruses, we investigated whether these 2 parameters correlate with CD4$^+$ T cell counts and HIV-1 RNA loads. Among HIV-1-infected patients, the presence of CMV DNA was associated with a lower median CD4$^+$ T cell count than in patients in whom no CMV DNA was detected: this difference was statistically significant for blood (178.5 [IQR, 163–296] vs 288.5 cells/mm^3 [IQR, 220–439]; p=0.03) but not for seminal plasma (265 [IQR, 180–397] vs 403 cells/mm^3 [IQR, 219–611]; p=0.08). No significant difference was found in the CD4$^+$ T cell counts between HIV-1-infected patients with and those without EBV DNA in blood or seminal plasma.

On the basis of the pattern of detection of EBV and CMV DNA in semen, we divided the HIV-1-infected patients into 4 subgroups: (1) EBV-CMV dual shedders (17/50; 34%), (2) EBV shedders (11/50; 22%), (3) CMV shedders (18/50; 36%), and (4) non-shedders (neither EBV nor CMV). The HIV-1 seminal loads were significantly different among these 4 groups (p=0.01): in particular, dual shedders had a significantly higher HIV-1 seminal load than non-shedders (p<0.05) (Fig. 3.3A). A similar pattern was found in the levels of blood plasma HIV-1 for these 4 groups of patients (p=0.008) (Fig. 3.3B). Thus, in HIV-1-infected individuals, the concomitant shedding of EBV and CMV in semen is associated with HIV-1 RNA loads in both blood and seminal plasma that are higher than the loads in HIV-1-infected individuals shedding neither of these 2 herpesviruses.

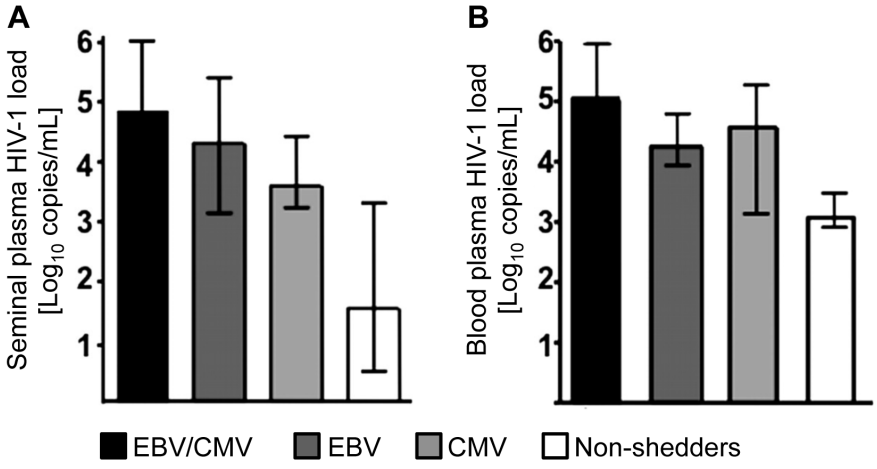

*Figure 3.3 EBV and CMV seminal shedding is associated with higher HIV-1 loads in seminal (**A**) and blood plasma (**B**). Presented are medians and interquartile ranges. On the basis of the pattern of seminal EBV and CMV, the HIV-1-infected patients were divided into 4 subgroups: (black) EBV/CMV dual shedders, (dark grey) EBV shedders, (grey) CMV shedders, and (white) non-shedders (neither EBV nor CMV).*

3.3 Cytokine levels in blood and semen

To evaluate the immunological profile of the blood and seminal plasma of HIV-1-infected and HIV-uninfected individuals, we measured the concentrations of 21 cytokines. HIV-1 infection resulted in aberrant production of the cytokines in both seminal and blood plasma samples compared with those from HIV-uninfected individuals. In seminal plasma of HIV-1-infected individuals, there was a significant up-regulation of 16 of the 21 measured cytokines: IL-1α, IL-1β, IL-6, IL-7, CXCL8, IL-16, CCL2, CCL11, CCL3, CCL4, CCL5, CCL20, CXCL9, CXCL10, CXCL12b, and TGF-β (p<0.05) (Fig.3.4A and B). In blood plasma, HIV-1 infection was associated with an altered production of 9 of the 20 analyzed cytokines: whereas CCL4 and CXCL12b were significantly down-regulated, we found a significant up-regulation of IL-16, CCL20, CCL2, CCL11, CXCL9, CXCL10, and TNF-α (p<0.05) (Fig. 3.5A and B).

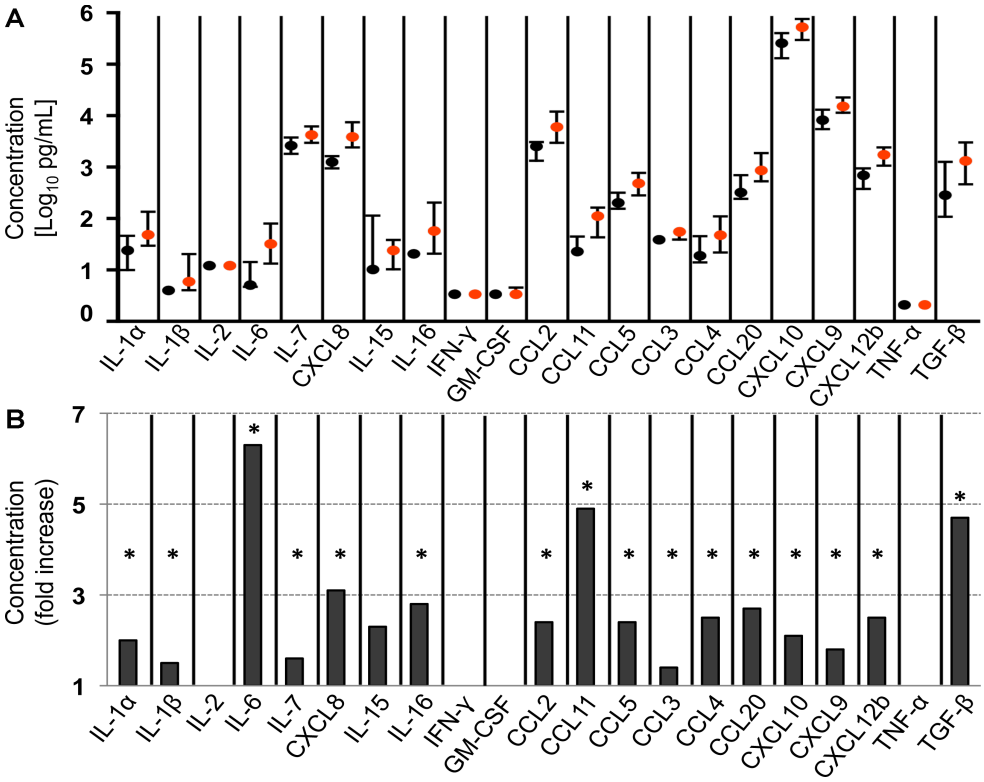

*Figure 3.4 HIV-1 affects the cytokine levels in seminal plasma. (**A**) Absolute concentration of cytokines in seminal plasma of HIV-uninfected (black dots) and HIV-1-infected (red dots) individuals. Values are presented as medians and interquartile ranges. (**B**) Average increases of the cytokine concentration in seminal plasma of HIV-1-infected compared to HIV-uninfected individuals. Values are presented as ratio of the medians. Asterisks denote a significant difference between median levels of cytokine in HIV-uninfected and HIV-1-infected individuals.*

