Deficiencies in Myeloid Antigen-Presenting Cells in Women With Cervical Squamous Intraepithelial Lesions

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BACKGROUND. There is little information on the function of dendritic cells in women with human papillomavirus (HPV)-related cervical squamous intraepithelial lesions (SILs). In the current study the functions of dendritic cells in the development of T-cell immunity in women with cervical SILs were assessed.

METHODS. The percentage of myeloid dendritic cells (MDCs) and plasmacytoid dendritic cells (PDCs) in peripheral blood were enumerated of 44 patients with SIL (low-grade, 19; high-grade, 25), 19 patients with atypical squamous cells of undetermined significance (ASCUS), and 18 controls. The expression of costimulatory receptors was assessed and the ability of monocyte-derived dendritic cells (MDDC) to present HPV16-E6 and HPV16-E7 antigens to autologous T cells.

RESULTS. Patients with either low (L)-grade or high (H)-grade SIL had significantly lower median plasma levels of interferon-γ than did the controls ($P = .038$ and .031, respectively). Compared with the controls, patients with ASCUS or LSILs had significantly lower median percentages of MDCs ($P = .002$ and $P < .001$, respectively), and significantly lower median percentages of MDDCs that expressed CD86 ($P < .001$ and $P = .003$, respectively) and major histocompatability complex class-II antigen human leukocyte antigen DR (HLA-DR) ($P = .012$ and $P < .001$, respectively). T cells of patients with ASCUS or LSILs proliferated less than those of the controls in response to HPV16-E7 ($P = .002$ and .046, respectively).

CONCLUSIONS. Low levels of peripheral blood MDCs and of MDDCs expressing CD86 and HLA-DR suggest that deficiencies in the ability of MDDC to present antigen to autologous T cells may lead to persistent infection with HPV and the development of cervical SILs in HPV-infected women. *Cancer* 2006;107:999–1007. © *2006 American Cancer Society.*

KEYWORDS: human papillomavirus, cervical squamous intraepithelial lesions, dendritic cells, T-cell proliferation.

Human papillomavirus (HPV) DNA is detected in more than 99% of all tumors of the uterine cervix and is implicated in the etiology of precancerous cervical lesions.[1] The clinical manifestation of HPV infection depends on the type of HPV, the location of the epithelial lesion, and the host's immune status.[2] HPV DNA is integrated into the chromosome with disruption of the E2 gene, which promotes expression of the E6 and E7 oncoproteins, leading to the accumulation of DNA damage and subsequent cervical cancer. E6 and E7 are the HPV-coded proteins overexpressed in cervical cancer; they are involved in the malignant transformation of HPV-infected cells; their presence is necessary for the maintenance of the malignant phenotype of the cells.

HPV infection of the cervix can be transient, with 70% to 90% of women having spontaneous resolution of the infection within 12–30 months.[3,4] Both cellular[5,6] and humoral[7] immunity play a role in controlling HPV infection. Whereas humoral immunity is not likely to predict the spontaneous regression of cervical intraepithelial neoplasia (CIN),[8] an increased cellular immunity correlates with good clinical prognosis.[9] Although systemic immunity develops through the presentation of antigens to T cells by antigen-presenting cells (APC) or dendritic cells (DCs) in the regional lymphoid organs,[10] priming of antiviral immunity in situ depends on the cross-presentation of viral antigen to T cells by epithelial DCs, the Langerhans cells, in the cervix.

Two subsets of DCs are present in peripheral blood (PB)—myeloid DCs (MDCs) and plasmacytoid DCs (PDCs). Freshly isolated PDCs and unmethylated CG oligonucleotide-matured PDCs can bind HPV16 capsid protein-derived virus-like particles (VLPs) to induce secretion of interferon (IFN)-α and interleukin (IL)-6, cytokines that are involved in the generation of antibody responses.[11] Similarly, HPV VLPs can activate PB monocyte-derived dendritic cells (MDDCs),[12] and VLPs presented by MDDCs are capable of priming naive CD8+ T cells and inducing cytotoxic T lymphocytes (CTLs) in vitro.[13] In contrast, HPV VLPs are not able to activate monocyte-derived Langerhans cells.[14]

An earlier study using MDDC pulsed with HPV16-E7 peptides indicated that patients with CIN lesions generated higher E7-specific immunologic responses than patients with cervical cancer.[15] Moreover, cervical cancer progression is linked to an undesirable shift from a Th1 type response to that of a Th2 type response in CD4+ T cells exposed to 2 novel E7-derived epitopes presented by MDDC.[16] To better understand the development of immunity to HPV-related cervical neoplasia, we examined several aspects of cellular immunity in patients with HPV-associated squamous intraepithelial lesions (SILs). In particular, we measured plasma cytokines; enumerated peripheral blood MDCs and PDCs; assessed the expression of costimulatory receptors (CD86 and CD80) by activated MDDCs; determined the ability of activated MDDC to present HPV16-E6 and HPV16-E7 antigens to autologous T cells; and measured the proliferation of T cells after exposure to HPV antigens.

