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Synthesis of IFN-γ by CD8$^+$ T Cells Is Preserved in HIV-Infected Women with HPV-Related Cervical Squamous Intraepithelial Lesions[1]

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Objective. **The aim of this study was to investigate whether coinfection with HIV affects the synthesis of Th1 and Th2 cytokines by peripheral blood T cells of women infected with human papillomavirus (HPV).**

Methods. **Cervical swabs and peripheral blood were obtained from women referred for colposcopy. HPV DNA by Digene's hybrid capture assay, HIV RNA by Roche's Amplicor assay, and cytokine synthesis of T-cell subsets by flow cytometry were assessed. HPV-associated cervical and HIV-associated immune deficiency diseases were staged using the Bethesda System and the Centers for Disease Control criteria, respectively.**

Results. **Patients with HIV and/or HPV infections had lower percentages of IL-2$^+$ and higher percentages of IL-10$^+$ T cells than healthy women. Furthermore, women with both virus infections (HIV$^+$/HPV$^+$) had significantly fewer IL-2$^+$ CD4$^+$, IFN-γ^+ CD4$^+$, and TNF-α^+ CD4$^+$ T cells than women with HPV infection alone (HPV$^+$). Whereas HIV$^+$ and healthy women had similar numbers of IFN-γ^+ CD8$^+$ T cells, HPV$^+$ women had significantly fewer IFN-γ^+ CD8$^+$ T cells than healthy women.**

Conclusion. **HIV infection adversely affects the synthesis of Th1 cytokines by CD4$^+$, but not IFN-γ synthesis by CD8$^+$ T cells of women with active HPV infection. The increase in IFNγ^+ CD8$^+$ T cells, a phenotype consistent with cytotoxic T lymphocytes, may account for the stable HIV disease of the women studied. However, the increase in IFN-γ^+ CD8$^+$ T cells is less likely to be HPV-specific as there was a higher incidence of HPV-related cervical SIL in HIV$^+$/HPV$^+$ women compared with HPV$^+$ women.**

Key Words: **human papillomavirus (HPV); human immunodeficiency virus-1 (HIV); squamous intraepithelial lesions (SIL); cytokine.**

INTRODUCTION

Cervical squamous intraepithelial lesions (SIL) precede the development of cervical cancer, the third leading cause of cancer mortality in women worldwide [1]. Carcinoma of the cervix is causally related to infection with the human papillomavirus (HPV), a double-stranded DNA virus [2] of more than 100 distinct genotypes [3]. Susceptibility to infection with HPV [4, 5], the development of HPV-associated malignancies, and clinical outcome are influenced by the immune competence of the host [6]. Cervical SIL are more likely to occur among individuals with compromised immunity [4, 7].

In general, antibody (humoral) responses are protective against initial viral infection whereas cytotoxic T lymphocytes (CTL) (cellular) responses are protective against established infection. Th1 and Th2 cytokines modulate cellular and humoral immune responses, respectively [8]. Whereas the Th1 cytokines, interleukin-2 (IL-2) and interferon-γ (IFN-γ), enhance cellular immunity, IL-4 and IL-10 (Th2 cytokines) inhibit cellular immunity and stimulate humoral immunity. Th1 and Th2 cytokines are important cofactors in the natural history of HPV-related cervical diseases [9, 10]. Patients with HPV-associated neoplasms with a Th1 cytokine profile are purported to have a better clinical outcome compared with others exhibiting a Th2 cytokine profile [9, 10]. Moreover, a predominance of Th2 cytokines, either locally or in the peripheral circulation, may promote the development of cervical SIL and neoplastic change [9].

HPV may evade host immune surveillance in a cervical microenvironment of Th2 cytokines [11, 12] that are capable of down-regulating the expression of MHC class I antigens and β_2-microglobulin [13, 14] and diminishing the function of intraepithelial antigen-presenting cells [15]. While HIV-specific peripheral CTL in HIV-infected patients may be functional, high levels of Th2 cytokines in cervicovaginal secretions [16] can down-regulate the activity of cervical CTL [17] and lead to persistent HPV infection [18, 19].

