

# **Immune Correlates of Protection in HIV-exposed but Uninfected Heterosexual Males**

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## **ABSTRACT**

**Background:** Despite multiple, repeated exposures to HIV, some individuals never seroconvert. Mucosal and systemic immune correlates of protection have been analysed in HIV-exposed women but no data are available concerning HIV-specific immune responses in exposed but uninfected heterosexual males.

**Methods:** To address this issue, we analysed cellular and humoral immune parameters in peripheral lymphocytes, seminal fluid samples and urethral swab samples of 14 recently HIV-1-exposed uninfected heterosexual (ESN), 7 HIV-infected individuals (HIV) and 7 healthy controls (HC). Concomitant infectious or sexually-transmitted diseases were not present in any of the individuals included in the study.

**Results:** Results show that: 1) HIV-specific IgA were detected in urethral swabs of 11/16 ESN and of 6/7 HIV but not in HC (mean OD: ESN= .37; HIV= .51; HC= .006); 2) HIV primary isolates could be neutralised by seminal fluid of two ESN; 3) env- and gag-specific IFN $\gamma$ -producing CD4+ and CD8+ peripheral T lymphocytes were present in ESN and HIV patients; 4) seminal lymphocytes, but not peripheral blood lymphocytes, of ESN were enriched in activated populations (CD8+38+RO and CD4+25+). p24-specific CTL were correlated to the percentage of CD4+ in the HIV partners. High urethral concentration of HIV-1-specific IgA and in vitro neutralisation of HIV-1 by seminal fluid were seen in those ESN with the most recent unprotected sexual episode.

**Conclusions:** In conclusion our results show that exposure to HIV results in the activation of mucosal and systemic immune mechanisms in heterosexual males in whom infection cannot be detected by virological or clinical parameters. Because these ESN are uninfected despite continuous and repeated exposure, activation of HIV-specific humoral and cellular mucosal and systemic immunity is likely to be protective. These immune mechanisms should be reproduced in vaccine design.

## **BACKGROUND**

- **HIV-specific IgA are detected in vaginal washes and in plasma of HIV-exposed seronegative women, (Mazzoli et al. Nat. Med. 1997; Kaul et al. AIDS 2001).**
- **Mucosal and plasma IgA from HIV-exposed seronegative individuals neutralize different HIV clades, (Mazzoli et al. J. Infect. Dis. 1999; Devito et al. AIDS 2000).**
- **Mucosal and plasma IgA from HIV-exposed uninfected women inhibit HIV-1 transcytosis across human epithelial cells, (Devito et al. J. Immunol. 2000).**

# **OBJECTIVES**

**To analyse the presence and the quality of HIV-specific immune responses in HIV-exposed but uninfected heterosexual males.**

## **MATERIALS AND METHODS (I)**

- Immune parameters were analyzed in urethral swabs, seminal fluids mononuclear cells (SFMC) and PBMC of:

- 14 heterosexual healthy men with repeated and recent (within 3 months and up to one day before the study) sexual exposure to HIV (ESN); concomitant infectious or sexually-transmitted diseases were not present in any of the individuals included in the study.

- 7 HIV-infected individuals (HIV)

- 7 healthy controls (HC)

