Mucosal and Systemic Immune Responses in HIV/HPV co-infected males

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OBJECTIVE

We investigated anal inflammation, systemic T cell phenotype/activation, and HPV-specific immunity in HIV/HPV co-infected patients (pts) with and without HPV-driven dysplasia; we also studied the association with HPV disease progression and/or clearance at anal site.

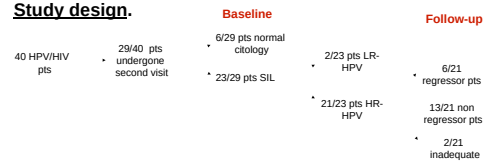
PATIENTS AND METHODS

We consecutively enrolled HIV-positive men asymptomatic for genital distress, who underwent anorectal swabs for HPV-PCR detection and genotyping (high-risk genotypes: HR) and for cytologic abnormalities (according to Bethesda 2001: Low and High Grade Squamous Intraepithelial Lesions, LSIL-HSIL). A subgroup was re-examined at a 18 months follow-up (FU) visit. The following parameters were measured at baseline (BL) and associated with anorectal cytology at follow-up: TNF-alpha, IFN-gamma and IL-2 mRNA were tested at anal site by RT-PCR; CD4 and CD8 T-cell activation (CD25, CD25/HLADRII, CD45R0, CD38/CD45R0), maturation (CCR7/CD45RA) and HPV-16-specific (major capsid protein L1) cytokine production (IL-2, IFN-γ, TNF-α, granzyme, perforin) (flow cytometry).

Comparisons were assessed by Mann-Whitney, Wilcoxon and chi-square test.

RESULTS 1

Study design.



40 HIV-positive male subjects were studied. 27 patients had a SIL (26 LSIL) at baseline while 13 had a normal cytology. 29/40 patients underwent a follow-up visit. 21/29 were diagnosed HR-HPV and LSIL at baseline. At follow-up, 13/21 displayed persistence of LSIL (non-regressor, nR), 6/21 normalised cytology (regressors, R), 2/21 had inadequate results, and were therefore excluded from the analyses.

RESULTS 2

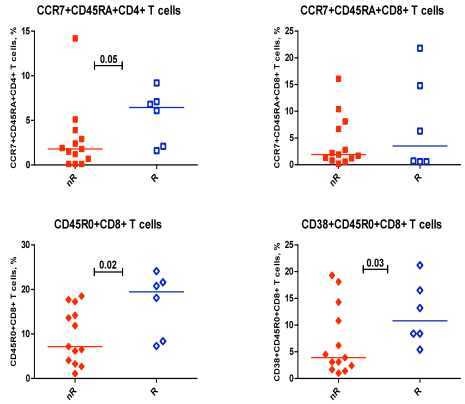
Study population. Table 1 shows the demographic, clinical and immunologic characteristics of the 40 HIV/HPV coinfected patients, divided according to anal cytology. Patients with SIL were more frequently MSM (88% *vs* 46%, p=0.0065) and harboured more frequently HR-HPV genotypes (92% *vs* 53%, p=0.0085). Patients with anal dysplasia showed a tendency to higher proportion of CD8+ circulating T cell count (44.2% IQR 39.1-51.1 *vs* 38.8% IQR36.1-43.9; p=0.056). No significant differences in the peripheral T-cell immunophenotype, in the proportion of HPV-specific T-cell subpopulations and in mucosal TNFα, IFNγ and IL-2 production was shown between patients with normal cytology and patients with SIL (Table 1).