Since HIV-specific peripheral and cervical CTL of HIV-

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infected patients recognize the same epitopes [20], it is reasonable to suggest that cervical CTL are recruited from the peripheral circulation [11, 12]. Hence, one might hypothesize that the competence of the peripheral blood T cell is a reliable measure of the type and quality of the immune responses that are present at the cervical lesion. What is unclear from these studies is whether coinfection with HIV affects the synthesis of Th1 and Th2 cytokines in women infected with HPV to the extent that it contributes to the development of cervical SIL. Therefore, we evaluated the syntheses of Th1 and Th2 cytokines by peripheral blood T cell subsets as a measure of cellular immunity of women coinfected with HPV and HIV.

MATERIALS AND METHODS

Clinical Characteristics of Patients

We studied 57 nonpregnant women above the age of 18 years who were referred to the colposcopy clinics at the The University of Texas M. D. Anderson Cancer Center (UT-MDACC) and the Harris County Hospital District Lyndon Baines Johnson (LBJ) Hospital in Houston, Texas. Among the 57 women, 23 were HIV seropositive. Twelve HIV-seronegative healthy women served as control subjects. The age and ethnicity of these women were comparable with those of patients even though the controls were of a higher socioeconomic status.

Patients underwent a physical examination of the pelvis at which time a smear of the cervix for Pap stain and a swab of the cervix were obtained for the detection of HPV DNA in exfoliated cells. If atypical cells were present on the Pap smear, a colposcopy-directed biopsy was obtained for the purpose of establishing a histological diagnosis. To determine whether the atypical cells were due to infection with HPV, exfoliated cervical cells attached to the swab obtained at the time of the physical examination were tested for the presence of HPV DNA using the first generation hybrid capture (HC-1) assay (Digene Diagnostic, Silver Springs, MD). In addition, 15 ml of peripheral blood was obtained from each woman to determine the synthesis of Th1 or Th2 cytokines by T-cell subsets. Plasma HIV load was measured by the Amplicor assay with a lower detection limit of 400 copies per μl (Roche Diagnostics, Branchburg, NJ). Patients were staged for their cervical disease using the Bethesda System [21] and for their HIV disease by criteria set forth by the Centers for Disease Control and Prevention [22]. Anti-retroviral therapy consisting of nucleoside analogs, nonnucleosides, and protease inhibitors was known for 18 HIV-infected women. One HIV^+ woman was off therapy at the time of study and the nature of therapy was unknown for the remaining four HIV^+ women. All HIV^- patients were untreated or were off therapy at the time of study.

The Human Experimentation Committees of the UT-MDACC and LBJ Hospital approved the study, and all patients were required to provide written consent.

Phenotype of Peripheral Blood Lymphoctyes

The phenotype of peripheral blood lymphocytes was determined by the whole blood staining method for three-color FACS analysis [23]. Briefly, blood was incubated with monoclonal antibody reagents to detect the total leukocytes (anti-CD45), monocytes (anti-CD14), total T cells (anti-CD3), and T-cell subsets, helper/inducer (anti-CD4), cytotoxic/suppressor (anti-CD8), memory (anti-CD45RO), and naive (anti-CD45RA) T cells. Mouse immunoglobulin isotype controls were used to account for nonspecific binding of mouse monoclonal antibodies to human leukocytes; lymphocyte purity was assessed by setting a gate around cells that were positive for CD45 and negative for CD14 antigens. All reagents were purchased from Becton–Dickinson Immunocytometry Systems Inc. (San Jose, CA).

Cytokine Synthesis by T Cells

Assays for the activation of lymphocytes by phorbol 12-myristate 13-acetate (PMA) and detection of cytokines in the cytoplasm of T cells were performed according to our published procedure [23]. Cytokine synthesis was evaluated on $CD3^+$ cells among the 10,000 total events acquired. List-mode multiparameter data files (each file with forward scatter, side scatter, and three fluorescence parameters) were analyzed using the CellQuest software program (Becton–Dickinson). Isotype controls were used to verify the staining specificity of experimental conditions and as a guide for setting markers to delineate positive and negative populations. Reaction of blood lymphocytes with anti-CD8 defined two T-cell subsets, $CD8^+$ and $CD8^-$ (or $CD4^+$), and permitted the evaluation of Th1 (IL-2, IFN-γ, and TNF-α) or Th2 (IL-10) cytokine syntheses by T-cell subsets.