## **MATERIALS AND METHODS (II)**

- ***HIV-specific IgA:***

  - ELISA (modified Calypte HIV-1 EIA)**

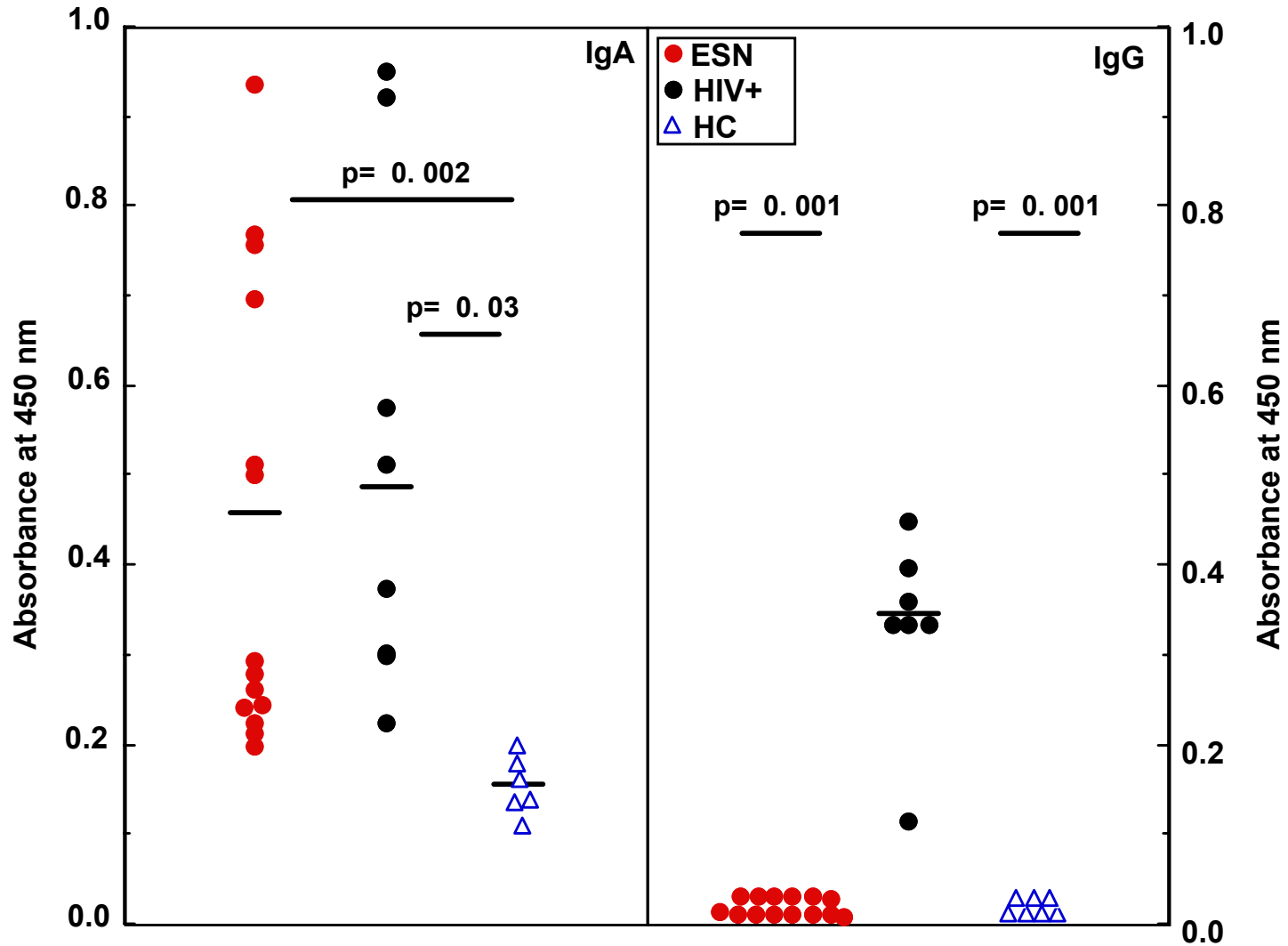
  - Neutralization assay (Resting Cell assay)**