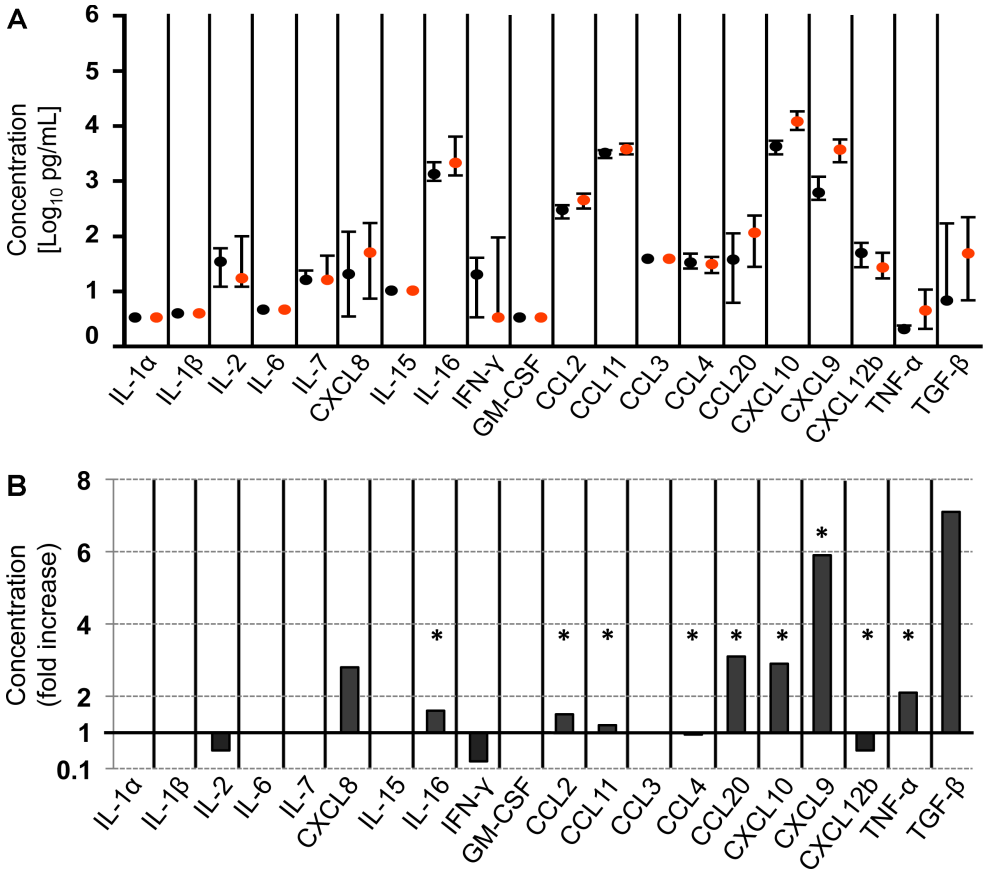

*Figure 3.5 HIV-1 affects the cytokine levels in blood plasma. (**A**) Absolute concentration of cytokines in blood plasma of HIV-uninfected (black dots) and HIV-1-infected (red dots) individuals. Values are presented as medians and interquartile ranges. (**B**) Average increases of the cytokine concentration in blood plasma of HIV-1-infected compared to HIV-uninfected individuals. Values are presented as ratio of the medians. Asterisks denote a significant difference between median levels of cytokine in HIV-uninfected and HIV-1-infected individuals.*

Even in the absence of HIV-1 infection, we found that the cytokine spectra in blood and semen were profoundly different because seminal plasma of HIV-uninfected individuals was enriched in IL-1α, IL-7, CXCL8, CCL2, CCL20, CXCL9, CXCL10, CXCL12b, and TGF-β (p<0.0001), whereas blood plasma was enriched in IL-2, IL-16, CCL4, and CCL11 (p<0.0001) (CCL5 was excluded from the analysis because the nonspecific spontaneous degranulation of this chemokine from platelets upon venipuncture renders its measurement not representative of its specific release [221]. Figure 3.6 displays the compartmentalization of cytokines by paired sample ratios of seminal plasma to blood concentrations. HIV-1 infection was associated with an increased compartmentalization of most of the cytokines. In samples from HIV-1-infected patients, the median ratios of seminal plasma to blood plasma concentrations for 11 cytokines were significantly different from those in HIV-uninfected individuals: IL-1α (10.2 vs 4.6), IL-1β (1.2 vs 1.0), IL-6 (6.0 vs 1.0), IL-7 (204.0 vs 110.0), CCL2 (14.9 vs 7.5), CCL11 (0.03 vs 0.01), CCL3 (1.4 vs 1.0), CCL4 (0.7 vs 0.2), CXCL9 (5.0 vs 10.3), CXCL12b (44.5 vs 7.9), and TNF-α (0.4 vs 0.9) (p<0.05).

In summary, HIV-1 infection had a differential effect on cytokines in blood and seminal plasma, resulting in profound changes in the levels and the compartmentalization of the majority of the evaluated cytokines.

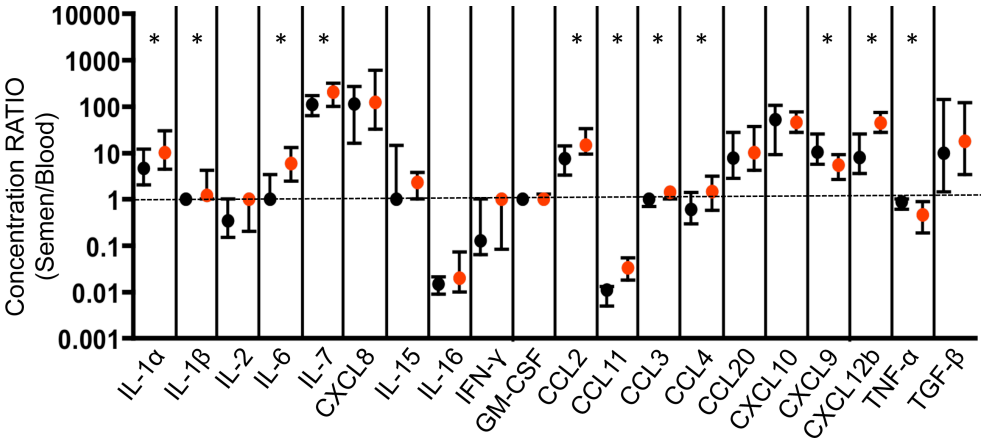

Figure 3.6 HIV-1 infection changes the compartmentalization of cytokines between seminal and blood plasma. Median and interquartile range of ratios of seminal plasma to blood plasma cytokine concentrations (S:B) are shown for HIV-uninfected (black dots) and HIV-1-infected individuals (red dots). Asterisks denote a significant difference between the S:B ratios in HIV-uninfected versus HIV-1-infected individuals. Ratios >1 or <1 indicate enrichment of a cytokine in semen or blood plasma, respectively. Note that HIV-1 infection significantly changes the compartmentalization of 11 of the 20 cytokines measured.

3.4 Effect of shedding of herpesviruses on the cytokine profile in semen

Among the 47 HIV-1-infected individuals whose seminal cytokine levels were measured, 34 (72%) were CMV shedders. In the seminal plasma of these individuals, we found higher levels of CCL5, CCL11, and CXCL9 than in HIV-1-infected individuals not shedding CMV in semen (2.2-fold, 1.8-fold, and 1.2-fold, respectively; $p<0.05$) (Fig. 3.7). Analysis of cytokines in seminal plasma of HIV-1-infected patients revealed no significant differences between 25 patients (53%) who shed EBV in seminal plasma and 22 patients without EBV DNA seminal shedding ($p>0.1$).

Finally, the seminal levels of CCL5 were significantly different ($p<0.03$) among the 4 subgroups: 571 pg/mL (IQR, 325–751) for EBV/CMV dual shedders, 192 pg/mL (IQR, 147–649) for EBV shedders, 622 pg/mL (IQR, 460–767) for CMV shedders, and 267 pg/mL (IQR, 234–292) for non-shedders. Although the post hoc analysis did not reach statistical significance for the pairwise comparisons of the median CCL5 seminal levels in these 4 groups, these data indicate that in semen of HIV-1-infected individuals CMV shedding, independently of EBV shedding, is associated with higher levels of CCL5. No significant differences were found among the 4 subgroups in the seminal levels of CCL11 and CXCL9.

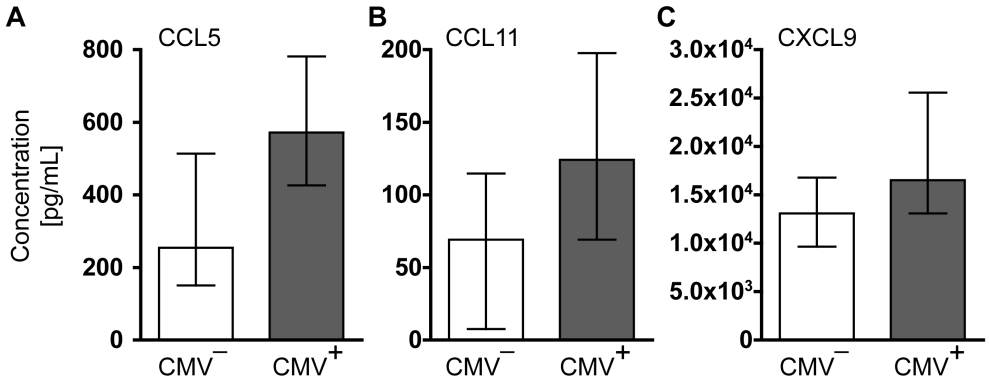

*Figure 3.7 CMV seminal shedding is associated with higher levels of CCL5, CCL11 and CXCL9 in semen. Median and interquartile range of CCL5 (**A**), CCL11 (**B**), and CXCL9 (**C**) are shown for HIV-1-infected individuals who shed CMV (grey) and do not shed CMV (white) in semen.*

3.5 Relations between cytokines, HIV-1 load and CD4$^+$ T cell count

We performed a correlation analyses to reveal possible links between the concentrations of cytokines, HIV-1 viral load, and CD4$^+$ T cell count. We found that the CD4$^+$ T cell count negatively correlates with the blood plasma concentrations of IL-7, CXCL9, CXCL10, and TNF-α (ρ = -0.26, ρ = −0.34, ρ = −0.51, ρ = −0.26, respectively; $p \leq 0.04$). A significant negative correlation was also revealed between the CD4$^+$ T cell count and the seminal plasma levels of CCL2 (ρ = −0.39; p=0.006). In regard to HIV-1 viral load, we found that the seminal HIV-1 RNA levels correlated positively with the seminal concentrations of IL-6, IL-16, CCL2, CCL11, and CXCL12b (respectively, ρ = 0.31, ρ = 0.30, ρ = 0.32, ρ = 0.35, ρ = 0.34; $p \leq 0.05$), while the blood plasma HIV-1 RNA levels correlated positively with the blood plasma concentrations of IL-7, CXCL9, and CXCL10 (ρ = 0.29, ρ = 0.28, ρ = 0.38; $p \leq 0.02$) and negatively with that of IFN-γ (r = −0.32; p=0.008).