MATERIALS AND METHODS
Patient Recruitment
Women attending colposcopy clinics at three different hospitals in Houston, Texas, including: Memorial-Hermann Hospital, Lyndon Baines Johnson Hospital, and the University of Texas M. D. Anderson Cancer Center were recruited for this study over a 12-month period. The Institutional Review Boards of the 3 hospitals approved the study protocols and patients provided written informed consent before enrollment. Informed consent was obtained from each participant in accordance with the US Department of Health and Human Services guidelines and the Institutional Review Board of each participating institution. Each patient consented in writing to answer a questionnaire and phlebotomy to provide 30 mL of peripheral blood for immunologic studies before undergoing a physical examination to obtain cervical smears for Papanicolaou (Pap) stain and cytologic examination, and a cervical brush specimen for HPV molecular testing. The Bethesda system for reporting cervical smears was used to classify the patients as having atypical squamous cells of undetermined significance (ASCUS) and low-grade or high-grade squamous intraepithelial lesions (LSIL and HSIL, respectively).[17] Whenever clinically indicated, a cervical biopsy was obtained for histologic staging of disease. A pathologist reviewed all available cervical biopsy specimens to provide consistent histologic staging with respect to either LSIL or HSIL.

To be eligible for this study, women had to be 18 years or older, not pregnant, and not infected with human immunodeficiency virus type 1 (HIV-1). Each patient was handed a questionnaire that requested information on lifestyle, personal information, and limited medical history.

Eighteen healthy women who worked in research laboratories were recruited by word-of-mouth to be control subjects. Control women were not required to take the lifestyle questionnaire nor were they subjected to a pelvic examination. In lieu of a pelvic examination for the study, each control subject attested to having a normal Pap smear during her recent visit to her gynecologist and to not smoke cigarettes.

Detection of HPV DNA in Cervical Specimens
Exfoliated epithelial cells were collected with a cervical brush and placed in the transport medium included in the Hybrid Capture 2 (HC-2) assay specimen collection kit (Digene Diagnostics, Gaithersburg, MD). HC-2 is a nucleic acid hybridization assay that uses RNA probes to detect 18 HPV types, including 13 high-risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low-risk types (types 6, 11, 42, 43, and 44). Only subjects with high-risk HPV types were included in this study.

Enumeration of Peripheral Blood DCs

MDCs and PDCs can be distinguished from one another by their mutually exclusive expression of β-integrin/CD11c and IL-3 receptor-alpha/CD123, respectively.[18] Peripheral blood obtained from all participants was incubated with monoclonal antibodies (BD Biosciences, Mountain View, CA) to detect the lineage-specific (Lin) antigens (CD3, CD19, CD14, CD16, and CD56), IL-3 receptor-alpha (CD123), major histocompatability complex class-II antigen (human leukocyte antigen DR, HLA-DR), and CD11c on the surface of leukocytes. The stained leukocytes were analyzed by a FACSCalibur flow cytometer equipped with CellQuest software (Becton-Dickinson Immunocytometry Systems, San Jose, CA). A minimum of 5×10^3 Lin$^-$ HLA-DR$^+$ cells from each participant was analyzed to enumerate MDC and PDC subsets based on their mutually exclusive expression of CD11c and CD123, respectively.

Isolation of CD14$^+$ Monocytes from Peripheral Blood

Peripheral blood mononuclear cells (PBMCs) from participants were prepared by Ficoll (Sigma-Aldrich, St. Louis, MO) density gradient centrifugation of whole blood. PBMCs were washed twice with phosphate-buffered saline and suspended in complete tissue culture medium consisting of RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (J.R. Scientific, Woodland, CA), 100 U/mL of penicillin (Whittaker Bioproducts), 100 μg/mL of streptomycin (Whittaker Bioproducts), and 2 mM of glutamine (GIBCO, Grand Island, NY).

PBMCs were incubated with anti-CD14 microbeads (Miltenyi Biotec, Auburn, CA) and passed through a magnetic field to isolate CD14$^+$ monocytes by positive selection using magnetic cell sorting (MACS) (autoMACS separation system, Miltenyi Biotec) that yielded a monocyte population of >80% purity. The CD14$^-$ cell population was used to isolate CD3$^+$ T cells.

