

THE CCL28/CCR3/CCR10 AND THE CCL25/CCR9 CIRCUITS ARE UPREGULATED IN HIV-EXPOSED BUT UNINFECTED INDIVIDUALS

MANUELA BORELLI¹, SERGIO LO CAPUTO², AMBRA HERNIS¹, FRANCESCA VICHI², FRANCESCA FASANO¹, DARIA TRABATTONI¹,
 FRANCESCO MAZZOTTA² AND MARIO CLERICI¹

¹ CHAIR OF IMMUNOLOGY, UNIVERSITY OF MILANO, ITALY, ² OSPEDALE SM ANNUNZIATA, FIRENZE, ITALY.

ABSTRACT

Background: CXCL12, CCL25 and CCL28 are chemokines that play important roles in mucosal immunity by recruiting plasma cells. In particular, CCL25 and CCL28 recruit IgA-secreting cells (ASC) in the mucosal lamina propria (MLP). CXCL12 uses CXCR4 as its receptor whereas ASC respond to CCL25 and CCL28 and migrate into the MLP via the surface receptors CCR9 (CCL25), CCR3 and CCR10 (CCL28). HIV-exposed but uninfected individuals (ESN) are characterized by the presence of HIV-specific IgA in mucosal secretions. We analysed the CXCL12 and the CCL25/28 systems in ESN as well as in healthy, HIV unexposed controls (HC), and HIV-infected individuals.

Methods: CXCL12, CCL25 and CCL28 were quantified in serum, saliva, as well as vaginal or urethral washes of 15 ESN, 10 HC, and 13 HIV patients. CXCR4, CCR9, CCR3, and CCR10 expression was measured in CD3, CD19, and CD14-expressing peripheral blood cells of the same individuals.

Results: HIV-specific CCL25 and CCL28 production was significantly increased in PBMCs of ESN and HIV compared to HC. The percentage of CXCR4, CCR3, CCR9 and CCR10-expressing peripheral CD19+ cells was similarly augmented in ESN and HIV compared to HC. Interestingly, CD19+ cells of ESN expressed a higher density of CXCR4, CCR9 and CCR10 on a per cell basis as compared to both HIV and HC.

Conclusions: The expression of CCR9, CCR3, CCR10 on B lymphocytes as well as the production of their ligand CCL25 and CCL28 were upregulated in HIV-exposed uninfected individuals. A positive correlation was detected between mucosal CCL28 production and mucosal HIV-specific IgA concentration in ESN, suggesting a role of this chemokine in the recruitment of protective antibodies at mucosal sites. These results could therefore explain the IgA expression that characterizes the HIV-exposed but uninfected condition.

BACKGROUND

- CCL25 (TECK) and CCL28 (MEC) play important roles in mucosal immunity by recruiting IgA Ab-secreting cells (ASCs) into mucosal lamina propria.

- Despite multiple repeated exposure to HIV-1 some individuals do not become infected (HIV-Exposed Seronegative Individuals, ESN).

- HIV-specific mucosal IgA were reported to be involved in the generation of the HIV-exposed uninfected status.

- No data are available in ESN on the chemokines involved in mucosal migration of IgA-secreting cells.

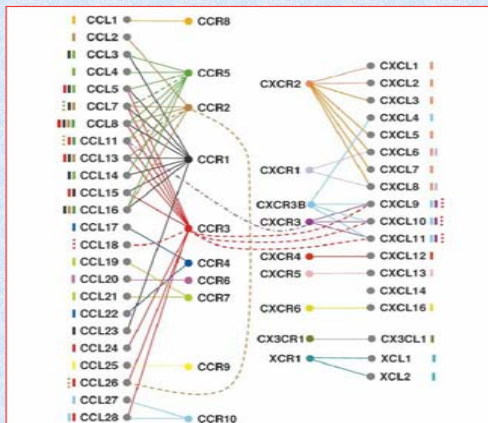
MATERIALS and METHODS

We enrolled in the study:

- 15 HIV-exposed but uninfected individuals, heterosexual partners of HIV-infected patients (ESN)
- 13 HIV-infected patients (HIV)
- 10 healthy controls (HC)

We evaluated:

- the expression of CXCR4, CCR9, CCR3 and CCR10 on CD19, CD3 and CD14 cells
- the amount of CCL25 and CCL28 in PBMCs, saliva, vaginal washes and seminal samples
- CCL25, CCL28, CCR9, CCR3 and CCR10 specific mRNA



AIMS

To verify the involvement of the CXCL12, CCL28 and CCL25 systems in the recruitment of HIV-specific IgA secreting cells in the mucosa of ESN individuals.

Table 1. Epidemiological and clinical characterization of 15 HIV-exposed seronegative individuals and their partners

ID	EXPOSED SERONEGATIVES			HIV-INFECTED PARTNERS				
	Duration of unprotected sex	sex	Last at-risk episode (prior to enrollment)	CD4/ μ l	CD4/CD8 ratio	Viral load (copies/ml)	therapy	CDC stage
1	> 3 years	F	2 weeks	759	0.66	< 50	yes	A2
2	< 1 year	F	1 week	552	0.33	12.000	yes	A1
3	1-3 years	M	1 week	408	0.90	630	yes	A2
4	> 3 years	M	1 week	342	0.47	< 50	yes	A2
5	1-3 years	M	4 months	492	0.63	< 50	yes	A2
6	> 3 years	M	3 days	944	1.05	< 50	yes	A2
7	1-3 years	M	4 days	485	1.57	360	yes	A2
8	> 3 years	F	1 week	598	1.67	< 50	no	A2
9	> 3 years	F	1 week	388	0.24	15.000	yes	A3
10	< 1 year	F	2 months	1060	0.91	22.000	no	A1
11	> 3 years	F	1 week	516	0.60	< 50	yes	A2
12	> 3 years	M	2 days	145	0.00	260	yes	A2
13	> 3 years	F	2 months	387	0.51	< 50	yes	C3
14	> 3 years	F	2 months	98	0.19	220.000	no	A3
15	1-3 years	M	1 month	320	0.89	25.000	yes	A2