3.5.1 Cytokine network in semen and blood

To quantify the interactions between cytokines, we determined whether individual cytokines changed in a coordinated way by measuring the correlations between the production of one cytokine and the production of each of the other cytokines. We deduced the organization of the cytokine network from (i) the Spearman's rank correlation coefficient (ρ) values, which indicate the signs and strengths of correlations between individual cytokines, and (ii) the total number of statistically significant correlations, which reflects the global pattern of cytokine correlations. Figure 3.8 shows the results of our analysis as a 'heat map' in which the signs (positive or negative) and the strengths [weak ($0.3 \leq \rho \leq 0.5$), strong ($0.5 \leq \rho \leq 0.7$), or very strong ($\rho \geq 0.7$)] of the statistically significant correlations are presented.

In both blood and seminal plasmas, HIV-1 infection resulted in a significant modification of the interconnections between cytokines: the midpoints of the cumulative distribution functions of all the correlation coefficients were significantly different in HIV-1-infected and -uninfected individuals (p<0.05). Furthermore, in blood and seminal plasmas of uninfected individuals, there were, respectively, 18 and 21 statistically significant correlations between the levels of individual cytokines. In contrast, in HIV-1-infected patients, there were, respectively, 68 and 72 such correlations (p<0.01). In blood and seminal plasmas of these individuals, respectively, 54 and 57 new correlations were established, and 3 and 4 pre-existing correlations increased their strengths. In contrast, only 10 correlations were lost in both blood and seminal plasmas in the course of HIV-1 infection, respectively 3 and 0 decreased their strength, and 8 and 7 did not change their strength. Manova multivariate analysis confirmed that the measured correlations did not result from random error (p<0.01).

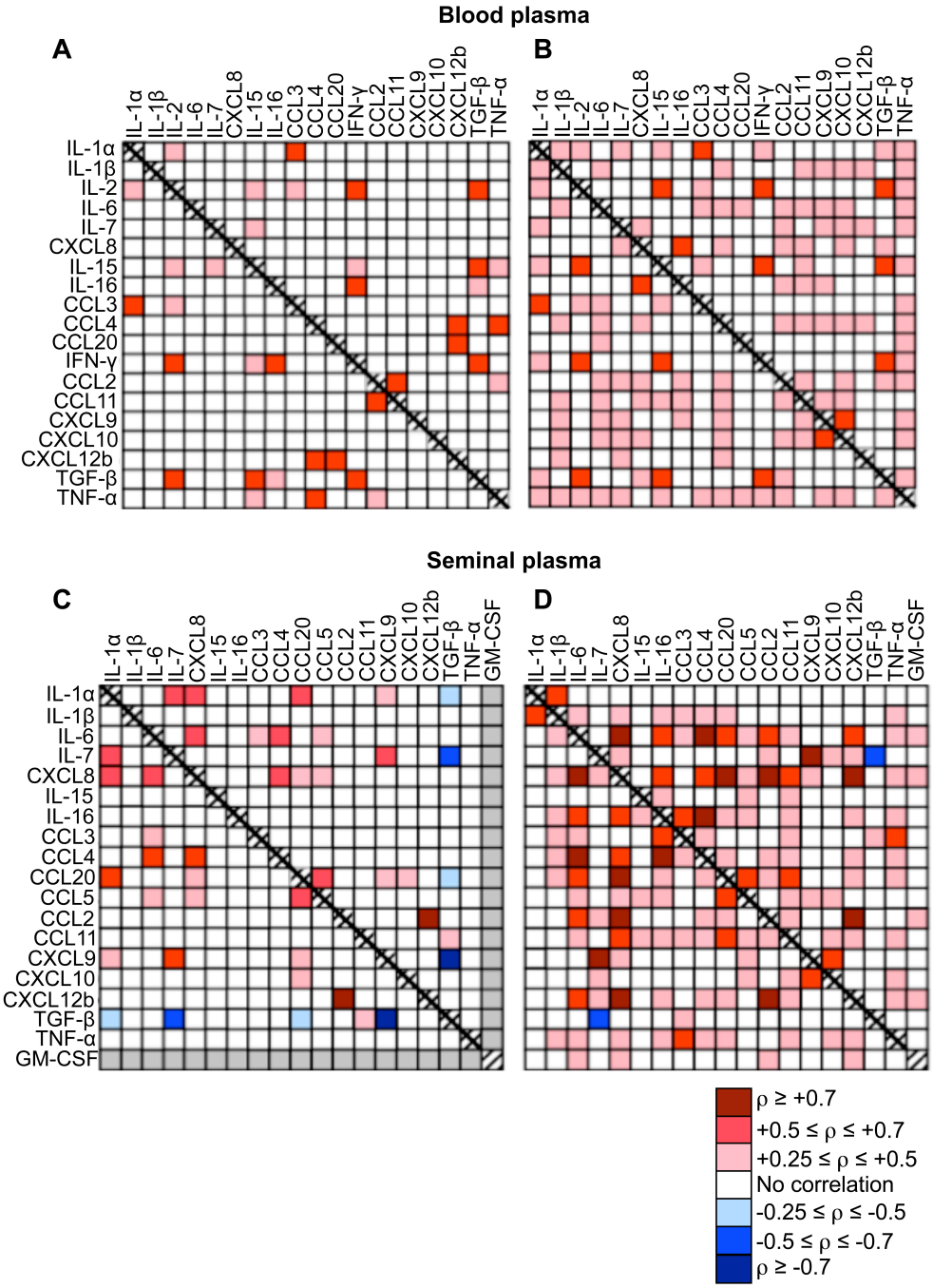

*Figure 3.8 Heat maps of cytokine networks in blood and seminal plasma of HIV-uninfected individuals (**A** and **C**) and of HIV-1-infected (**B** and **D**). Red indicates a positive correlation and blue a negative correlation, calculated as* Spearman`s rank correlation coefficient (ρ). *Strength of correlation is defined by color intensity.*

3.7 Interleukin-7 enhances HIV-1 replication in human tissue explants

The analysis of the cytokine profile of HIV-uninfected individuals showed that IL-7 is 100-fold higher in concentration in semen compared to blood (Fig. 3.6). Moreover a 2-fold increase in IL-7 concentration was observed in semen of HIV-1-infected compared to HIV-uninfected individuals (Fig. 3.4B). Because IL-7 is a key regulator of the central development and peripheral maintenance of T cells, we decided to investigate the effect of IL-7 on $CD4^+$ T cells, the primary target of HIV-1 during vaginal transmission, employing a system of human cervico-vaginal and lymphoid tissue explants. Cervico-vaginal and tonsillar tissues were infected *ex vivo* with HIV-1 and maintained in the presence of IL-7 at concentrations of 5 and 25 ng/mL, values comparable with the median and 2-fold the highest concentrations of IL-7 found in semen of HIV-1-infected individuals (Fig. 3.9).

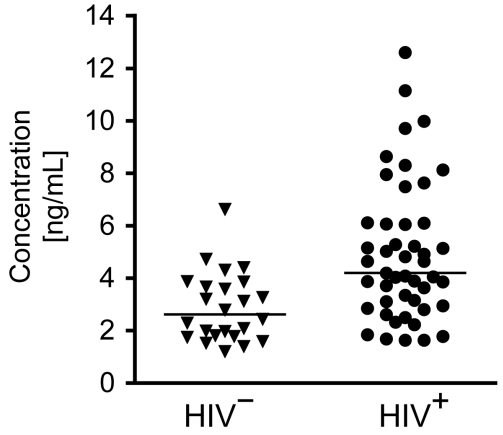

Figure 3.9 Interleukin-7 in semen. The line indicates the median value of IL-7 concentration in semen of HIV-1-infected (4.2 ng/mL, n=47) and HIV-uninfected individuals (2.6 ng/mL, n=24). Note that the highest concentration detected in semen of HIV-1-infected individuals is 12.6 ng/mL.

3.7.1 Lymphoid tissue

Donor-matched lymphoid tissue blocks were inoculated with a CXCR4- or CCR5-utilizing HIV-1 variant, HIV-$1_{LAI.04}$ or HIV-1_{BaL}, respectively, and maintained in culture for 9 days in the absence or presence of recombinant human IL-7 at a concentration of 5 or 25 ng/mL. IL-7 enhanced replication of both HIV-1 variants in tonsillar tissues compared with donor-matched HIV-1-infected control tissues not exposed to IL-7. This enhancement was dose-dependent. Figure 3.10 (A-C) demonstrates the increase of replication of HIV-1_{BaL} and HIV-$1_{LAI.04}$ in tonsillar tissues in the presence of IL-7 at 5 and 25 ng/mL. The absolute cumulative production of HIV-1 in controls varied in tissues from different donors and on average was 12.7 ± 3.1 ng/mL for HIV-$1_{LAI.04}$ and 7.3 ± 0.9 ng/mL for HIV-1_{BaL}. On average, 5 ng/mL of IL-7 significantly enhanced production of HIV-$1_{LAI.04}$ (2.8 ± 0.3-fold; n=3, p<0.01). Also, the production of HIV-1_{BaL} was increased (1.7 ± 0.3-fold; n=3), but did not reach statistical significance (p=0.094). For 25 ng/mL IL-7, this

increase was 10.8 ± 1.8 and 4.4 ± 0.9-fold, respectively (n=13 and 9, p<0.001) (Fig. 3.10C). Also, a similar IL-7-mediated enhancement of HIV-1 replication was observed when the virus inoculum was diluted 100-fold. On average, in these experiments cumulative productions of HIV-$1_{LAI.04}$ and HIV-1_{BaL} were 4.1 ± 2.2 ng/mL and 2.7 ± 1.3 ng/mL, respectively. When these tissues were treated with IL-7 at 25 ng/mL, replication of HIV-$1_{LAI.04}$ increased 10.6 ± 3.4-fold (n=6, p<0.001) and replication of HIV-1_{BaL} 4.2 ± 2.0-fold (n=6, p<0.05).