Differentiation of Monocytes into MDDCs

To differentiate the monocytes into MDDCs, the CD14$^+$ monocytes were dispensed into 25 mm^2 flasks at a density of 3×10^6 per flask in 3 mL of DC culture medium (DC medium) that consisted of RPMI-1640 supplemented with 1,000 U/mL of granulocyte-macrophage colony-stimulating factor (GM-CSF; Immunex, Seattle, WA) and 500 U/mL of IL-4 (R&D Systems, Minneapolis, MN) and cultured for 6 days at 37°C in a humidified atmosphere containing 5% CO$_2$. On each of the first 5 days of culturing, 1 mL of fresh DC medium

was added. On the sixth day of culturing, immature MDDCs were harvested and activated with 10 pg/mL of lipopolysaccharide (Sigma-Aldrich) for an additional day to induce maturation of the immature MDDCs. On the seventh day, mature MDDCs were harvested and assessed for their expression of costimulatory receptors CD80, CD86, and HLA-DR and for their ability to present HPV antigen to autologous T cells.

Isolation of CD3$^+$ T Cells

Twenty microliters of anti-CD3 microbeads (Miltenyi Biotec) were added to 10^7 CD14$^-$ cells that were suspended in 80 μL of MACS running buffer. The cell suspension was incubated at 4°C for 15 minutes before being passed through the autoMACS cell separator (Miltenyi Biotec) using the positive selection protocol to isolate CD3$^+$ T cells (>95% purity). CD3$^+$ T cells were cryopreserved in liquid nitrogen for future co-culturing with autologous MDDCs.

Expression of Costimulatory Molecules by MDDCs

MDDCs (2.5×10^5) were suspended in 100 μL of culture medium and allowed to react for 20 minutes with a panel of monoclonal antibodies directly conjugated with a fluorochrome. The cocktail consisted of mouse monoclonal antibodies directed against human CD86, CD80, and HLA-DR surface antigens. Cells were then washed once in PBS for 5 minutes at 400g and fixed with 1% paraformaldehyde and analyzed using flow cytometer (FACS Calibur, BD, Mountain View, CA). MDDCs were also allowed to react with the appropriate mouse immunoglobulin isotype control at the same protein concentration as the test antibody to detect nonspecific or background fluorescence. Stained cells were analyzed by the FACSCalibur flow cytometer.

Presentation of HPV Antigens by MDDCs to Autologous T Cells

MDDCs (10^4) were incubated with glutathione S-transferase/HPV16-E6 or glutathione S-transferase/HPV16-E7 protein (10 μg/mL, ProtoProbe, Milwaukee, WI) in a 96-well plate for 30 minutes before co-culturing with 1×10^5 autologous T cells for 5 days. On Day 5 of culturing, 1.0 μCi of ^{3}H-thymidine (specific activity, 6.7 Ci/mmol; DuPont-New England Nuclear, Boston, MA) in RPMI-1640 medium was added to each well. After incubation of the culture plate for an additional 16 hours at 37°C, the contents were harvested onto glass fiber filters and the amount of radioactive incorporation of ^{3}H-thymidine into DNA synthesis was determined in a liquid scintillation counter (TopCount, Beckman Coulter, Fuller-

ton, CA). The levels of DNA synthesis by T cells are expressed as the mean count per minute (cpm) $\pm$ standard error of the mean. A stimulation index was calculated by determining a quotient between the average cpm of the quadruplicate wells of T cells activated with antigen-pulsed MDDCs and the average cpm of the ones without HPV antigen-pulsed MDDCs.

Plasma Levels of Interferon (IFN)-γ and Interleukin (IL)-10

Plasma was obtained from each participant and stored at $-70°$C until samples were analyzed in batches for the presence of IFN-γ and IL-10 by enzyme-linked immunosorbent assay (BD Pharmingen, San Diego, CA).

Statistical Analysis

The median levels of plasma cytokines and median percentages of MDCs, PDCs, and MDDCs expressing specific surface antigens, and the median T-cell proliferative responses, were obtained for each study group. The Kruskal–Wallis test was used to detect any significant differences among the study groups. The non-parametric Mann–Whitney test was used to determine the difference in the median values between study group pairs. P values less than .05 were considered statistically significant.

RESULTS
Study Population Characteristics

We collected cervical specimens from all patients for the HC-2 assay and only included subjects with high-risk HPV types for analysis in this report. Two women with ASCUS, 3 women with LSIL, and 5 women HSIL also had detectable levels of low-risk types of HPV. The control group consisted of healthy women who had normal Pap smears, and the patients included 19 women with ASCUS, 19 with LSIL, and 25 with HSIL. Women with biopsy-proven CIN were excluded from the ASCUS group for data analysis.