Statistical Analysis

Study subjects were organized into five groups (Groups A–E) based on their HIV serological status and the presence of HPV DNA in cervical cells. Group A consisted of "healthy controls," Group B of HIV^-/HPV^-, Group C of HIV^-/HPV^+, Group D of HIV^+/HPV^-, and Group E of HIV^+/HPV^+. The data are presented as the mean percentage and mean number of T-cell subsets synthesizing Th1 and Th2 cytokines for each group. Whereas statistical differences in percentages and numbers of cytokine-producing T cells between study groups were determined using the Mann–Whitney test, differences between disease status and other patient characteristics were assessed by Pearson's χ^2 test. Statistical significance was set at a P value of less than 0.05.

RESULTS

Patient Groups

Patients (Groups B–E) had a median age of 32 years (range of 18–63 years); 25 (44%) were African American, 19 (33%)

TABLE 1
Clinical Characteristics of HIV-Seropositive Patients

PID	Disease stage[a]	HPV-RLU[b]	CDC stage[c]	CD4/μl	HIV RNA	Therapy[d]
1	ASCUS	Neg	A2	481	1179	1BE
2	ASCUS	Neg	A2	534	25181	1DE
3	ASCUS	Neg	A2	230	2010	1AE
4	ASCUS	Neg	A1	589	<400	1AE
5	ASCUS	Neg	A3	225	552	1AE
6	ASCUS	Neg	A2	484	500	1AE, 3
7	ASCUS	Neg	A3	172	<400	1AE, 2A
8	ASCUS	Neg	A1	138	<400	1AE
9	ASCUS	Neg	N/A	1526	N/A	N/A
10	ASCUS	713967	A2	644	<400	1BC
11	ASCUS	128167	A2	177	2128	1BD
12	LSIL	67557	C3	131	9907	1AE
13	LSIL	97805	A1	938	6607	1A
14	LSIL	702555	B3	233	<400	N/A
15	LSIL	853926	A1	410	8413	N/A
16	LSIL	338634	A2	235	<400	N/A
17	LSIL	N/A	A2	156	1170	1B
18	LSIL	77545	A2	465	4543	1AE, 3
19	LSIL	400218	A2	440	<400	1AE
20	HSIL	27492	A2	702	5152	1AE, 2B
21	HSIL	1968296	A2	267	70466	None
22	HSIL	99084	B2	367	12891	1AE
23	HSIL	1996111	B2	255	12000	1AE

[a] Staging according to the Bethesda System for HPV-related cervical disease [21]. ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

[b] RLU, relative light unit as determined by Digene's HPV hybrid capture assay that detects high-risk HPV (types 16, 18, 31, 33, 35, 45, 51, 52, and 56); Neg refers to the absence of HPV DNA or to the presence of HPV DNA at a concentration below the sensitivity of the Digene's HPV hybrid capture (HC-1) assay. N/A, not available.

[c] Staging according to the revised criteria for HIV/AIDS set by the CDC [22].

[d] 1, Nucleoside analog: A, zidovidine (ZDV); B, stavudine (d4T); C, didanosine (ddI); D, zalcitabine (ddC); E, lamivudine (3TC). 2, Nonnucleoside: A, nevirapine; B, delavirdine. 3, protease inhibitor: indinavir.

were Hispanic, and 13 (23%) were Caucasian women. There were 40 (70%) patients with atypical squamous cells of undetermined significance (ASCUS) and 17 (30%) with cervical SIL. The overall prevalence of HPV was 42%. No HPV testing was conducted among subjects of Group A. Twenty-four patients were classified as Group B (HIV$^-$/HPV$^-$) with ASCUS; 10 patients as Group C (HIV$^-$/HPV$^+$), 5 ASCUS and 5 cervical SIL; 9 patients as Group D (HIV$^+$/HPV$^-$) with ASCUS;