- ***PBMC:***

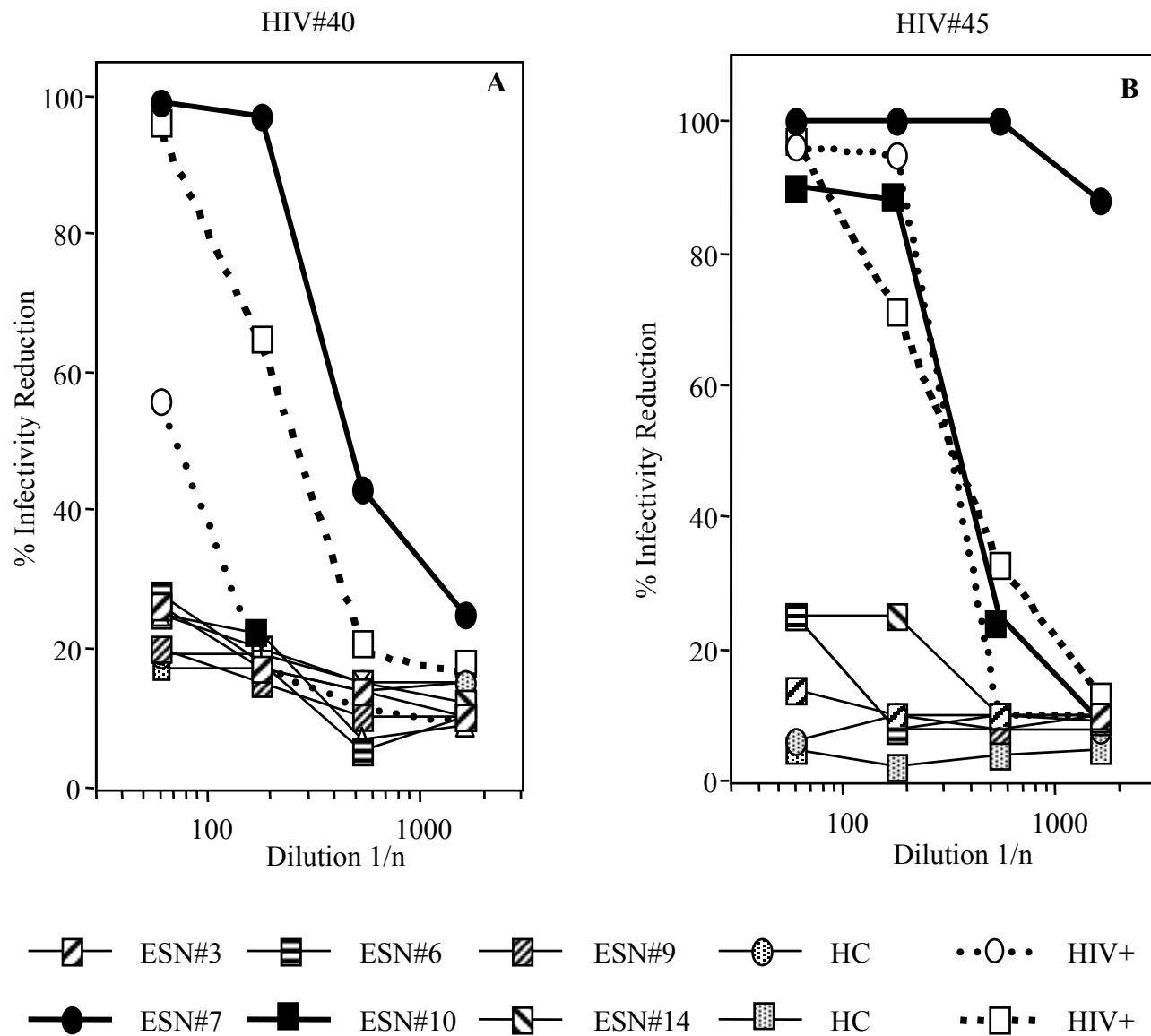
  - Intracellular cytokine production (FACS)**