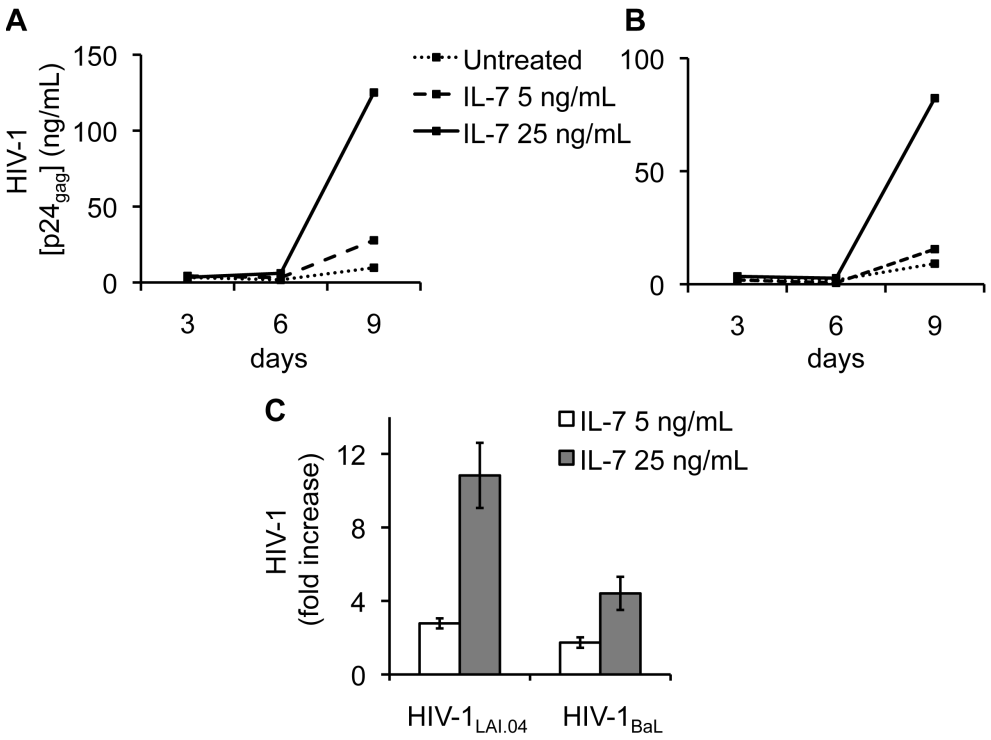

*Figure 3.10 IL-7 enhances HIV-1 production in lymphoid tissue explants. Presented are kinetics of the release of HIV-1 $p24_{gag}$ in culture media of tissue blocks inoculated with HIV-$1_{LAI.04}$ (**A**) and HIV-1_{BaL} (**B**) of one representative donor. Each point represents pooled viral release from 27 tissue blocks over a 3-day period. (**C**) Average increases of the cumulative release of HIV-1 $p24_{gag}$ in culture media of tissue blocks infected with HIV-$1_{LAI.04}$ or HIV-1_{BaL} and treated with IL-7 at 5 ng/mL (n=3) or 25 ng/mL (n=13 and 9), compared with untreated donor-matched tissue blocks (means ± s.e.m.).*

Consistently with IL-7-mediated up-regulation of HIV-1 replication, IL-7 increased the number of HIV-1-infected $CD4^+$ T cells as revealed by flow cytometric analysis of tissue $CD8^-$ T cells stained intracellularly for $p24_{gag}$ antigen (Fig. 3.11A). As we previously described [222], we gated here on $CD8^-$ T cells to account for the HIV-1-induced down-regulation of CD4. At day 9 post-infection, 25 ng/mL of IL-7 increased the number of $CD8^-$ $p24^+$ T cells in HIV-$1_{LAI.04}$- and HIV-1_{BaL}-infected tissues on average 4.1 ± 0.4-fold and 7.7 ± 1.8-fold, respectively (n=8 and 6, p<0.001) (Fig. 3.11B).

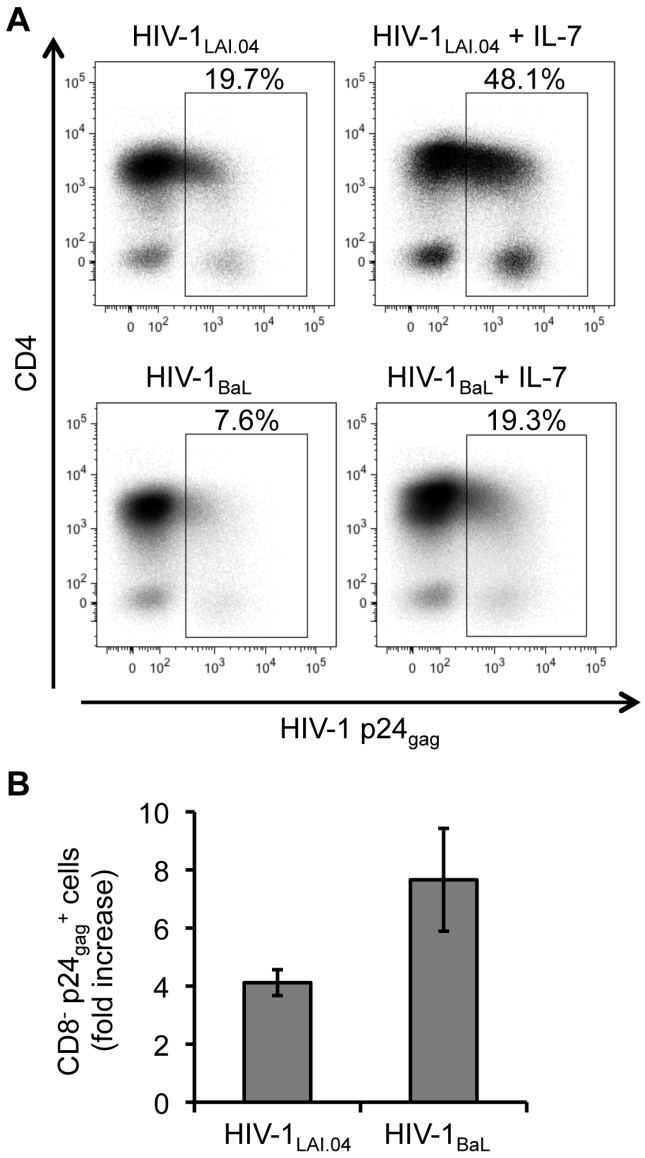

*Figure 3.11 IL-7 increases the number of HIV-1-infected $CD4^+$ T cells in lymphoid tissue explants. (**A**) Presented are dot plots reporting the distribution of $CD4^+$ T cells isolated from tissue blocks treated with IL-7 at 25 ng/mL and untreated donor-matched tissue blocks of one representative donor. The amount of $p24_{gag}^+$ cells is expressed as percentage of total $CD3^+$ $CD8^-$ cells. (**B**) Presented are the average increases in the number of $CD8^-$ $p24_{gag}^+$ cells isolated from tissue blocks infected with $HIV\text{-}1_{LAI.04}$ (n=8) or $HIV\text{-}1_{BaL}$ (n=6) and treated with IL-7 compared with untreated donor-matched tissue blocks (means ± s.e.m.).*

3.7.1.1 Time dependency of IL-7-mediated enhancement of HIV-1 replication

In the above-described experiments, IL-7 was present throughout the entire culture period. Next, we investigated whether a short exposure of the tissue to IL-7 was sufficient to enhance HIV-1 replication. In lymphoid tissue treated with 25 ng/mL of IL-7 overnight prior to infection with HIV-$1_{LAI.04}$ and subsequently maintained in the absence of IL-7, HIV-1 replication was increased 3.1 ± 0.6-fold (n=4, p<0.05) (Fig. 3). In lymphoid tissue pre-treated with IL-7 and maintained in the presence of IL-7 until day 3 post-infection, HIV-$1_{LAI.04}$ replication was increased by 6.3 ± 1.4-fold (n=4, p<0.01) (Fig. 3). Also, a 3-day treatment or pre-treatment with IL-7 enhanced the subsequent replication of HIV-1_{BaL} (respectively 2.8 ± 0.8-fold and 1.6 ± 0.2-fold; n=5, p<0.05) (Fig. 3.12).