TABLE 2
Phenotype of Peripheral Blood Lymphocytes of the Study Groups

Phenotype	Healthy (A)	HIV$^-$/HPV$^-$ (B)	HIV$^-$/HPV$^+$ (C)	HIV$^+$/HPV$^-$ (D)	HIV$^+$/HPV$^+$ (E)
% CD4$^+$	45.4 ± 2.0 (46.5)	41.3 ± 1.6 (40.6)	43.4 ± 2.0 (44.8)	28.9 ± 4.8 (30.0)[a]	22.7 ± 2.7 (23.3)[a]
% CD8$^+$	27.0 ± 1.9 (24.7)	27.5 ± 1.7 (27.3)	28.1 ± 2.4 (25.8)	48.1 ± 4.7 (48.6)[b]	53.2 ± 3.2 (53.5)[b]
% CD8$^+$45RO$^+$	N/A	14.5 ± 1.4 (12.7)	14.8 ± 2.1 (13.7)	26.0 ± 4.9 (24.7)[c]	31.9 ± 3.2 (28.7)[c]
% CD8$^+$45RA$^+$	N/A	13.2 ± 1.3 (13.7)	13.3 ± 1.0 (12.1)	22.1 ± 3.3 (23.6)[c]	21.3 ± 1.7 (29.0)[c]
% CD56$^+$	11.7 ± 2.2 (9.3)	12.2 ± 1.4 (11.5)	10.7 ± 2.2 (8.4)	5.0 ± 1.1 (5.0)[a]	5.2 ± 1.1 (3.6)[a]

Note. Data are presented as the mean percentage or number/μl ± SEM. Number in parentheses indicates median percentage or number/μl for each phenotype. N/A, not available.

[a] HIV$^+$ (Groups D and E) women have significantly lower percentages of CD4$^+$ T cells and CD56$^+$ NK cells then those of healthy (Group A) and HIV$^-$ (Groups B and C) women.

[b] HIV$^+$ (Groups D and E) women have significantly higher percentages of CD8$^+$ T cells than those of healthy (Group A) and HIV$^-$ (Groups B and C) women.

[c] HIV$^+$ (Groups D and E) women have significantly higher percentages of CD8$^+$ CD45RO$^+$ and CD8$^+$ CD45RA$^+$ T cells than those HIV$^-$/HPV$^-$ (Group B) women.

TABLE 3
IL-2 Synthesis by PMA-Activated T-Cell Subsets

Phenotype	Healthy (A)	HIV^-/HPV^- (B)	HIV^-/HPV^+ (C)	HIV^+/HPV^- (D)	HIV^+/HPV^+ (E)
% IL-2^+ $CD4^+$	45.4 ± 4.7 (46.5)[a]	31.7 ± 4.5 (31.3)	28.5 ± 5.5 (22.8)	22.1 ± 5.9 (15.8)	19.9 ± 4.8 (22.3)
IL-2^+ $CD4^+/\mu l$	411 ± 65 (409)[b]	284 ± 58 (202)[c]	246 ± 52 (175)[d]	72 ± 29 (41)	81 ± 23 (60)
% IL-2^+ $CD8^+$	11.9 ± 1.8 (10.7)[e]	11.6 ± 2.4 (7.7)[f]	8.4 ± 2.2 (7.1)[g]	4.3 ± 1.1 (4.0)	5.2 ± 2.0 (2.3)
IL-2^+ $CD8^+/\mu l$	58 ± 11 (45)	69 ± 16 (60)	51 ± 18 (27)	31 ± 9 (25)	38 ± 11 (21)

Note. Data are presented as the mean percentage or number/μl ± SEM. Number in parentheses indicates median percentage or number/μl for each phenotype.
[a] A vs B, C, D, E, $P < 0.02$.
[b] A vs D, E, $P < 0.01$.
[c] B vs D, E, $P < 0.01$.
[d] C vs D, E, $P < 0.01$.
[e] A vs D, E, $P < 0.01$.
[f] B vs E, $P < 0.05$.
[g] C vs E, $P < 0.05$.

and 14 patients as Group E (HIV^+/HPV^+), 2 ASCUS and 12 cervical SIL, respectively.