Table 1

Demographic characteristics	Normal cytology (n=13)	SIL (n=27)	Tot (n=40)	p
Age *	51 (34-58)	41 (34-45)	41 (34-49.5)	0.21
MSM°	6 (46%)	24 (88%)	30 (73%)	0.0065
Smoke °	4 (30%)	13 (48%)	18 (45%)	0.33
>25 sexual partners lifelong °	7/9 (77%)	7/16 (43%)	14/25 (56%)	0.20
HAART °	11 (84%)	15 (55%)	26 (66%)	0.08
HAART duration (mths)*	43 (29-74)	65 (7-139)	70 (7.5-137)	0.79
HIV duration (mths) *	43 (28.5-106)	38 (17-120)	41.5 (18-118)	0.76
CD4+ T cells/mmc *	480 (263-548)	518 (417-713)	504 (357-713)	0.10
Nadir CD4+ T cells/mmc *	294 (98-344)	304 (189-460)	304 (189-360)	0.35
CDC C°	3 (23%)	2 (7%)	5 (14%)	0.3
HR-HPV°	7 (53%)	25 (92%)	32 (77%)	0.0085
HCVAb +°	2 (15%)	4 (14%)	6 (17%)	1
HBsAg +°	0 (0%)	1 (3%)	1 (2%)	1
Mucosal TNF-α, n-Fold*	1.6 (0.2-3.6)	0.9 (0.4-3.9)	1.0 (0.3-3.6)	0.81
Mucosal IFN-γ, n-Fold*	1.4 (0.5-4.6)	0.8 (0.3-2.2)	1.1 (0.3-2.5)	0.40
Mucosal IL-2, n-Fold*	1.4 (0.3-5.0)	0.6 (0.3-2.2)	0.7 (0.4-3.2)	0.45
CD4+ %*	18.9 (13.6-25.5)	18.6 (14.9-25.4)	18.6 (14.8-25.4)	0.69
CD8+ %*	38.8 (36.1-43.9)	44.2 (39.1-51.1)	41.8 (36.9-50.7)	0.056
CD4+CD25+ %*	1.9 (1.4-4.7)	1.8 (1.1-3.8)	1.8 (1.2-3.8)	0.58
CD8+CD25+ %*	0.9 (0.5-2.6)	0.8 (0.4-1.2)	0.8 (0.4-1.5)	0.22
CD4+CD25+HLADRII %*	0.7 (0.5-1.7)	0.8 (0.3-1.7)	0.8 (0.4-1.7)	0.95
CD8+CD25+HLADRII %*	0.8 (0.4-1.4)	0.4 (0.3-0.9)	0.5 (0.3-1.1)	0.20
CD8+CD45R0 %*	5.7 (1.9-13.3)	10.2 (4.8-17.7)	8.05 (4.6-15.8)	0.21
CD8+CD38+CD45R0 %*	3.6 (1.8-9.3)	6.5 (3.1-13.2)	5.0 (2.4-10.6)	0.14
CD4+CCR7+CD45RA+ %*	2.9 (0.2-5.3)	2.1 (0.8-6.8)	2.6 (0.7-6.5)	0.78
CD4+CCR7+CD45RA- %*	1.1 (0.4-4.5)	1.5 (0.7-4.7)	1.2 (0.6-4.6)	0.77
CD4+CCR7-CD45RA- %*	60.3 (28.3-94.4)	62.5 (34.7-86)	61.4 (34.2-90.5)	0.95
CD8+CCR7+CD45RA+ %*	1.4 (0.8-2.6)	1.7 (0.6-6.7)	1.5 (0.6-6.3)	0.80
CD8+CCR7+CD45RA- %*	0.3 (0.2- 2.0)	0.4 (0.1-5.6)	0.3 (0.1-2.9)	0.92
CD8+CCR7-CD45RA- %*	37.1 (21.2-55.2)	38.0 (24.6-63.3)	37.3 (22.0-62.1)	0.64
CD8+CCR7-CD45RA+ %*	59.4 (41.9-67.1)	43.9 (23.4-66.2)	48.1 (25.4-65.9)	0.69
CD4+/IL-2 %*	3.1 (1.5-6.9)	3.7 (2.3-6.4)	3.5 (1.9-6.4)	0.56
CD4+/IFN-γ %*	0.8 (0.2-1.6)	0.3 (0.1-1.2)	0.3 (0.2-1.4)	0.44
CD4+/IL-2/IFN-γ %*	0.2 (0.1-1.7)	0.2 (0.1-0.4)	0.2 (0.1-0.7)	0.45
CD8+/TNF-α %*	1.8 (0.3-4.1)	1.2 (0.6-1.9)	1.3 (0.6-2.2)	0.69
CD8+/ IFN-γ %*	1.1 (0.2-2.1)	0.6 (0.4-1.4)	0.6 (0.3-1.8)	0.98
CD8+/TNF-α/IFN-γ %*	0.8 (0.15-1.6)	0.4 (0.2-0.8)	0.4 (0.20-1.1)	0.60
CD8+/Granyme %*	60.9 (46.3-64.5)	79.1 (65.4-86.6)	77.5 (56.8-85.7)	0.15
CD8+/Perforin %*	19.3 (11-28.9)	27.6 (19.1-46.2)	19.2 (12.7-27.4)	0.78
CD8+/Granzyme/Perforin %*	6.5 (3.2-17.5)	11.9 (3.1-20.4)	10.8 (3.2-17.8)	0.62