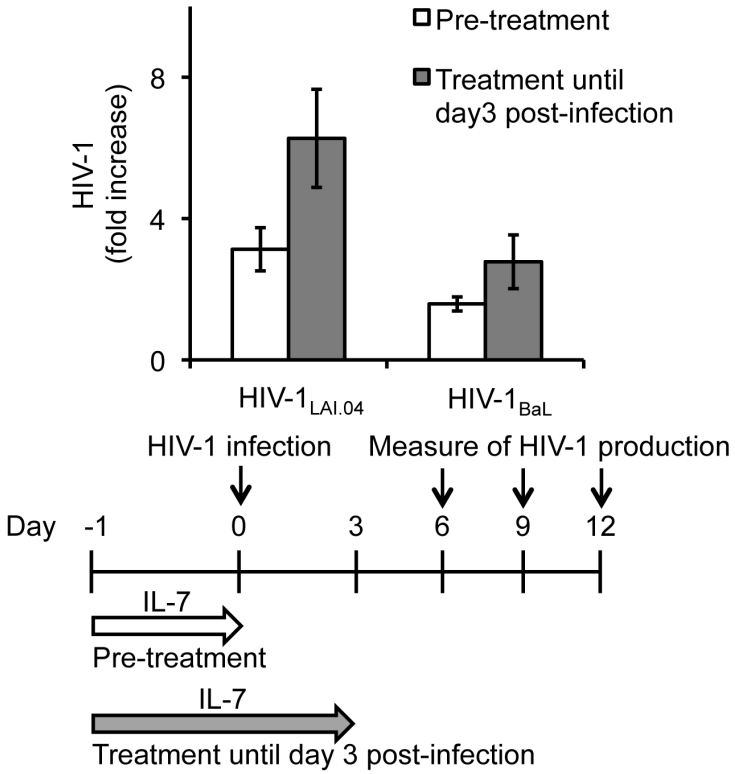

Figure 3.12 Exposure to IL-7 for a short time is sufficient to enhance HIV-1 production. Presented are the average increases of the cumulative release of HIV-1 $p24_{gag}$ antigen in culture media of tissue blocks infected with HIV-$1_{LAI.04}$ (n=4) or HIV-1_{BaL} (n=5) and treated with IL-7 at 25 ng/mL compared with untreated donor-matched tissue blocks (means ± s.e.m.).

3.7.2 Cervico-vaginal tissue

Donor-matched cervico-vaginal tissue blocks were inoculated with the CCR5-utilizing HIV-1 variant HIV-1_{BaL} and maintained in culture for 12 days in the absence or presence of recombinant human IL-7 at 5 or 25 ng/mL. IL-7 enhanced HIV-1_{BaL} replication in human cervico-vaginal tissues, which predominantly support R5 rather than X4 HIV-1 productive infection [143]. This enhancement was first observed on day 9 post-infection and became more prominent on day 12 (Fig. 3.13A). On average, 25 ng/mL of IL-7 increased production of HIV-1_{BaL} 5.5 ± 1.4-fold (n=5, p<0.01). IL-7 at 5 ng/mL also increased HIV-1_{BaL} production (2.1 ± 0.5-fold), but this increase did not reach statistical significance (n=5, p=0.129) (Fig. 3.13B). This enhancement of HIV-1 replication by IL-7 was consistent for cervico-vaginal tissues from different donors and was observed even in tissue in which HIV-1 replication without IL-7 was as small as 75 pg/mL.

As with tonsillar tissues, IL-7 increased the number of $CD4^+$ T cells infected with HIV-1_{BaL}, as revealed by flow cytometric analysis of tissue $CD8^-$ T cells stained intracellularly for $p24_{gag}$ antigen (Fig. 3.13C). On average, on day 9 post-infection IL-7 at 25 ng/mL enhanced the numbers of $CD8^-$ $p24^+$ T cells in HIV-1_{BaL}-infected tissues by 3.0 ± 0.9-fold (n=5, p<0.05).

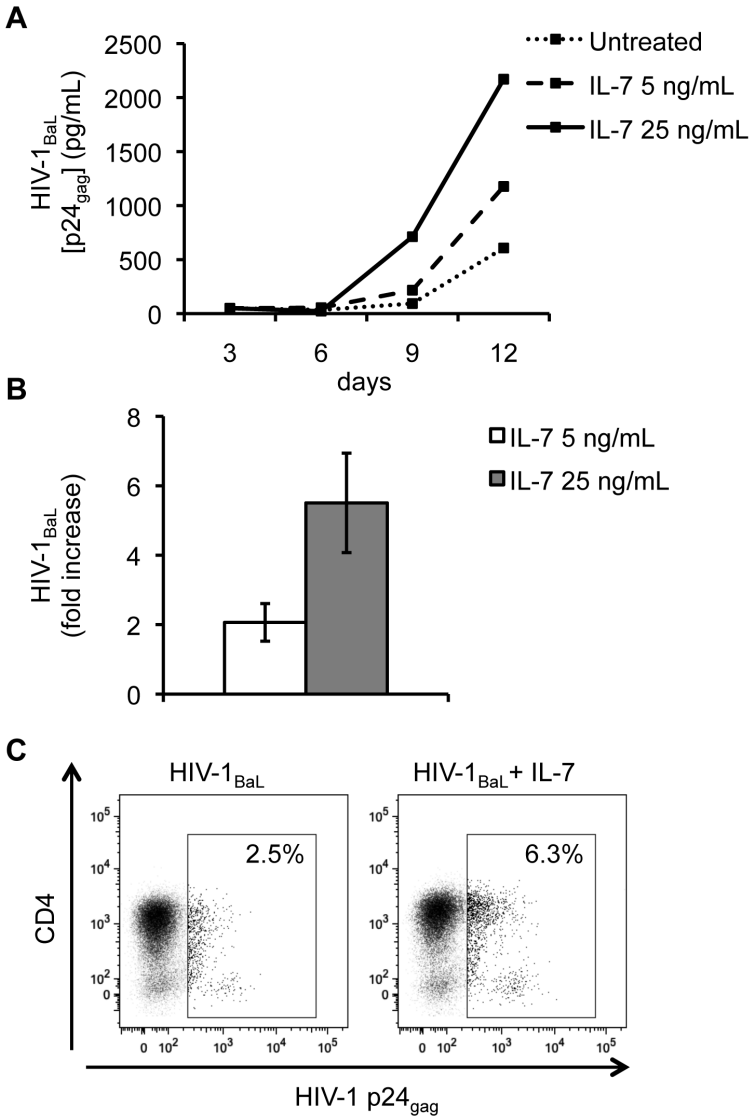

*Figure 3.13 IL-7 enhances HIV-1 production and the number of HIV-1-infected $CD4^+$ T cells in cervico-vaginal tissue explants. (**A**) Presented are kinetics of the release of HIV-1 $p24_{gag}$ antigen in culture media of tissue blocks inoculated with $HIV\text{-}1_{BaL}$ of one representative donor. Each point represents pooled viral release from 24 tissue blocks over a 3-day period. (**B**) Presented are the average increases of the cumulative release of HIV-1 $p24_{gag}$ in culture media of tissue blocks infected with $HIV\text{-}1_{BaL}$ and treated with IL-7 at 5 ng/mL or 25 ng/mL compared with untreated donor-matched tissue blocks (means ± s.e.m. n=5). (**C**) Presented are dot plots reporting the distribution of $CD4^+$ T cells isolated from tissue blocks treated with IL-7at 25 ng/mL and untreated donor-matched tissue blocks of one representative donor. The amount of $p24_{gag}^+$ cells is expressed as percentage of total $CD3^+$ $CD8^-$ cells.*

3.8 IL-7 prevents the death of CD4$^+$ T cells in HIV-1-infected tissues

3.8.1 Lymphoid tissue

We evaluated the effect of IL-7 on the HIV-mediated depletion of tissue CD4$^+$ T cells by comparing the numbers of CD8$^-$ T cells in HIV-1-infected IL-7-treated lymphoid tissue blocks and in untreated controls. Flow cytometric analysis revealed that, on average, HIV-$1_{LAI.04}$ depleted 48.0 ± 5.6% of CD8$^-$ T cells after 9 days of infection, while 25 ng/mL IL-7 treatment for 9 days reduced CD8$^-$ T cell depletion approximately 3-fold to 16.7 ± 7.4% (n=7, p<0.0001).

To investigate whether the decreased depletion in IL-7-treated HIV-1-infected tissues was associated with a lower incidence of CD4$^+$ T cell apoptosis, we compared the expression of the apoptotic marker APO2.7 and the anti-apoptotic protein Bcl-2 in IL-7-treated lymphoid tissues, infected either with HIV-$1_{LAI.04}$ or with HIV-1_{BaL}, with that in infected untreated donor-matched tissues. In tissues treated with IL-7 at 25 ng/mL there was a decrease in the fraction of APO2.7-positive HIV-$1_{LAI.04}$-infected CD4$^+$ T cells (CD8$^-$/p24$^+$) from 10.9 ± 1.0% to 5.8 ± 0.6% and from 7.7 ± 1.1% to 5.0 ± 0.7% at day 6 and 9 post-infection, respectively (n=8, p<0.001) (Fig. 3.14A). Also, a decrease in the fractions of APO2.7-positive CD8$^-$/p24$^+$ T cells was observed in tissues infected with HIV-1_{BaL} from 13.0 ± 1.3% to 8.8 ± 1.3% and from 5.7 ± 1.0% to 4.1 ± 1.1% at day 6 and 9 post-infection, respectively (n=6, p<0.05) (Fig. 3.14B).