Clinical characteristics of the HIV-serpositive women are listed in Table 1. HPV was detected more frequently in HIV^+ (14/23) than in HIV^- (10/34) patients ($\chi^2 = 5.569$; $P = 0.0183$). Moreover, the risk of cervical SIL was significantly higher among HIV^+ patients (OR = 6.327; 95% CI, 1.807–22.16) compared with HIV^- patients. Patients with fewer than 500 $CD4^+$ T cells/μl had a higher risk of developing cervical SIL than patients with CD4 count above 500/μl ($\chi^2 = 7.083$; $P = 0.0078$). HIV^+ patients with ASCUS had comparable plasma HIV RNA levels to HIV^+ patients with cervical SIL. HIV RNA was not available for one HIV^+ patient.

Phenotypes of Peripheral Blood Lymphocytes

HIV-infected patients (Groups D and E) had significantly lower percentages of $CD4^+$ T cells ($P < 0.01$) and $CD56^+$ NK cells ($P < 0.05$) and significantly higher percentages of $CD8^+$ ($P < 0.01$) T cells than healthy and HIV^- (Groups B and C) women. In addition, the percentages of $CD8^+$ ($P < 0.001$), $CD8^+$ $CD45RO^+$ memory ($P < 0.001$), and $CD8^+$ $CD45RA^+$ naive ($P < 0.001$) T cells were significantly higher in (HIV^+/HPV^+) women than others with HPV infection alone (Group C) (Table 2). There were no differences in the percentages of the T-cell subsets of healthy women and HIV^- (Groups B and C) patients.

Cytokine Synthesis by PMA-Activated $CD4^+$ and $CD8^+$ Cells

Significantly fewer $CD4^+$ T cells from HIV^+ (Groups D and E) patients synthesized IL-2 ($P < 0.01$), IFN-γ ($P < 0.01$), and TNF-α ($P < 0.03$) than those of HPV-infected (Group C) women. Conversely, significantly more $CD8^+$ ($P < 0.03$) T cells of HIV^+ (Groups D and E) patients synthesized IL-10 than those of women with undetectable viruses (Group B).

a. Synthesis of cytoplasmic IL-2 by PMA-activated $CD4^+$ and $CD8^+$ T-cell subsets. All of the patient groups had a significantly lower percentage ($P < 0.02$) of IL-2^+ $CD4^+$ T cells than healthy women (Table 3). In addition, HIV^+ women, irrespective of infection with HPV, had a significantly lower

TABLE 4
IFN-γ Synthesis by PMA-Activated T-Cell Subsets

Phenotype	Healthy (A)	HIV^-/HPV^- (B)	HIV^-/HPV^+ (C)	HIV^+/HPV^- (D)	HIV^+/HPV^+ (E)
% IFN-γ^+ $CD4^+$	23.6 ± 2.9 (22.7)[a]	16.9 ± 2.4 (16.3)	19.8 ± 4.5 (14.0)	15.9 ± 4.9 (13.8)	14.9 ± 2.7 (12.4)
IFN-γ^+ $CD4^+/\mu l$	226 ± 44 (231)[b]	149 ± 29 (112)[c]	168 ± 37 (125)[d]	66 ± 34 (35)	56 ± 14 (39)
% IFN-γ^+ $CD8^+$	39.4 ± 4.8 (34.8)[e]	23.0 ± 3.7 (18.5)	20.7 ± 4.2 (15.7)	34.6 ± 8.5 (32.9)	35.3 ± 7.5 (31.4)
IFN-γ^+ $CD8^+/\mu l$	238 ± 54 (162)[f]	140 ± 35 (132)[g]	120 ± 35 (91)	283 ± 96 (170)	340 ± 94 (330)

Note. Data are presented as the mean percentage or number/μl ± SEM. Number in parentheses indicates median percentage or number/μl for each phenotype.
[a] A vs D, E, $P < 0.03$.
[b] A vs D, E, $P < 0.01$.
[c] B vs D, E, $P < 0.03$.
[d] C vs D, E, $P < 0.01$.
[e] A vs B, C, $P < 0.01$.
[f] A vs C, $P < 0.05$.
[g] B vs E, $P < 0.03$.

