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Genital-plasma cytokine gradients in HIV+ pregnant women & systemic T cell activation

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BACKGROUND

•The aim of this work is to explore genital mucosa and systemic immunology in HIV-infected pregnant women and how this correlates with gestational age at delivery, in order to delineate potential mechanisms behind the excess of preterm birth in this group.

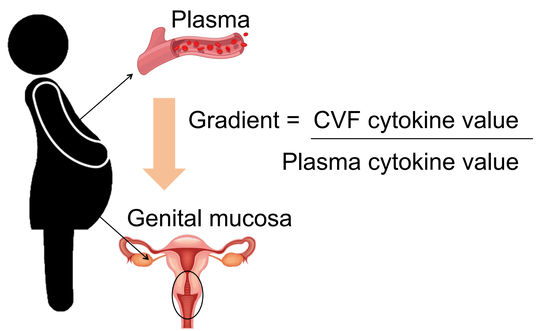
•Term and preterm labour are inflammatory events characterised by up regulation of Th1/pro-inflammatory cytokines at the maternal foetal interface[1].

•Early data indicate that peripheral blood mononuclear cells (PBMCs) migrate to the genital tract to influence inflammation[2].

•The genital mucosa-systemic cytokine gradient has been proposed as a surrogate for the migration of PBMCs to the genital mucosa[3].

•No data in HIV-infected pregnant women are available.

•We characterized the gradient of cervicovaginal (CVF) cytokines to plasma cytokines in HIV-infected and uninfected pregnant women.



METHODS

•A prospective observational multi-site study. CVF and whole blood (for plasma and PBMCs) were isolated from HIV uninfected (n=27) and HIV-1 infected (n=48) pregnant women during the 2nd trimester.

•Exclusion criteria were CD4 count <350 cells/mm^3, multiple or IVF pregnancy and injecting drug user.

•Multiplex immunoassays were used to measure concentrations of 10 cytokines in CVF and plasma.

•Flow cytometry was performed for T cell surface markers: CD4, CD8, HLA-DR and CD25.

•Maternal characteristics, immunovirologic parameters and pregnancy outcome were recorded. All HIV-infected pregnant women received cART.

•Genital-plasma cytokine gradients were calculated and compared by HIV status, cART exposure, prematurity and correlations with T cell subsets and gestational age at delivery were explored.

•HIV-infected pregnant women have **high** levels of ***genital inflammation*** v. uninfected pregnant women

•There is a **genital mucosal-plasma cytokine gradient** which may be *modulated* by circulating adaptive immune cells

•Higher **CD25+ T cells** correlate with lower **genital IL-1B** cytokine gradients suggesting a *regulatory role* in local inflammation

•The importance of **T regulatory cells** in *preterm birth* in women with HIV-infection warrants further investigation

RESULTS

•Genital-plasma gradients were greater in HIV-infected versus uninfected pregnant women, see table 1.

•The greatest gradients observed in HIV-infected women were for pro-inflammatory cytokine IL-1β and chemokine IL-8, followed by IL-2. This was primarily driven by elevated CVF cytokines in HIV-infected women compared to uninfected women, see figure 1.

Table 1 Genital-plasma cytokine gradients in HIV+ vs. HIV- pregnant women

Cytokine gradient Median (IQR)	HIV-infected pregnant	Uninfected pregnant	p value
IFN-γ	63 (9-468)	1 (0-4)	<0.0005
IL-1β	17294 (5076-115968)	5675 (663-14654)	<0.0001
IL-2	1068 (325-2304)	5 (3-29)	<0.0001
IL-8	15788 (4347-64995)	1812 (219-7160)	<0.0001
IL-12	464 (86-949)	4 (1-28)	<0.0001
TNF-α	99 (11-316)	1 (0-8)	<0.0001
IL-4	353 (97-9790	2 (1-6)	<0.0001
IL-6	262 (50-742)	49 (9-143)	<0.0001
IL-10	152 (61-467)	2 (0-20)	<0.0001
IL-13	579 (226-1328)	6 (1-34)	<0.0001