The 63 patients (median age, 32 years; range, 20–64 years) (Table 1) were age-matched to the 18 controls (nonsmokers; median age, 27 years; range, 23–50 years). There were no significant differences in ethnicity ($\chi^2 = 5.04$, $P = .54$), proportions of smokers ($\chi^2 = 1.25$, $P = .54$), oral contraceptive users ($\chi^2 = 1.10$, $P = .58$), or number of past cervicovaginal bacterial or yeast infections and sexually transmitted diseases ($\chi^2 = 0.49$, $P = .78$) between the patient groups. However, there were significant differences in ethnicity and proportions of smokers between

TABLE 1
Demographic Characteristics of the Study Population

Groups	ASCUS (n = 19)	LSIL (n = 19)	HSIL (n = 25)	Controls (n = 18)
Ethnicity:				
Caucasian	7	4	10	5
Hispanic	5	9	9	6
African American	6	6	6	0
Asian	1	0	0	7
Median age				
Years (range)	42 (21–64)	26 (20–46)	31 (20–54)	27 (23–50)
Median parity (range)	2 (0–5)	1 (0–6)	2 (0–4)	1 (0–2)
Smokers	4	5	9	0
Oral contraceptive use	5	8	8	N/A[‡]
Past infection[†]	11	9	14	N/A[‡]

ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intra-epithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

[*] Patients were grouped according to their cytological cervical squamous cell abnormalities as defined by the Bethesda system.[17]

[†] Past cervicovaginal bacterial or yeast infections and/or sexually transmitted diseases were considered.

[‡] Not available.

patients and controls. Specifically, the proportion of individuals of Asian descent was significantly higher among controls than among patient groups (7/18 versus 1/63, $P < .0001$, Fisher exact test) and there were no smokers among controls (0/18 versus 18/63, $P < .01$ by Fisher exact test). Concomitant cervicovaginal infections such as gonorrhea and chlamydia in patients were rare at the time of study. Although cultures were not done routinely for cervicovaginal infections, 1 woman with ASCUS had *Trichomonas vaginalis* and another with LSIL had bacterial vaginosis.

Enumeration of MDCs and PDCs

The proportions of MDCs and PDCs in the population of mononuclear cells devoid of lineage-specific markers but with HLA-DR expression, i.e., Lin⁻ HLA-DR⁺ cells, were recorded for each participant. Compared with the median percentage of MDCs of the controls (65.5%), the proportion of MDCs in patients with ASCUS (40.2%; $P = .002$), LSILs (30.5%; $P < .001$), or HSILs (38.4%; $P < .001$) were significantly lower (Fig. 1A). In contrast, compared with the median percentages of PDCs of the controls (18.5%), the proportion of PDCs in patients with ASCUS (30.8%; $P < .001$) or HSIL (25.5%; $P = .047$) were significantly higher (Fig. 1B).
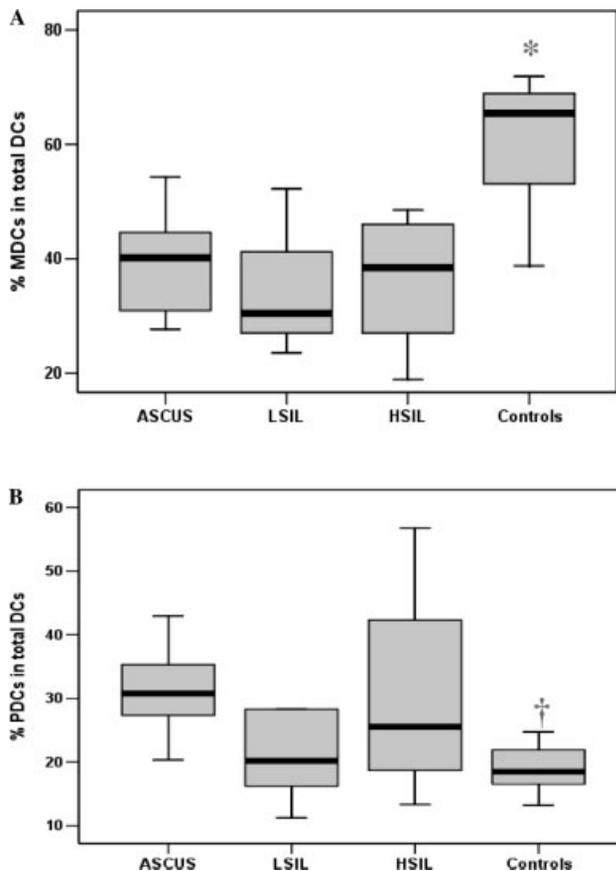
FIGURE 1. (A) Peripheral blood myeloid and (B) plasmacytoid dendritic cells of women with human papillomavirus (HPV)-related cytological cervical squamous cell abnormalities. The boxes represent the interquartile range, which extended from the 25th percentile to the 75th percentile, and the line across the box indicates the median (50th percentile); error bars represent 95% confidence intervals. DCs, dendritic cells; ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion. *Statistical differences between controls and ASCUS ($P = .002$), LSIL ($P < .001$), or HSIL ($P < .001$). †Statistical differences between controls and ASCUS ($P < .001$) or HSIL ($P = .047$).