TABLE 5
TNF-α Synthesis by PMA-Activated T-Cell Subsets

Phenotype	Healthy (A)	HIV−/HPV− (B)	HIV−/HPV+ (C)	HIV+/HPV− (D)	HIV+/HPV+ (E)
% TNF-α+ CD4+	39.7 ± 8.2 (34.2)	36.3 ± 4.4 (40.5)	36.7 ± 6.5 (28.1)	34.9 ± 6.8 (28.8)	28.0 ± 5.9 (27.3)
TNF-α+ CD4+/μl	355 ± 105 (217)[a]	332 ± 58 (297)[b]	279 ± 54 (198)[c]	134 ± 48 (82)	102 ± 23 (96)
% TNF-α+ CD8+	28.6 ± 9.1 (25.6)	20.0 ± 3.3 (16.7)	19.9 ± 6.3 (10.5)	21.0 ± 6.5 (13.1)	21.1 ± 6.1 (13.5)
TNF-α+ CD8+/μl	171 ± 67 (95)	126 ± 27 (134)	78 ± 22 (47)	181 ± 75 (73)	169 ± 46 (118)

Note. Data are presented as the mean percentage or number/μl ± SEM. Number in parentheses indicates median percentage or number/μl for each phenotype.
[a] A vs D, E, $P < 0.04$.
[b] B vs D, E, $P < 0.04$.
[c] C vs D, E, $P < 0.02$.

percentage ($P < 0.01$) of IL-2+ CD4+ T cells than healthy and HIV− (Groups B and C) women (Table 3). HIV+ patients (Groups D and E) also had a significantly lower percentage ($P < 0.01$) of IL-2+ CD8+ T cells than healthy women. Patients not infected with HIV (Groups B and C) had a significantly higher percentage ($P < 0.05$) of IL-2+ CD8+ T cells than that of HIV+ patients (Groups D and E).

b. Synthesis of cytoplasmic IFN-γ by PMA activated T-cell subsets. HIV+ patients, irrespective of infection with HPV, had significantly lower percentages of IFN-γ+ CD4+ T cells than healthy women ($P < 0.03$) and significantly fewer IFN-γ+ CD4+ T cells than HIV− ($P < 0.03$) and healthy women ($P < 0.01$) (Table 4). Moreover, women with HPV infection (Group C) had significantly lower percentages ($P < 0.01$) and lower numbers ($P < 0.05$) of IFN-γ+ CD8+ T cells than healthy women. In contrast, patients infected with both viruses (Group E) had significantly more IFN-γ+ CD8+ T cells ($P < 0.05$) than patients with undetectable virus infections (Group B).

c. Synthesis of cytoplasmic TNF-α by PMA-activated T-cell subsets. All study groups had similar percentages of CD4+ and CD8+ T cells synthesizing TNF-α (Table 5). The numbers of TNF-α+ CD8+ T cells were also similar for all groups. In contrast, HIV+ patients had significantly fewer TNF-α+ CD4+ T cells compared with HIV− (Group B and C) patients ($P < 0.04$) and healthy women ($P < 0.04$).

d. Synthesis of cytoplasmic IL-10 by PMA-activated T-cell subsets. Patients infected with either virus (Groups C and D) or both viruses (Group E) had higher percentages of IL-10+ CD4+ ($P < 0.04$) and IL-10+ CD8+ ($P < 0.05$) T cells compared with patients in whom no virus was detected (Group B) (Table 6). Furthermore, HIV+/HPV+ patients had significantly more IL-10+ CD8+ T cells than women in Group B ($P < 0.01$) or healthy women ($P < 0.03$).