RESULTS

CCL25/CCR9 system

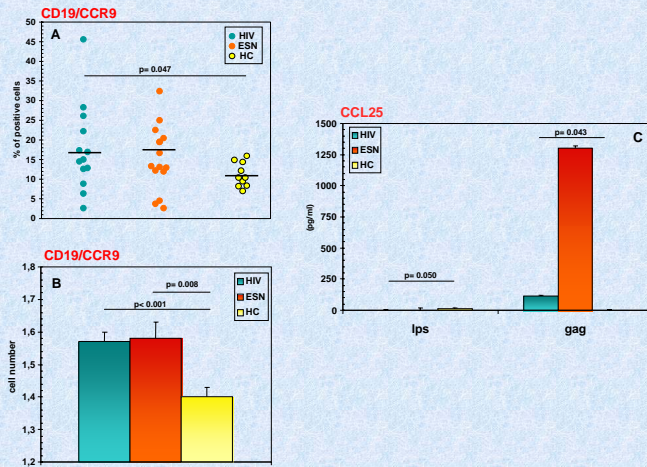


Figure 2. CCR9 expression on CD19+ B cells in HIV-infected patients (HIV), Exposed Uninfected Individuals (ESN) and Healthy Controls (HC). **Panel A:** percentage of positive cells (horizontal lines: mean values); **Panel B:** mean fluorescence intensity (mean values and S.E.). **Panel C:** CCL25 production by PBMC of HIV, ESN and HC upon stimulation with lipopolysaccharides(lps) and HIV-specific antigen (gag) (mean values and S.E., background subtracted).

CCL28/CCR3/CCR10 system

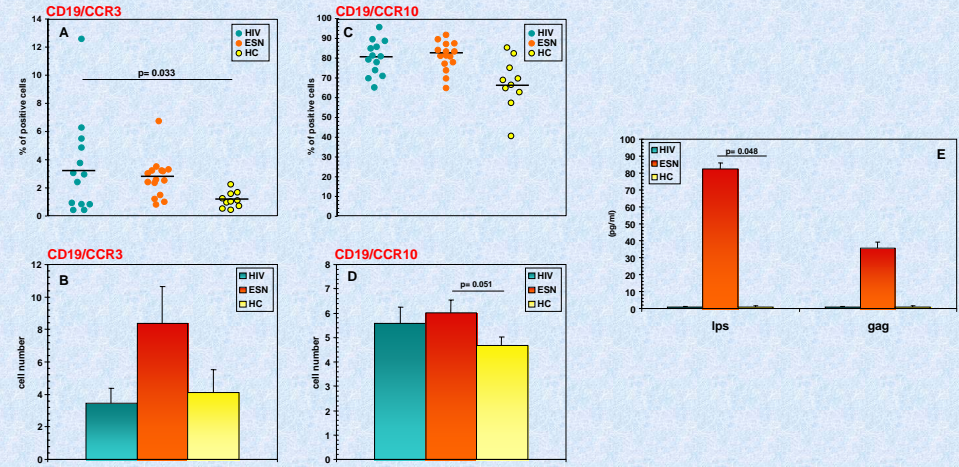


Figure 3. CCR3 and CCR10 expression on CD19+ B cells in HIV-infected patients (HIV), Exposed Uninfected Individuals (ESN) and in Healthy Controls (HC). **Panels A and C:** percentage of positive cells (horizontal lines: mean values); **Panels B and D:** mean fluorescence intensity (mean values and S.E.). **Panel E:** CCL28 production by PBMC of HIV, ESN and HC upon stimulation with lipopolysaccharides (lps) and HIV-specific antigen (gag) (mean values and S.E., background subtracted).

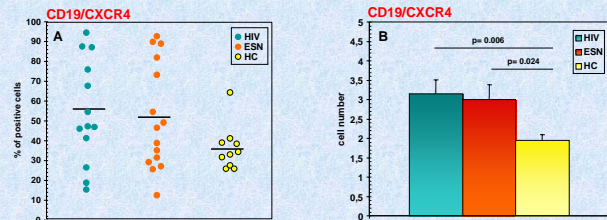


Figure 4. CXCR4 expression on CD19+ B cells in HIV-infected patients (HIV), Exposed Uninfected Individuals (ESN) and in Healthy Controls (HC). **Panel A:** percentage of positive cells (horizontal lines: mean values); **Panel B:** mean fluorescence intensity (mean values and S.E.).

A positive correlation was observed between mucosal CCL28 production and mucosal HIV-specific IgA concentration in Exposed Uninfected Individuals (*Pearson Correlation: $r = 0.908$, $p > 0.01$*)

DISCUSSION

- The expression of CCR9, CCR3, CCR10 on B lymphocytes as well as the production of their ligands, CCL25 and CCL28, is upregulated in HIV-exposed uninfected individuals.
- A positive correlation was detected between mucosal CCL28 production and mucosal HIV-specific IgA concentration in ESN, suggesting a role for this chemokine in the recruitment of potentially protective antibodies at mucosal sites.
- These results could explain the IgA expression that characterizes the HIV-exposed but uninfected condition.