Table 2 Baseline immuno-phenotype in HIV+ vs. HIV- pregnant women

Characteristic	HIV- infected pregnant	Uninfected pregnant	p value
CD4+ cell count at entry /mcL, median (IQR)	632 (495-751)	930 (732-1058)	<0.0001
CD8+ cell count at entry /mcL, median (IQR)	665 (526-891)	603 (439-728)	0.089
HIV viral load at entry copies /mL, median (range)	<20 (<20-18522)	NA	-
CD4+CD25+%	25.5 (19.0-30.0)	22.5 (19.0-28.0)	0.337
CD4+HLA-DR+%	8.0 (5.0-10.0)	5.0 (4.0-6.8)	<0.0001
CD8+CD25+ %	5.5 (5.0-7.0)	6.0 (5.0-7.8)	0.440
CD8+HLA-DR+ %	26.0 (18.0-34.8)	13.0 (8.0-19.5)	<0.0001

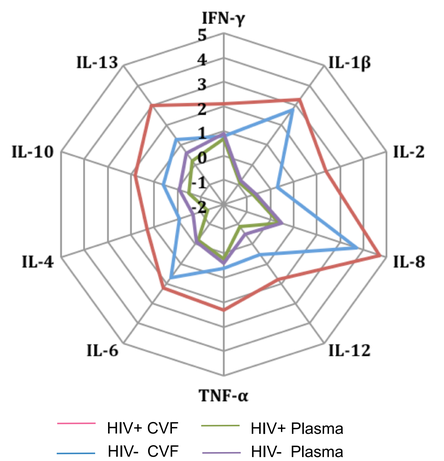


Figure 1. Spider-chart to demonstrate log^{10} cytokine concentration by mucosal and systemic compartment and HIV status

Associations with T cell subsets

CD4% cells were positively correlated with inflammatory IL-2 gradient and immune-regulatory IL-13 in HIV-infected pregnant women. CD4/CD8 ratios were positively correlated with IL-2 gradient in the same women, see figure 2A

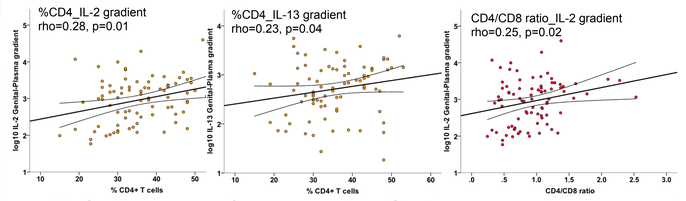


Fig 2A. Scatter graphs of T cell subsets v. Genital-plasma cytokine gradients

CD25+T cell subsets were inversely associated with IL-1β gradient and CD4+HLA-DR+cell% was inversely correlated with IL-2 gradient, see figure 2B.

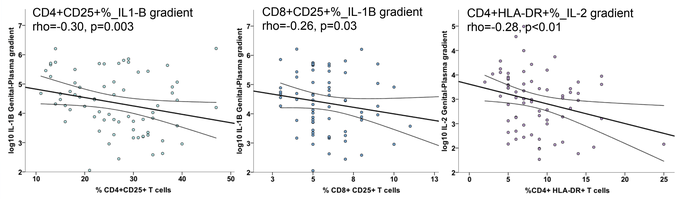


Fig 2B. Scatter graphs of T cell subsets v. Genital-plasma cytokine gradients

No association between genital-plasma gradient with cART or gestational age at delivery was observed, possibly as a result of small sample size.

CONCLUSIONS

•HIV-infected women have elevated genital-plasma cytokine gradients compared to uninfected women, the most prominent gradients being IL-1β and IL-8; both known for their role in the trigger of preterm labour.

•Activated CD25+T cell subsets were inversely correlated with IL-1β gradient suggesting a regulatory role in genital inflammation.

•These data along with other groups[4,5] showing a reduction in T regulatory cells in HIV-infected v. uninfected pregnant women indicate that further study of how this cell type associates gestational age at delivery is desirable.

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