DISCUSSION

Although clinical HPV infection is often detected by cytological examination of cervical smears and/or colposcopy-directed cervical biopsy [24], active HPV infection can be confirmed by the presence of HPV DNA in exfoliated cervical epithelial cells. The low frequency of HPV DNA among patients with ASCUS in this study is consistent with previous experience in women with abnormal Pap smears [25, 26]. The detection of HPV DNA can be highly variable within individuals [27, 28] and may require multiple samplings to confidently establish HPV infection in an individual. Despite the lower sensitivity of the HC-1 assay in comparison with routine cytological screening for diagnosing high-grade SIL [25, 29], there was an association between the detection of HPV DNA and cervical SIL ($\chi^2 = 3.601$, $P = 0.0289$).

TABLE 6
IL-10 Synthesis by PMA-Activated T-Cell Subsets

Phenotype	Healthy (A)	HIV−/HPV− (B)	HIV−/HPV+ (C)	HIV+/HPV− (D)	HIV+/HPV+ (E)
% IL-10+ CD4+	11.3 ± 4.5 (6.5)	11.2 ± 4.4 (2.3)[a]	26.9 ± 11.0 (21.7)	20.4 ± 4.0 (14.8)	22.7 ± 5.6 (16.0)
IL-10+ CD4+/μl	82 ± 28 (55)	120 ± 52 (19)[b]	223 ± 88 (198)	75 ± 15 (68)	85 ± 27 (49)
% IL-10+ CD8+	3.7 ± 0.9 (4.1)	3.2 ± 1.0 (1.2)[c]	6.9 ± 2.5 (5.6)	6.3 ± 1.7 (5.0)	7.8 ± 1.9 (6.7)
IL-10+ CD8+/μl	20 ± 5 (28)[d]	20 ± 7 (9)[e]	35 ± 11 (35)	42 ± 11 (40)	52 ± 9 (40)

Note. Data are presented as the mean percentage or number/μl ± SEM. Number in parentheses indicates median percentage or number/μl for each phenotype.
[a] B vs C, D, E, $P < 0.04$.
[b] B vs D, $P < 0.03$.
[c] B vs D, E, $P < 0.05$.
[d] A vs E, $P < 0.03$.
[e] B vs D, E, $P < 0.01$.

Although there is little evidence to support a higher rate of invasive cervical cancer among HIV^+ patients [30], the increased risk for anal cancer in severely immune-compromised AIDS patients is well documented [31]. HIV^+ women have a higher prevalence of HPV infection in the cervix and anus, as well as SIL at these sites, than do HIV^- women matched for age and risk factors [32]. Consistent with these earlier studies, we found cervical SIL to be significantly higher among HIV^+ than among HIV^- women ($\chi^2 = 9.202$, $P = 0.0024$).

HPV infection in HIV^+ homosexual men has been shown to correlate with a low number of $CD4^+$ T cells [33]. As expected, HIV^+ women had decreased $CD4^+$ T cells accompanied by an increase in $CD8^+$ T cells (Table 2), yet the $CD4^+$ count was similar for those ASCUS and SIL patients. Although there was in increase in $CD8^+$ memory cells in HIV^+ patients, the increase in naive $CD8^+$ T cells was unexpected and could account for the HIV-infected patients being clinically healthy [34]. On the other hand, the distribution of $CD4^+$ T cells in HPV^+ women is more controversial. Women with condyloma have been described as having normal [35] or decreased [36] $CD4^+$ T cells accompanied by increases in the percentage and number of $CD8^+$ T cells [35, 36]. We found the percentages of $CD4^+$ and $CD8^+$ T cells in HPV-infected (Group C) and uninfected (Group B) women to be similar.