  - ELISPOT (env, gag p17, gag p24)**

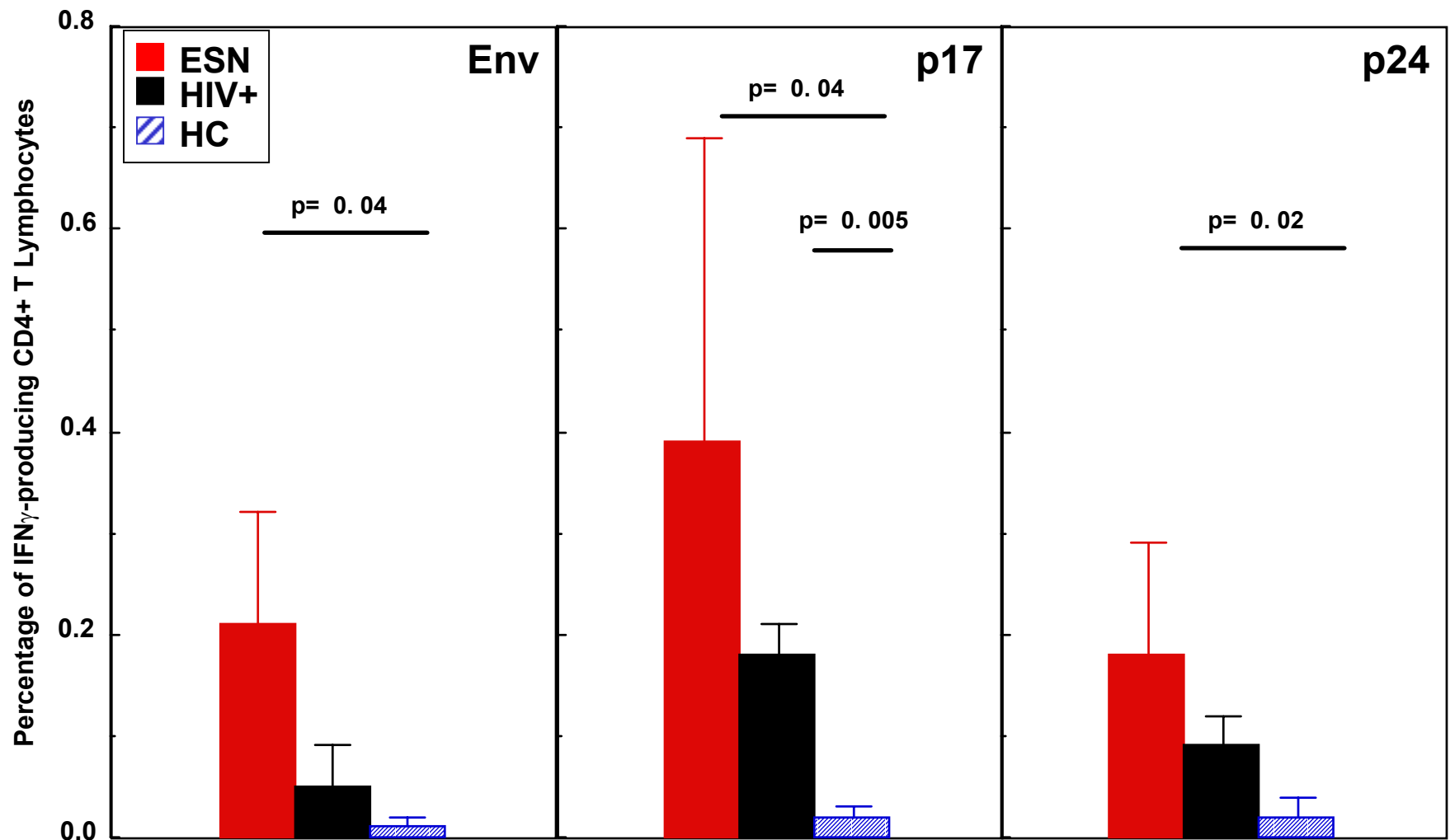
  - Phenotypic markers (PBMC and SFMC)**



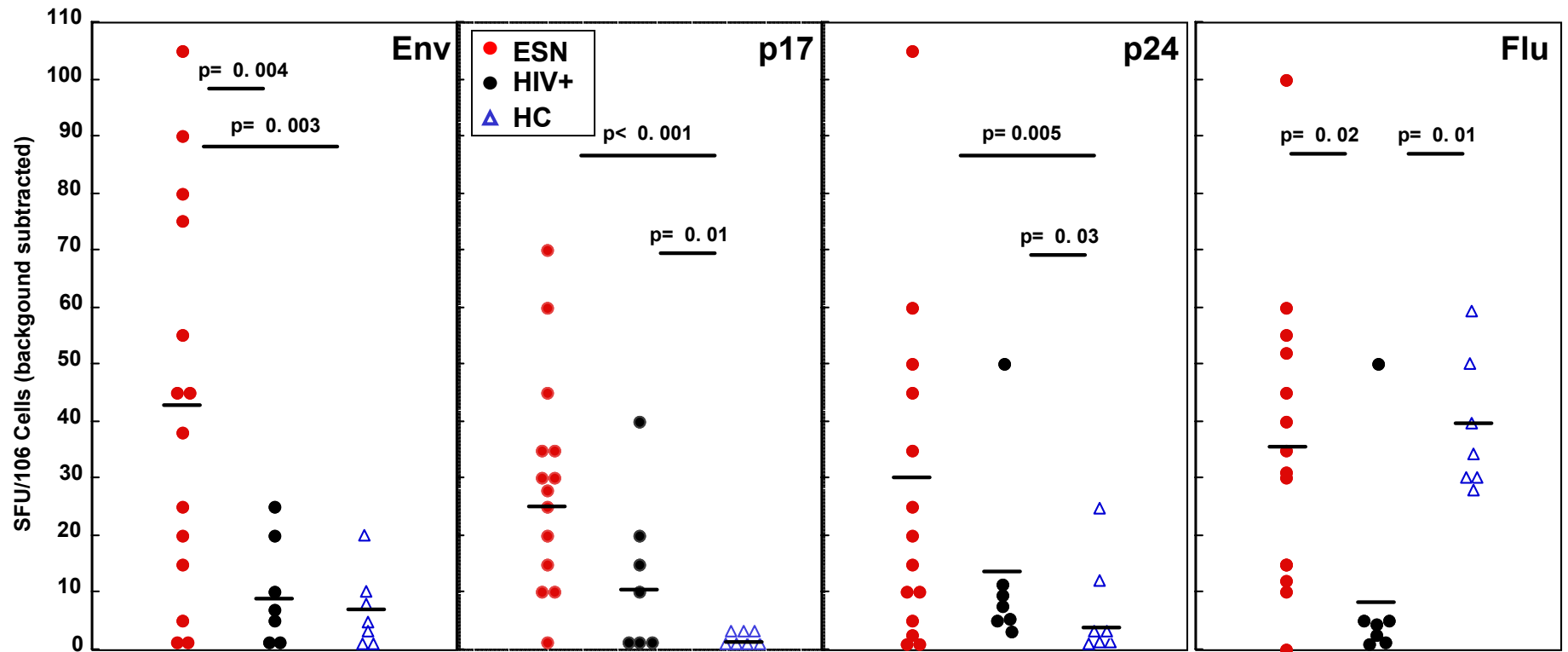
**Fig. 1:** HIV-specific IgA and IgG in urethral swabs of ESN, HIV+ and HC.