Expression of Costimulatory Receptors on MDDCs

The induction of tumor-specific cytotoxic T lymphocytes (CTL) requires activated DCs that express costimulatory molecules (CD86/CD80) with the ability to present antigens to T cells. Whereas CD86 is thought to act early in an immune response and in the primary lymphoid tissue, CD80 acts later and in the periphery at the site of inflammation.[19] Therefore, we assessed the expression of CD86 and CD80 costimulatory molecules by activated MDDCs from women with HPV-related cervical neoplasia. Compared with the median percentage of activated MDDCs that expressed CD86 from the controls (81.7%), the

median percentages of activated MDDC of patients with ASCUS (47.7%, $P < .001$), LSILs (50.6%, $P = .003$), or HSILs (62.8%, $P = .042$) were significantly lower (Fig. 2A). Patients with ASCUS had a significantly higher median percentage (84.4%) of activated MDDCs that expressed CD80 than did the controls (49.6%, $P < .001$), patients with LSIL (60.0%, $P = .010$), or patients with HSIL (59.7%, $P = .040$) (Fig. 2B). Patients with ASCUS or LSIL had a significantly lower median percentage of activated MDDCs that expressed HLA-DR than did the controls (80.9%, $P = .012$ and 76.6%, $P < .001$, respectively, versus 89.6%) (Fig. 2C). In addition, patients with LSIL had a significantly lower median percentage of activated MDDC that expressed HLA-DR than did patients with HSIL (76.6% versus 84.9%, $P = .013$).

T-Cell Proliferation in Response to Activation with Autologous MDDCs Pulsed with Either HPV16-E6 or HPV16-E7 Antigens

The measurement of T-cell proliferation in response to activation with autologous MDDCs pulsed with either HPV16-E6 or HPV16-E7 antigens is presented as a stimulation index (SI) (Fig. 3). The median SI of T cells proliferating in response to activation with autologous MDDC pulsed with HPV16-E6 antigen was significantly higher in the controls than it was in women with HPV-related LSIL (29.5 versus 16.6, $P = .006$) but similar to those of women with ASCUS (18.9) or HSIL (25.3) (Fig. 3A). Similarly, the median SI of T cells pulsed with HPV16-E7 antigen proliferating in response to autologous MDDC from the controls (27.3) was significantly higher than that of patients with ASCUS (12.7, $P = .002$) or LSIL (21.9, $P = .046$) but similar to that of patients with HSIL (23.9, $P = .065$) (Fig. 3B).

Plasma Levels of IFN-γ and IL-10

The median plasma IFN-γ level, in descending order, was 9.89 pg/mL in the patients with ASCUS, 7.85 pg/mL in the control subjects, 4.94 pg/mL in patients with LSIL, and 4.82 pg/mL in patients with HSIL (Fig. 4A). Patients with LSIL or HSIL had significantly lower IFN-γ plasma levels than did the controls ($P = .038$ and $.031$, respectively) or patients with ASCUS ($P < .001$ and $P < .001$, respectively).