A defect in IL-2 production by T cells of HPV-infected women with high-grade SIL and cervical cancer in response to activation of the T-cell receptor (TCR) by HPV-specific peptides has been reported [26]. By activating T cells with PMA, the current study demonstrates that the defect in cytokine synthesis is not restricted to activation through the TCR and that the defect can be detected even at an earlier stage of HPV disease. Abnormal cytokine synthesis by T cells of patients without detectable HPV infection (Group B) was unexpected as others [9, 37] have described a defect in cellular immune function only in women with advanced SIL. Our data describing a significantly higher number of $CD8^+$ T cells accompanied by an impairment in IL-2 synthesis in HIV^+ women identify additional deterioration of the cellular compartment of the immune system to explain the higher incidence of cervical SIL in these patients. In an earlier study, we found the production of IFN-γ and not IL-2 by peripheral blood mononuclear cells to be a prognosticator of a favorable outcome in pediatric long-term survivors of HIV infection [38]. The production of IFN-γ by patients with cervical carcinoma [37] or with HIV disease [39] has been associated with good prognosis; our observation of the preservation of IFN-γ^+ $CD8^+$ T cells in HIV^+/HPV^+ patients further supports this association.

TNF-α is capable of arresting [40] and modulating [41] the growth of $HPV16^+$ keratinocytes *in vitro* and suppressing the expression of E6/E7 mRNA [42]. Apart from deficiencies in the syntheses of IL-2^+ and IFN-γ^+ by $CD4^+$ T cells, HIV^+ (Groups D and E) women had significantly fewer TNF-α^+ $CD4^+$ T cells than healthy women. Moreover, HPV^+ patients had fewer TNF-α^+ $CD8^+$ T cells than healthy controls. Thus, it is conceivable that women infected with both viruses (Group E) will be more prone to persistent HPV-related cervical lesions as a consequence of undeterred oncogene expression.

Although the measurement of HPV-specific antibodies was beyond the scope of this study, we assessed the synthesis of IL-10, a known inducer of antibody production [43], by T-cell subsets. Patients infected with HIV and/or HPV had increased percentages of IL-10^+ $CD4^+$ and IL-10^+ $CD8^+$ T cells which, in turn, can have a negative impact on the synthesis of Th1 cytokines [43]. Moreover, an increase in the proportion and number of IL-10^+ T cells by patients infected with both viruses may mediate the predominance of Th2 cytokines found in the cervical mucosa [44]. IL-10 can also modulate anti-tumor responses by preventing the presentation of tumor antigen to $CD8^+$ CTL [45, 46]. Thus, the increased number of IL-10^+ T cells of patients infected with either or both viruses may compromise immune surveillance mechanisms to prevent disease progression.

A switch in production from IL-2 to IL-10 was found not only among HIV^+ patients but also among HIV^- patients with active HPV infection. Our data are consistent with previous observations of an association between a switch from a Th1 to a Th2 cytokine profile and cervical disease progression in HPV-infected patients [9, 37]. However, the present study is the first to report an increase in IFN-γ^+ $CD8^+$ T cells in the peripheral blood of HPV^+/HIV^+ women compared to women with HPV infection alone (Table 4). As the majority of $CD8^+$ T cells produced IFN-γ and TNF-α and not IL-2, the phenotype of effector CTL [47], it is plausible that these cells were capable of HIV-specific CTL activity associated with the relative good health of the HIV^+ women in this study. Although HIV-specific peripheral and cervical CTLs recognize the same HIV-specific epitope [20], the higher incidence of HPV-related cervical SIL in women with both viruses suggests that these IFN-γ^+ $CD8^+$ T cells are less effective at controlling the pathogenesis associated with HPV disease.

In conclusion, the significant decrease in IFN-γ^+ $CD8^+$ T cells may contribute to the development of cervical lesions in women with HPV infection alone (Group C) and the ASCUS in women at risk for HPV infection (Group B). Furthermore, in HPV^+ women with HIV infection, the increase in IFN-γ^+ $CD8^+$ T cells may delay the onset of AIDS but not the development of cervical lesions. Further study is required to evaluate the cytotoxic potential of the IFN-γ^+ $CD8^+$ T cells described here before any clinical significance can be ascribed to the preservation of this phenotype in HIV^+ patients.

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