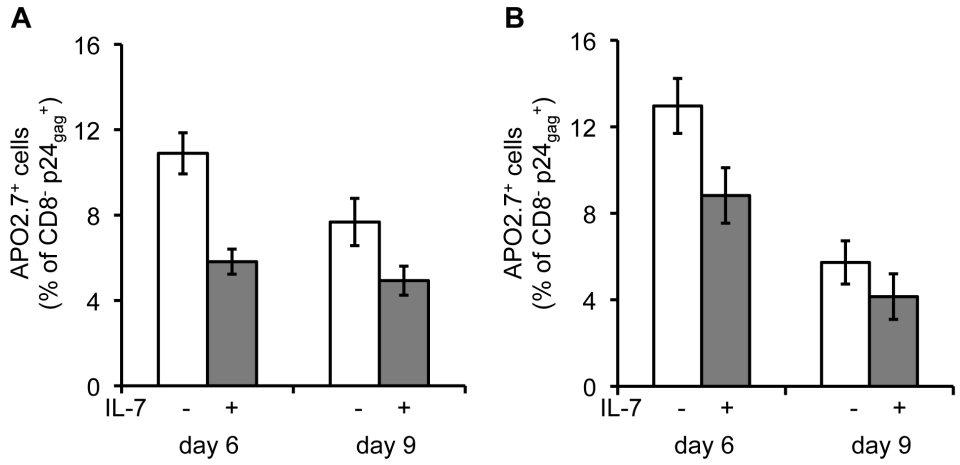

*Figure 3.14 IL-7 decreases apoptosis of HIV-1-infected CD4$^+$ T cells. Presented are the average distributions of HIV-1-infected CD4$^+$ T cells expressing the apoptotic marker APO2.7 isolated from lymphoid tissue blocks infected with HIV-$1_{LAI.04}$ (n=8) (**A**) or HIV-1_{BaL} (n=6) (**B**) treated with IL-7 and untreated donor-matched tissue blocks (means ± s.e.m.). The amount of APO2.7$^+$ cells is expressed as a percentage of total CD8$^-$ p24$^+$ cells.*

Consistently with the down-regulation of the apoptotic marker APO2.7, IL-7 increased the expression of the anti-apoptotic protein Bcl-2 in HIV-1-infected CD4$^+$ T cells. Since Bcl-2 is highly expressed by all mature T cells [223], we compared

the levels of its expression in HIV-1-infected tissues treated or not treated with IL-7 at 25 ng/mL by measuring the median fluorescence intensity (MFI) (Fig. 3.15A). In HIV-$1_{LAI.04}$-infected lymphoid tissues, IL-7 increased Bcl-2 expression in $CD8^-/p24^+$ T cells on average 2.3 ± 0.1-fold and 2.4 ± 0.1-fold on days 6 and 9 post-infection, respectively (n=8, p<0.0001) (Fig. 3.15B). For HIV-1_{BaL}-infected tissues, IL-7 increased Bcl-2 expression in $CD8^-/p24^+$ T cells on average 2.2 ± 0.2-fold and 2.6 ± 0.2-fold on day 6 and 9 post-infection, respectively (n=6, p<0.001) (Fig. 3.15B).

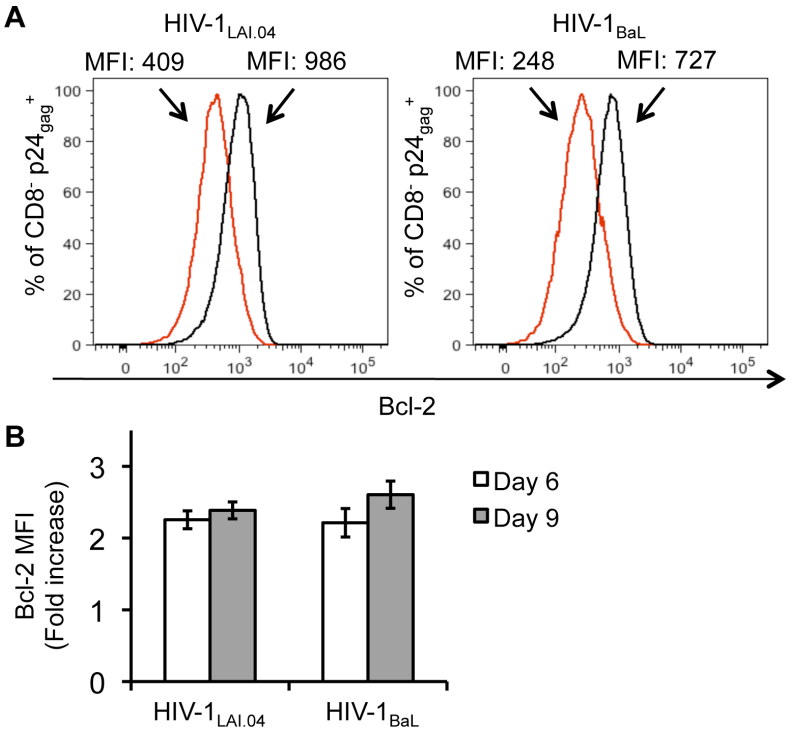

*Figure 3.15 IL-7 up-regulates the expression of the anti-apoptotic factor Bcl-2. (**A**) Presented are the amounts of Bcl-2 expressed by HIV-1-infected $CD4^+$ T cells isolated from lymphoid tissue blocks infected with HIV-$1_{LAI.04}$ or HIV-1_{BaL} and treated with IL-7 (black line) vs. untreated tissue blocks (red line) of one representative donor at day 9 post-infection. The amount of Bcl-2 is expressed as median fluorescence intensity (MFI) value. (**B**) Presented are the average increases of Bcl-2 expression in HIV-1-infected $CD4^+$ T cells isolated from lymphoid tissue blocks infected with HIV-$1_{LAI.04}$ (n=8) or HIV-1_{BaL} (n=6) and treated with IL-7 compared with untreated donor-matched tissue blocks (means ± s.e.m.).*

IL-7 treatment increased the levels of Bcl-2 expression in uninfected $CD4^+$ T cells ($CD8^-/p24^-$) on average 2.5 ± 0.2-fold and 2.7 ± 0.1-fold, at day 6 and 9 respectively, in HIV-$1_{LAI.04}$-infected lymphoid tissues (n=8, p<0.0001) and 2.6 ± 0.1-fold and 3.0 ± 0.1-fold, respectively, in HIV-1_{BaL}-infected tissues compared to untreated donor-matched tissues (n=6, p<0.0001). Consistently, in IL-7 treated tissues the fraction of uninfected cells expressing the apoptotic marker APO2.7

decreased from 10.8 ± 0.9% to 6.6 ± 0.6% and from 11.0 ± 1.2% to 8.3 ± 0.8%, at day 6 and 9 respectively, in HIV-$1_{LAI.04}$-infected tissues (n=8, p<0.001) and from 8.0 ± 0.8% to 5.5 ± 0.7% and from 4.6 ± 0.8% to 3.6 ± 0.8%, at day 6 and 9 respectively, in HIV-1_{BaL}-infected tissues (n=6, p<0.05).

3.8.2 Cervico-vaginal tissue

A similar effect was observed in cervico-vaginal tissues: in tissues infected with HIV-1_{BaL} and incubated with 25 ng/mL of IL-7 the amount of Bcl-2 in $CD8^-/p24^+$ T cells increased on average 1.5 ± 0.1-fold by day 9 post-infection (n=4, p<0.05) (Fig. 3.16 A & B). The anti-apoptotic effect of IL-7 was not limited to HIV-1-infected cells, as Bcl-2 expression was increased 2.0 ± 0.3-fold in uninfected $CD4^+$ T cells ($CD8^-/p24^-$) from HIV-1_{BaL}-infected cervico-vaginal tissues treated with IL-7 (n=4, p<0.05) (Fig. 3.16 A & B).