The median plasma IL-10 levels, in descending order, were 11.93 pg/mL in patients with ASCUS, 6.16 pg/mL in patients with LSIL, 6.08 pg/mL in the controls, and 4.46 pg/mL in patients with HSIL (Fig. 4B). The median level of IL-10 was significantly lower in patients with HSIL than in patients with ASCUS ($P = .003$) or the controls ($P = .032$). In addition, the
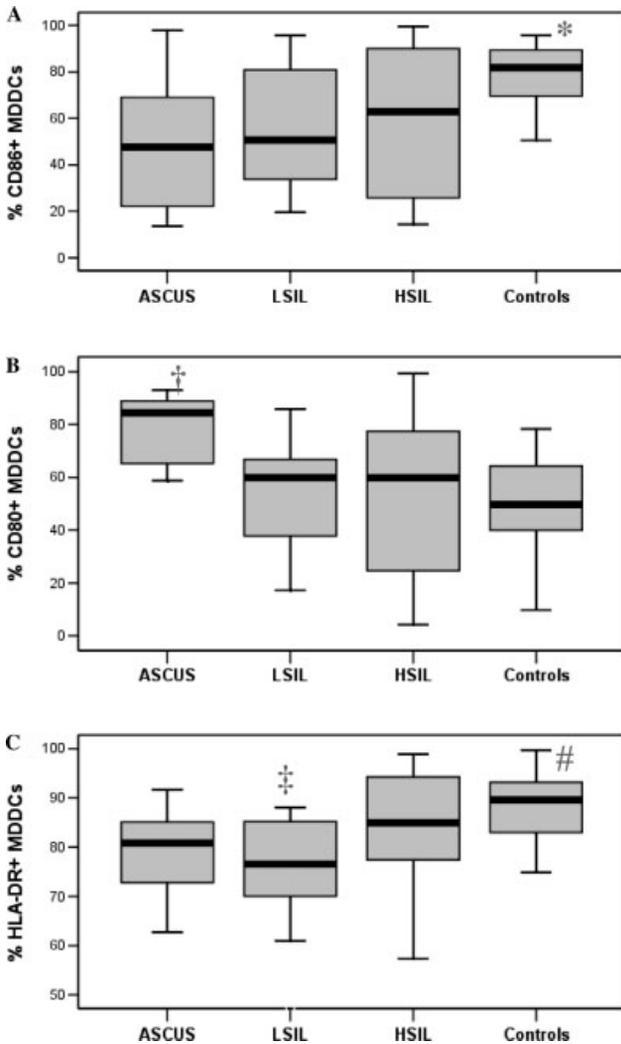
FIGURE 2. Immunophenotype of monocyte-derived dendritic cells (MDDCs) from women with human papillomavirus (HPV)-related cytological cervical squamous cell abnormalities. To determine the expression of costimulatory receptors and activation markers, MDDCs were stained with a panel of fluorescein-conjugated monoclonal antibodies against (A) CD86, (B) CD80, and (C) human leukocyte antigen DR (HLA-DR). The percentages of β-integrin$^+$ (CD11c$^+$) cells expressing these markers were recorded for each specimen. The boxes represent the interquartile range, which extended from the 25th percentile to the 75th percentile, and the line across the box indicates the median (50th percentile); error bars represent 95% confidence intervals. ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion. *Statistical differences between controls and ASCUS ($P <$ .001), LSIL ($P =$ .003), or HSIL ($P =$ .042). †Statistical differences between ASCUS and LSIL ($P =$ .010), HSIL ($P =$ .040), or controls ($P <$ .001). ‡Statistical differences between LSIL and HSIL ($P =$ .013). #Statistical differences between controls and ASCUS ($P =$ .012) or LSIL ($P <$ .001).
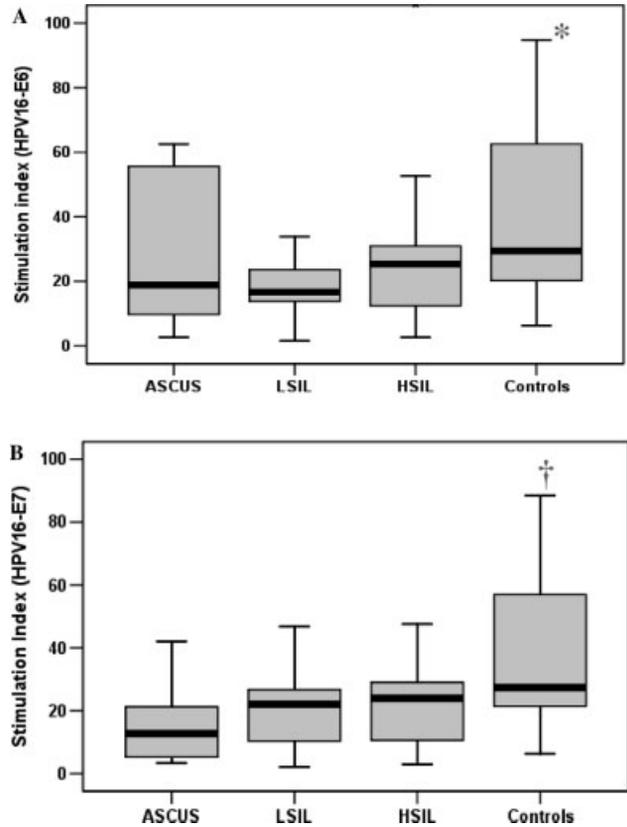

FIGURE 3. Proliferative response of the T cells from women with human papillomavirus (HPV)-related cytological cervical squamous cell abnormalities stimulated with autologous monocyte-derived dendritic cells pulsed with HPV antigens (A) HPV16-E6 and (B) HPV16-E7. The boxes represent the interquartile range, which extended from the 25th percentile to the 75th percentile, and the line across the box indicates the median (50th percentile); error bars represent 95% confidence intervals. ASCUS, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion. *Statistical differences between controls and LSIL ($P =$ .006). †Statistical differences between controls and ASCUS ($P =$ .002) or LSIL ($P =$ .046).

median IL-10 level was significantly higher in patients with ASCUS than in the control group ($P <$ .001), patients with LSIL ($P =$ .001), or patients with HSIL ($P =$ .003).