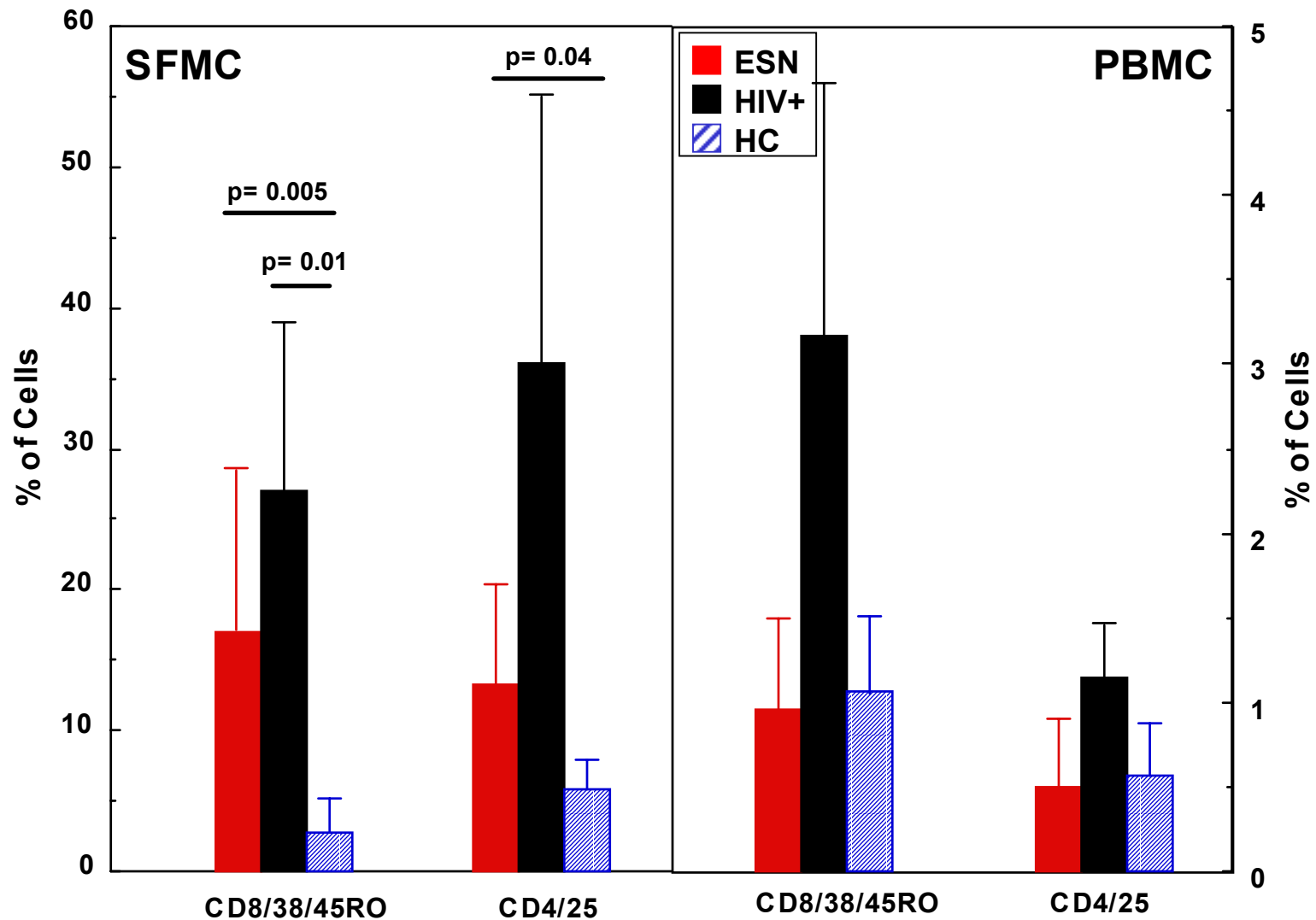
**Fig. 2:** Infectivity reduction of HIV-1 by seminal fluid samples of ESN, HIV+ and HC.