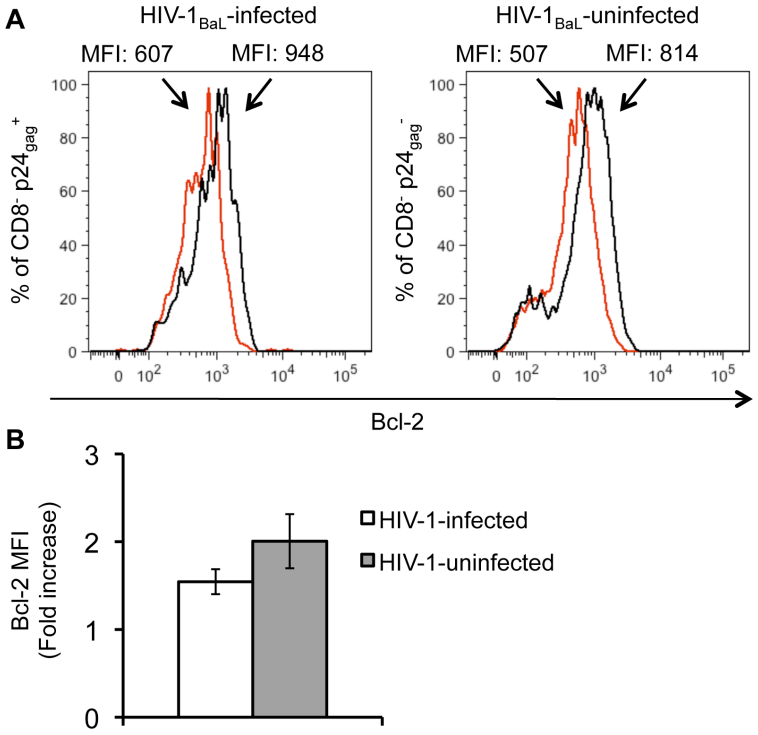

*Figure 3.16 IL-7 up-regulates the expression of the anti-apoptotic factor Bcl-2. (**A**) Presented are the amounts of Bcl-2 expressed by HIV-1-infected and -uninfected $CD4^+$ T cells isolated from cervico-vaginal tissue blocks infected with HIV-1_{BaL} and treated with IL-7 (black line) vs. untreated tissue blocks (red line) of one representative donor at day 9 post-infection. The amount of Bcl-2 is expressed as median fluorescence intensity (MFI) value. (**B**) Presented are the average increases of Bcl-2 expression in HIV-1-infected and -uninfected $CD4^+$ T cells isolated from cervico-vaginal tissue blocks infected with HIV-1_{BaL} and treated with IL-7 compared with untreated donor-matched tissue blocks (means ± s.e.m. n=5).*

4. DISCUSSION

4.1 Herpseviruses and cytokines in blood and semen

A deep understanding of the immunological and virological features of semen and their changes upon HIV-1 infection is of paramount importance for our knowledge of the biology of HIV-1 sexual transmission. To this purpose, we investigated the cytokine profile and presence of 6 herpesviruses, commonly associated with HIV-1 infection, in semen and blood from a cohort of HIV-1-chronically infected individuals naïve to therapy.

Our data indicate that reactivation of herpesviruses occurs as a local phenomenon, because 70% and 54% of HIV-1-infected individuals had CMV and EBV, respectively, in semen in the absence of viremia. Moreover, the median load of CMV in seminal plasma was more than 2 $\log_{10}$ copies/mL higher than in blood plasma. These findings are consistent with a previous report on CMV seminal shedding in HIV-1-infected therapy-naive individuals [186]. Our work indicates that the source of the EBV and CMV seminal shedding is not a systemic reactivation of the infection accompanied by a "spillover" of virus from the blood, but rather their compartmentalized reactivation in the male genital tract, further suggesting that HIV-1 infection may differentially affect semen and blood in their immunological characteristics. We showed that the reactivation of herpesviruses in the genital tract is associated with an increase of HIV-1 seminal load. Future experiments should reveal whether the high HIV-1 load and its immunological consequences promote the reactivation of herpesviruses or *vice versa*. Nevertheless, the association of dual EBV/CMV seminal shedding with a lower $CD4^+$ T cell count may indicate that the HIV-1-mediated loss of immune control over CMV and EBV infections may cause their reactivation, that could remain initially localized and become systemic only afterwards [224, 225].

Reactivation of CMV and EBV in the MGT was accompanied by changes in the cytokine spectrum of semen, which were different than those in blood. Our analysis of 21 cytokines in semen and blood, showed indeed that these two are separate immunological compartments, in which concentrations of cytokines and DNA loads of co-infecting herpesviruses are profoundly different. We found that semen is physiologically enriched with IL-1α, IL-7, CXCL8, CCL2, CCL2, CXCL9, CXCL10, CXCL12b, and TGF-β compared to blood, whereas blood is enriched in IL-2, IL-16, CCL4 and CCL11 compared to semen. With HIV infection, the levels of cytokines were significantly altered in both compartments, compared to HIV-uninfected individuals: in semen we observed up-regulation of 16 of the 21 measured cytokines (IL-1α, IL-1β, IL-6, IL-7, CXCL8, IL-16, CCL2, CCL3, CCL4, CCL5, CCL11, CCL20, CXCL9, CXCL10, CXCL12b, and TGF-β); in blood the levels of CCL4 and SDF-1β were down-regulated and the levels of IL-16, CCL2, CCL11, CCL20, CXCL9, CXCL10, and TNF-α were up-regulated. As a result of these changes, HIV-1 infection emphasized the semen/blood compartmentalization of some cytokines (IL-1α, IL-1β, IL-6, IL-7, CCL2, CXCL12b, and TNF-α) whereas it reduced that of others (CCL2, CCL4, and CXCL9).

Because the cytokine network in semen is different from that in blood and the increase in the levels of seminal cytokines is not always accompanied by an up-regulation of the same cytokines in the blood, it seems that changes in seminal

cytokine levels occur as a local phenomenon in the male genital tract. Thus, up-regulation of seminal cytokines upon HIV-1 infection may reflect a profound dysregulation of the functional state of immune cells resident in the male genital tract. In particular, the increase in pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, CXCL8, and TNF-α) and chemokines in semen could reflect the recruitment and activation of resident T cells, in addition to other leukocytes such as monocyte and dendritic cells. Of note, the levels of the type II interferon-induced chemokines CXCL9 and CXCL10 were very elevated in semen of HIV-uninfected individuals, although HIV-1 infection further increased their concentration. Our data is consistent with previously reported high concentration of CXCL9 in semen of healthy sperm donor [226], and its natural anti-microbial properties render this cytokine an interesting factor to investigate in transmission of sexual pathogens. Herpesviral genital reactivation is also accompanied by changes in seminal cytokine spectrum. Indeed, we found that the seminal shedding of CMV was associated with an increased concentration of the CCR5-ligands CCL5 and CCL11, as well as with an increase of CXCL9 in semen.

It is conceivable that the seminal cytokine spectra altered by local infection with HIV-1, CMV, EBV, and other pathogens that we did not consider, may favor the selection of particular HIV-1 variants. The occurrence of such phenomenon was demonstrated in pig-tailed macaques: in animals co-infected with HHV-6, which up-regulates CCL5, SIV acquired resistance to CCL5 [190]. Seminal cytokines may also play a role in the selection of transmitter/founder HIV-1, which is thought to be a multi-stage process starting in the male genital tract, where local infections and immunologic factors may play an important role in regulating the replication and the transmission of different HIV variants [84, 227].

Co-infecting viruses and cytokines may alter not only HIV-1 replication and evolution in the MGT but also the probability of HIV-1 transmission to the FGT: cytokines and high loads of pathogens in semen may alter the immunological landscape of the genital mucosa when delivered with the ejaculate, modifying the recruitment and activation status of immune cells therein [61]. For example, the β-chemokines CCL3, CCL4, and CCL5, and the CXCR4 ligand CXCL12b may play a dual role in HIV-1 transmission. Increased levels of these cytokines may initially reduce infection by both CCR5-tropic and CXCR4-tropic HIV-1 variants. At the same time, recruitment of immune cells at the site of infection with HIV-1 or other sexually transmitted co-pathogens may provide HIV-1 with more target cells or fuel local expansion of the founder pool of infected cells [228]. Increased levels of other chemokines, pro-inflammatory cytokines and TGF-β, considered the main mediator of the leukocytic reaction occurring in the FGM upon semen deposition, may further contribute to such phenomena.

4.2 Cytokine network in blood and semen

The net effect on HIV-1 replication of a complex network of cytokines is difficult to predict on the basis of the effects of individual cytokines, especially because their actions are often ambivalent. From our analysis, cytokine concentrations correlated with important hallmarks of HIV disease. We found correlations between the concentrations of various seminal cytokines and viral load/$CD4^+$ T cell counts in infected men. In particular, $CD4^+$ T cell count negatively correlated with the blood concentrations of IL-7, CXCL9, CXCL10 and TNF-α, and with the seminal plasma levels of CCL2. In contrast, blood HIV-1 RNA levels correlated positively with the concentrations of IL-7, CXCL9, and CXCL10 in blood, whereas seminal HIV-1 RNA levels correlated positively with the concentrations of IL-6, IL-16, CCL2, CCL11, and CXCL12b in semen. Some of these correlations have not been revealed before, and our data are in agreement with previous reports on the correlation between IL-7 concentration, $CD4^+$ T cell count, and HIV-1 load in blood [229, 230].
To further investigate the cytokine levels in blood and semen, providing a new insight into how the cytokine network is altered in the course of HIV-1 infection, we introduced a quantitative measure of the network deviation from normality: changes in the number of correlations between various cytokines irrespectively of their absolute amounts. For example in semen of both HIV-1-infected and HIV-uninfected individuals, the production of CCL5 positively correlates with the production of CCL20, which is a chemokine that was reported to facilitate HIV-1 infection in the FGT [79, 120]. We showed that, in the course of HIV-1 infection, CCL20 strongly positively correlates with CXCL8, which in its turn up-regulates IL-6, IL-16, CCL4, CCL20, CCL2, CCL11, and CXCL12b (Fig. 3.8). Thus, it appears that CCL5 production correlates with that of several pro-inflammatory cytokines and produces a positive feedback on CCL20 production. On the other hand, the production of a broad immuno-regulatory cytokine like TGF-β [231] negatively correlated with that of IL-1α, IL-7, CCL20, and CXCL9 in HIV-1-negative individuals, while with HIV-1 infection only the negative correlation with IL-7 was maintained. It appears from our results that in the course of HIV-1 infection more positive correlations among pro-inflammatory cytokines are built. This may be reflected in the general immuno-activation observed in HIV-1-infected individuals [32]. Although it is difficult to define the function of the changes induced by HIV-1 infection, our data show that in two immunologically separate compartments as blood and semen are HIV-1 infection induces not only quantitative changes in the levels of individual cytokines but also triggers dramatic qualitative changes in the cytokine network by imposing new and stronger correlations between its elements. We speculate that for efficient functioning, all complex multi-element systems have to be flexible and adaptable, allowing their elements to operate relatively independent. The rigidity of a system of strongly interdependent elements may result in diminished capacity to meet complex external challenges. In our case, HIV-1-triggered rigidity of the cytokine network may reflect the fact that the immune system is ‘globally’ engaged and therefore less adaptable to fight other infections or neoplasms that are frequently associated with HIV-1 disease and result fatal for patients with AIDS.