DISCUSSION

Host cell-mediated immunity plays a pivotal role in controlling HPV-related cervical intraepithelial neoplasia (SIL),[20,21] as half of the cases originally diagnosed with LSIL spontaneously regress to ASCUS or completely normalize within 6 months.[22] Several mechanisms by which HPV can escape the immune response of the host are acknowledged and include inadequate
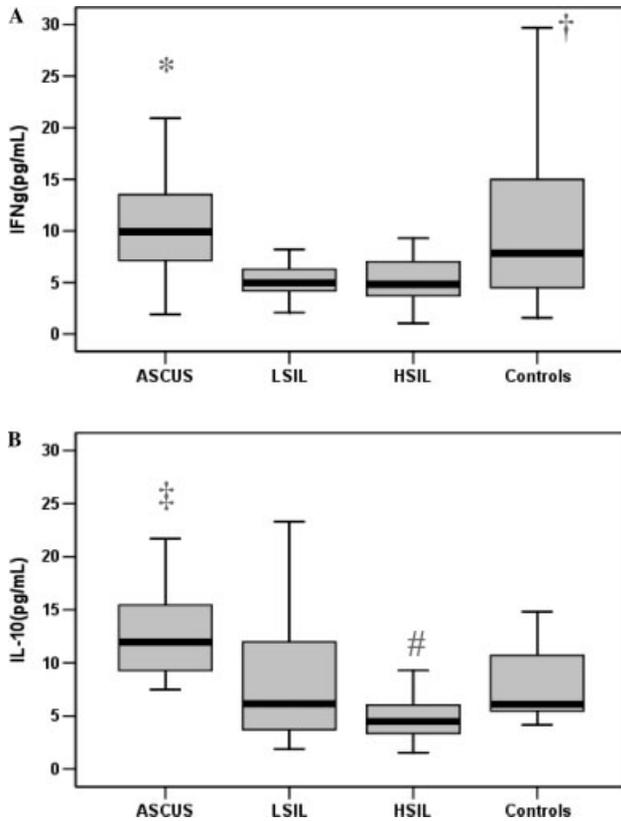
FIGURE 4. Plasma levels of (A) interferon-γ (IFN-γ) and (B) interleukin 10 (IL-10) from women with human papillomavirus (HPV)-related cytological cervical squamous cell abnormalities. The boxes represent the interquartile range, which extended from the 25th percentile to the 75th percentile, and the line across the box indicates the median (50th percentile); error bars represent 95% confidence intervals. ASCUS, atypical squamous cells of unknown significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion. *Statistical differences between ASCUS and LSIL ($P < .001$) or HSIL ($P < .001$). ‡Statistical differences between controls and LSIL ($P = .038$) or HSIL ($P = .031$). #Statistical differences between HSIL and ASCUS ($P = .003$) or the controls ($P = .032$). ‡Statistical differences between ASCUS and controls ($P < .001$), LSIL ($P < .001$), or HSIL ($P = .003$).

antigen presentation by immature myeloid dendritic cells, dysregulation of immunostimulatory cytokine signaling, and aberrant expression of major histocompatability complex (MHC) molecules.[23] Whereas PDCs in the peripheral blood are important for the innate immune defenses against viruses, they may suppress adaptive immunity by inducing $CD8^+$ T cells to produce IL-10.[24,25] Thus, it was not unexpected to find that women with HPV-related ASCUS had higher levels of PDC in peripheral blood and a higher IL-10 level in plasma than controls (Fig. 1B). These data are also consistent with an increased IL-10 level in cervical secretion of women with LSIL and it may be a useful

indicator of the stage of the cervical lesions induced by HPV infection.[25]

It is generally accepted that cervical SIL is due to persistent infection with high-risk type HPVs[26] and compromised host immunity. In this regard, we found patients with LSIL or HSIL to have significantly lower plasma levels of IFN-γ than controls (Fig. 4A). As IFN-γ is an essential cytokine for CTL activity, a lower level of this cytokine in patients with HSIL may account for the lack of HPV16-E6 specific CTL activity in women with persistent HPV infection.[27] In addition, the increase in PDC in the peripheral blood of patients with HSIL (Fig. 1B) may provide an immunosuppressive Th2 cytokine microenvironment that, in turn, is capable of down-regulating the expression of the MHC class-I antigens and $β_2$-microglobulin[28,29] and leading to impaired function of intraepithelial antigen-presenting cells.