**Fig. 3:** Env-, p17- and p24-IFN $\gamma$ -producing CD4+ T cells of ESN, HIV+ and HC, (mean values  $\pm$  S.D.)



**Fig. 4:** ELIspot analysis of the frequency of env-, p17-, p24-IFN $\gamma$ -producing CD8+ T lymphocytes of ESN, HIV+ and HC, (mean values are indicated by a horizontal line).



**Fig. 5:** Seminal fluid mononuclear cells (SFMC) and peripheral blood mononuclear cells (PBMC) expressing activation markers in ESN, HIV+ and HC, (mean values  $\pm$  S.D.).

ID	Exposed seronegatives males			HIV-infected female partners				
	Duration of unprotected sex	Last at-risk episode (prior to enrollment)	HIV-specific urethral IgA (OD)	CD4/ $\mu$ l	CD4/8 Ratio	Viral load (copies/ml)	Therapy	Route of infection <sup>a</sup>
1	8 Years	1 month	0.263	916	0.4	19440	none	Heterosex
2	11 Years	1 month	0.372	905	1.2	17730	none	CSW
3	7 years	2 months	0.279	385	0.9	<400	D4T+3TC+EFV	Heterosex
4	5 years	4 months	0.246	251	0.2	<400	D4T+3TC+NFV	IVDU
5	9 years	< 1 month	0.404	348	0.4	460	D4T+3TC+ABC	IVDU
6	10 years	4 months	0.211	704	0.7	1860	D4T+3TC+IDV	Heterosex
7	8 Years	1 day	0.696	824	0.7	<400	AZT+DDC+SQV	IVDU
8	8 Years	3 days	0.517	809	0.7	645	D4T+3TC+IDV	IVDU
9	8 Years	< 1 month	0.989	423	0.5	28500	D4T+DDI+ABC	IVDU
10	4 years	2 days	0.758	947	0.8	<400	D4T+3TC+NFV	IVDU
11	5 years	1 week	0.769	889	0.9	<400	D4T+DDI+NFV	Heterosex
12	5 years	1 week	0.933	1270	1.2	<400	none	Heterosex
13	7 years	10 days	0.224	1114	1.1	<400	AZT+3TC	Heterosex
14	8 years	4 months	0.198	1036	1.2	<400	D4T+3TC+IDV	IVDU

<sup>a</sup> CSW= commercial sex worker; IVDU= intravenous drug user

**Table 1. Epidemiological and clinical characterisation of 14 HIV-seronegative heterosexual males and their partners**

## **CONCLUSIONS**

- **Exposure to HIV results in the activation of mucosal and systemic immune mechanisms in heterosexual males in whom infection cannot be detected by virological or clinical parameters.**
- **Because these ESN are uninfected despite continuous and repeated exposure, activation of HIV-specific humoral and cellular mucosal and systemic immunity is likely to be protective.**
- **These immune mechanisms should be reproduced in vaccine design.**