4.3 The role of seminal interleukin-7 in HIV-1 transmission

As discussed above, we believe that a complex network of factors comprising multiple cytokines, co-infecting pathogens, and other determinants of donor infectivity and host susceptibility to HIV-1 infection account for the global effect that semen can play in HIV-1 transmission. However, we decided to focus our attention on a particular factor in semen due to the relevance of its biologic function in the context of HIV-1 infection. IL-7 indeed plays a major role in modulating T cell central development, and peripheral naïve and memory T cell homeostasis [64, 66]. For this reason, IL-7 is currently being evaluated as a preventative and a treatment for severe lymphopenia in lymphoablative chemo- and radiotherapies and in the course of HIV-1 infection [232].

In agreement with previous reports [55, 84], we found remarkably high levels of IL-7 in semen of HIV-uninfected individuals compared to blood (100 times higher), and that seminal IL-7 was further increased in HIV-1-infected individuals. Despite evidence of strikingly elevated IL-7 levels in seminal plasma little is known on the effect of IL-7 on HIV-1 vaginal transmission. Thus, we simulated the *in vivo* situation by infecting human cervico-vaginal tissue *ex vivo* with HIV-1 in the presence IL-7 at concentrations comparable with those that we found in semen of HIV-1-infected individuals.

We showed that IL-7 significantly enhanced HIV-1 replication in the cervico-vaginal explants from all the donors tested in this study, including one tissue sample in which, according to our criteria, there was no replication in the non-treated tissue. Similarly, we observed an enhancement of HIV-1 replication in HIV-1-infected tonsillar tissue (with both R5 and X4 HIV-1 variants, since unlike cervico-vaginal tissue *ex vivo* [143], tonsillar tissue supports replication of HIV-1 of both phenotypes). This enhancement is independent of the absolute level of viral replication, as it was also observed in tissues infected with a 100-fold diluted viral inoculum. Thus, IL-7 seems to be an enhancer of replication of different HIV-1 variants replicating at different levels in different human tissues. In general, our results are in agreement with the previous reports on IL-7-mediated enhancement of HIV-1 replication in primary mature thymocytes [223] and in PBMCs isolated from chronically infected patients, upon *in vitro* stimulation [233]. Also, it was reported that IL-7 is able to induce HIV-1 permissiveness in quiescent T cells [234] and can reactivate latent HIV-1 in resting $CD4^+$ T cells isolated from infected individuals [235, 236]. In our experiments, although the magnitude of HIV-1 enhancement was proportional to the length of exposure to IL-7, we found that IL-7 does not need to be present during the entire culture period to up-regulate HIV-1 infection. Moreover, when tissues were exposed to IL-7 prior to HIV-1 infection only, subsequent HIV-1 replication was enhanced. These data, extrapolated to male-to-female *in vivo* HIV-1 transmission, suggest that a high concentration of IL-7 in semen may render the female lower genital tract mucosa more susceptible to HIV-1 acquisition.

Our data indicate that IL-7 prevents $CD4^+$ T cell depletion, likely by suppressing apoptosis. Indeed, at day 9 post-HIV-1-infection *ex vivo*, 48.0 ± 5.6% of $CD4^+$ T cells were depleted, whereas in IL-7-treated lymphoid tissue only 16.7 ± 7.4% of $CD4^+$ T cells were depleted. Consistently with IL-7-mediated prolongation of the life of $CD4^+$ T cells [64], we observed a general and persistent increase in the expression of the anti-apoptotic protein Bcl-2 in $CD4^+$ T cells, both infected and

uninfected. Another evidence of suppression of apoptosis by IL-7 was the decreased number of infected $CD4^+$ T cells expressing the apototic marker APO2.7 compared to untreated tissues. IL-7 not only prolonged the life of the cells that replicate virus, thus allowing a continuous release of HIV-1, but it also suppressed apoptosis in the neighboring uninfected $CD4^+$ T cells, thus providing HIV-1 with more potential targets. These effects may be important for HIV-1 vaginal transmission since, unlike lymphoid tissue, the FGM contains a relatively low number of $CD4^+$ T cells [143]. Therefore, prolongation of the lifespan of HIV-1-infected cells, as well as uninfected cells, will result in expanding the pool of infected cells, thus increasing the risk of HIV-1 acquisition [228]. The increase in the number of HIV-1-producing cells by suppression of apoptosis may be not the only mechanism through which IL-7 facilitates HIV-1 replication in tissue *ex vivo*. IL-7 was reported to increase cell proliferation *in vivo* and in isolated cell cultures [230, 237, 238], induce HIV-1 LTR transcription [236], up-regulate co-stimulatory molecules [233], and increase the expression of CXCR4 on $CD4^+$ T cells [239-241].

Although we used a system of cervico-vaginal tissue *ex vivo* that preserves its cytoarchitecture and is more adequate than cultures of isolated cells, our study has obvious limitations. In particular, our study focused on IL-7, while other cytokines or seminal components may exacerbate or suppress the effect of IL-7 on HIV-1 transmission. Also, in *ex vivo* cervical tissue there is no recruitment of immune cells, which may play an important role in establishing HIV-1 infection. Cervico-vaginal explants are not polarized and both IL-7 and HIV have immediate access to the inner cervical cells (although *in vivo* HIV-1 also may have access to these cells through microabrasions of the FGM epithelium). Finally, in our model we used cell-free HIV-1, while according to some reports cell-associated HIV-1 also may be transmitted *in vivo* [88].

Administration of IL-7 to HIV-1-infected individuals under highly active antiretroviral treatment (HAART) resulted in increased blood T cell count, although transient blips of HIV-1 replication were observed in some patients [230, 242]. One of these studies reported that subcutaneous administration of IL-7 is well tolerated and leads to a dose-dependent $CD4^+$ T cell increase and a broadening of TCR diversity in HIV-1-infected subjects on HAART [242]. There is no apparent contradiction between these studies and our data, nevertheless, in light of the evidences of continuous shedding of HIV-1 in semen by some patients (see section 1.3.5.4), some concerns rise about the effect of IL-7 on the viral reservoir in the MGT, and consequently on the infectiousness of these individuals and the risk of HIV-1 acquisition for their partners.

5. CONCLUSION

In general, seminal cytokines together with viruses shed in semen may determine the efficiency of HIV-1 sexual transmission, and thus should be considered as a potential target in strategies aimed at decreasing seminal viral load and preventing HIV-1 transmission. Such strategies require a deeper understanding of the complex relationship between the cytokine milieu of semen, HIV-1, and co-infecting pathogens. Our data represent one of the first steps towards this goal. The present work provides new insights in the immunologic and virologic dynamics occurring in the MGT of HIV-1-infected individuals and their eventual role in HIV-1 transmission. Studying blood and seminal plasma samples from a cohort of HIV-1 chronically infected individuals, we showed that the herpesviruses Epstein-Barr virus and cytomegalovirus reactivate preferentially in the male genital tract and the cytokine profile in semen is dramatically altered by HIV-1 infection. Future studies designed to identify the molecular mechanisms of HIV-1-mediated increase in cytokine correlations, as well as multivariate analysis on cytokine production in larger cohorts of HIV-1-infected patients, may reveal critical common factors associated with the regulation of the cytokine network in the course of HIV-1 infection and indicate novel targets for antiviral strategies. Moreover, prospects for enhancing the effectiveness of the physiologic barriers in the female genital tract with preventive interventions will likely improve with a better understanding of the detailed biology of HIV transmission and early viral replication. Our investigation led us to identify the seminal concentration of interleukin-7 as a determinant of HIV-1 transmission to human cervico-vaginal tissue *ex vivo*. Although this data needs further verification using other models, our finding provides interesting perspectives on eventual outcomes of the clinical trials that currently employ IL-7 to reconstitute the T cell pool of HIV-1-infected individuals. Human tissue explants, with all their limitations, represent an extraordinary tool to study early events in HIV transmission, and we are working to develop a system of infection with HIV-1 in the context of seminal plasma or whole semen. It was shown that it is not possible to culture isolated cells in the presence of undiluted semen, and for long periods of time, because of its toxicity. This effect may depend on the inadequacy of such system, and may be overcome by using human tissues *ex vivo*, which retain their structure and cellular organization, although our system of cervico-vaginal tissue is non polarized and epithelium continuity is disrupted. Nevertheless, the possibility to infect tissue in the presence of seminal plasma will allow us to better mimic the events occurring during HIV-1 transmission in their natural complexity.

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