MDCs mediate adaptive immunity and antigen presentation by MDDC is dependent on the expression of MHC and costimulatory receptors. We report here that women with ASCUS, LSIL, or HSIL had a significantly lower proportion of peripheral blood MDC that is consistent with what has been observed in CINI lesions of women with high-risk type HPV infection.[30] Our study extends this observation to report a deficiency in CD86 expression on activated MDDC from women with HPV-related ASCUS, LSIL, or HSIL (Fig. 2A), a costimulatory receptor that can be down-regulated by HPV16-E6 and HPV16-E7 oncoproteins that interfere with the transcriptional activity of NF-κB.[31]

Earlier studies have shown that HPV-specific CTLs can be elicited in women with HPV16-associated cervical cancer after stimulation of T cells with autologous MDDCs transfected with HPV16 E6 or E7 expression vector[32] or in healthy women using autologous MDDCs pulsed with HPV16 E6/E7 fusion protein[33] or HPV16-E7 peptides.[34] In the current study, activated MDDCs from women with HPV-related cervical LSIL were less capable of presenting HPV16-E6 and HPV16-E7 proteins to autologous T cells, resulting in a lower SI of T cells (Fig. 3). As HPV early antigens are thought to be localized mainly in the nuclei of undifferentiated keratinocytes in the basal layers of stratified epithelium[35] accompanied by the integration of HPV16 into the host genome at rates of 28% and 58%, respectively, in low-grade and high-grade lesions,[36] the lower proliferation of T cells in women with ASCUS might be related to the lower frequency of effector memory $CD4^+$ ($CD45RA^-CCR7^-$) T cells[37] against HPV16-E7.

Previously, we reported that the percentage of activated $CD4^+$ T cells of women with HPV-related

HSIL that produced Th1 cytokines was suppressed when activated through a mechanism that did not require the presentation of antigen by APC.[38] Here we demonstrate that the proliferation of T cells in response to activation with autologous MDDC loaded with either HPV16-E6 or HPV16-E7 protein was similar in controls and women with HSIL. Our results are similar to T cells of CIN patients producing IFN-γ in response to activation with MDDC that were loaded with HPV L1L2-E7 VLP.[16] Whereas patients with LSIL had MDDC with low expression of HLA-DR and low T-cells proliferation to HPV16-E6/E7 antigens, T cells from patients with HSIL with normal expression of HLA-DR on MDDC proliferated normally. While the relation between HLA-DR expression and T-cell proliferation requires further study, some viruses are known to target molecules exclusive to the MHC Class II presentation pathway and to prevent the activation of naive CD4$^+$ T cells.[39] In addition to the defects in antigen presentation by MDDC, the progression of HPV-related SIL may be due to the immature myeloid cells that inhibit CTL induction in vivo through the production of transforming growth factor β.[40]

Our data demonstrate deficiencies in myeloid DC in women with HPV-related SIL when compared with control women with normal Pap smears. Nevertheless, we concede that the demographics of the patients and controls are not equivalent, nor were these groups enrolled in a similar way. Control women were not asked to undergo a gynecologic examination at the time of study, as each control attested to having a negative Pap smear at her last annual well-women check-up. The median age of women with ASCUS was greater than that of control women and of patients with HPV-related LSIL or HSIL. However, the differences in age among all groups including the controls were not statistically significant ($P = .065$ by Kruskal–Wallis test).

We are aware of the confounding variables such as ethnicity and cigarette smoking that may influence the study outcome. The ethnicity of patients and controls was somewhat different, as the latter group contained a significantly higher proportion of individuals of Asian descent. This problem is not easily remedied, as the pool of control women for such studies is limited. Cigarette smoke and its derivatives have been shown to adversely affect the differentiation and maturation of monocyte-derived DC[41,42] and to preferentially induce a type 2 helper T cell response.[43] We also analyzed the data between non-smoker patients and controls and did not see any significant differences from the previous analyses, except for the expression of CD80 on MDDC, which was similar for all groups. We recognize that smoking is a major cofactor

and will have to be critically evaluated in the future with more smokers, both patients and controls.

In summary, the current study introduces additional information regarding the host immune response to HPV infection and progression of disease. Our study confirms previous data regarding the possible mechanism of cervical cancer progression through T-cell responses against HPV16-E6/E7 epitopes presented by pan-HLA D region on MDDC.[16] The current study also provides information of additional immunologic deficiency, such as a higher level of peripheral blood PDC accompanied by a lower level of peripheral blood MDC and low percentages of activated MDDCs that express the costimulatory molecules CD86 and HLA-DR. Collectively, these factors could account for the lower T-cell immunity against HPV infection and provide a more suitable environment for the development of precancerous lesions in HPV-infected women.

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