SIL: squamous intraepithelial lesions; MSM: men who have sex with men; HAART: highly active antiretroviral therapy; CDC C: AIDS classification according to Atlanta Centers of Diseases Control; HR-HPV: High risk human papillomavirus; TNFα: tumor necrosis factor alpha; IFNγ: Interferon gamma; IL-2: interleukin 2. *data are presented as Median (IQR) °data are presented as absolute number (percentage)

Figure 1. T-cell immune phenotype



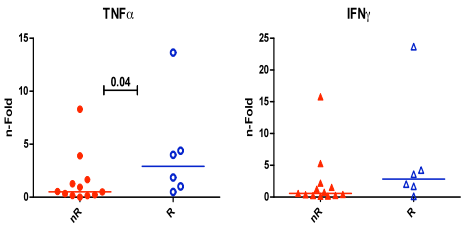
Naïve (CCR7+CD45RA+) CD4+ and CD8+ T cells in Regressors (R) and non Regressors (nR) patients. Memory (CD45R0+) and activated (CD38+CD45R0+) CD8+ T cells in R and nR patients.

RESULTS 3

As compared to nRs, R patients displayed higher naïve CCR7+CD45RA+CD4+ T-cells (6.45% IQR 1.9-7.6 *vs* 1.8% IQR 0.4-3.4, p=.05) and significantly higher memory activated CD45R0+CD8+(19.45% IQR 8.1-22.2 *vs* 7.2% IQR 3.7-15.7, p=.02) and CD38+CD45R0+CD8+ (10.8% IQR 7.6-17.6 *vs* 3.9% IQR 2.05-12.5, p=.03) (Figure 1).

At mucosal level, R patients tended to show higher anal TNFα (2.92% IQR 0.8-6.6 *vs* 0.53% IQR 0.19-2.8, p=.03) and IFNγ transcripts (2.81% IQR 1.2-9.1 *vs* 0.57% IQR 0.2-1.8, p=.08) compared to nR patients (Figure 2).

Figure 2. Cytokines at anal mucosa

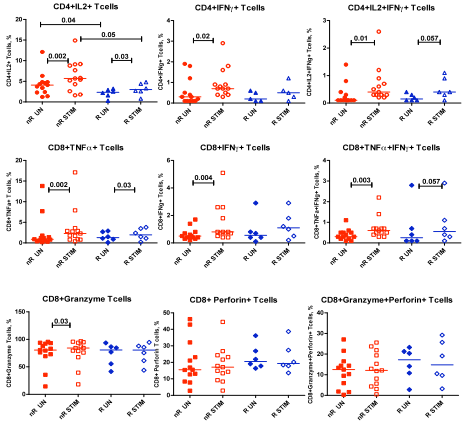


TNFα and IFNγ expression at mucosal site in Regressors (R) and non Regressors patients (nR).

RESULTS 4

Interestingly, nRs were characterized by a significant increase in CD4/CD8 single-positive (CD4+IL2+, CD4+IFNγ+, CD8+TNFα+, CD8+IFNγ+, CD8+Granzyme+), and double-positive (CD4+IL2+IFNγ+, CD8+TNFα+IFNγ+) cells upon HPV-specific stimulus, while R patients significantly expanded only CD4+IL2+ and CD8+TNFα+ populations (Figure 3).

Figure 3. Systemic ex-vivo response to HPV-specific stimulation



Impact of regression/persistence of anal dysplasia on ex-vivo HPV-specific T cell responses. Percentage of CD4+ T cells secreting IL-2, IFN-γ or both , before (UN) and after (STIM) HPV-16 specific stimulation; divided into regressors (R) and non regressors (nR) group. Percentage of CD8+ T cells secreting TNFα, IFNγ or both, UN and STIM; divided into R and nR group. Single and double positive Granzyme and Perforin secreting CD8+ T cells, divided into R and nR group.

CONCLUSIONS

In HIV/HPV coinfected patients, spontaneous clinical regression of HPV-related anal SIL correlate with the following baseline immune *milieu*:

- heightened local inflammation;
- systemic expansion of naïve/lymphonode-homing and memory/activated T-cells;
- HPV-specific T-cell function skewed toward IL-2 production, that has been proven to provide the strongest effect in the resolution of HPV infection and disease.

These findings suggest a most efficacious immunity both systemically and at the site of injury, that is not seen in patients with persistent dysplasia despite ex vivo reactivity to an HPV-specific trigger.