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Increased number of IL-2, IL-2 receptor and IL-10 positive cells in premalignant lesions of the cervix.

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Key words: IL-2, IL-2 receptor, IL-10, premalignant lesions, cervix.

Abstract. Previous studies have shown the involvement of the immune response in the progression of human uterine cervix cancer. The aim of this study was to determine the expression of Interleukin-2 (IL-2), IL-2 receptor (IL-2R) and Interleukin 10 (IL-10) in different grades of cervical intraepithelial neoplasias of the exocervix (CIN 1, 2 and 3), and its relationship with the serum cytokine profiles and human papilomavirus (HPV) infection status. Indirect immunofluorescence was used to study the expression of IL-2, IL-2R and IL-10 in human cervical samples from 50 patients and 9 normal controls. Serum IL-2, IL-2R and IL-10 were measured by ELISA and HPV DNA and HPV types were identified by PCR. Increased number of IL-2, IL-2R and IL-10 positive cells were observed in the cervix from patients with CIN, associated with the grades of dysplasia. A significant correlation was observed between IL-2 and IL-2R ($p>0.0001$), IL-2 and IL-10 ($p > 0,0001$), as well as IL-10 and IL-2R ($p> 0.0001$). Twenty percent of patients were HPV positive and 84 % of those patients were tissue cytokine positive. These results suggest that IL-2, IL-2R and IL-10 tissue expression may play a role in the development of cervical intraepithelial dysplasias.

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Incremento en el número de células positivas para IL-2, receptor de IL-2 e IL-10 en lesiones premalignas del cervix.
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Palabras claves: IL-2, receptor de IL-2, IL-10, lesiones premalignas, cervix.

Resumen. En previos estudios se ha determinado que la respuesta inmunitaria esta involucrada en la progresión del cáncer del cuello uterino humano. El propósito de este estudio fue determinar la expresión de Interleucina-2 (IL-2), del receptor de IL-2 (IL-2R) e Interleucina 10 (IL-10) en diferentes grados de la neoplasia intraepithelial cervical del exocervix (NIC 1, 2 y 3) y su relación con los niveles séricos de estas citocinas y el estado de infección con el virus del papiloma humano (VPH). Se usó la inmunofluorescencia indirecta para determinar la expresión de IL-2, IL-2R e IL-10 en muestras de tejido cervical procedentes de 50 pacientes y 9 controles. Las concentraciónes de IL-2, IL-2R e IL-10 sérica fueron determinadas mediante ELISA y el ADN del VPH y los tipos de virus mediante PCR. Se encontró aumento del número de células positivas para IL-2, IL-2R e IL-10 en el cervix de pacientes con NIC, asociado al grado de la lesión. Se encontró alta significacia cuando se correlacionó la expresión de IL-2 con IL-2R ($p>0.0001$), IL-2 con IL-10 ($p>0.0001$) y la IL-10 con IL-2R ($p>0.0001$). El 20% de los pacientes fueron VPH positivos y de estos pacientes el 84% tuvieron expresión de citocinas a nivel tisular. Estos resultados sugieren que la expresión tisular de la IL-2, IL-2R y la IL-10 pueden estar involucradas en la progresión de la displasia intraepitelial cervical.

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INTRODUCTION

Cancer of the cervix is a disease characterized by a long lead-time, with precancerous lesions gradually progressing through recognizable stages before developing into invasive disease. The term "cervical intraepithelial neoplasia" (CIN) was introduced by Richart (1) and implied the concept that a precursor lesion to squamous cell carcinoma represented a single, continuous disease process and makes clear the pre-invasive nature of lesions. The CIN nomenclature divides cervical cancer precursors into CIN 1, CIN 2 and CIN 3, corresponding to mild, moderate and severe dysplasia/carcinoma in situ (2). Cytokine production by T cells is a reliable measure of the quality of cell-mediated immunity (3). The local Th1 and Th2 immune response in tissues could be involved in the progression of cervical neoplasia from mild to severe status. The aim of this study was: 1) to assess the cellular expression of cytokines Th1 (Interleukin-2: IL-2) and Th2 (Interleukin-10: IL-10) in CIN 1, CIN 2 and CIN 3 lesions, normal tissues adjacent to the lesions and normal cervical tissues, and 2) to determine its association with the cellular expression of IL-2 receptor (IL-2R), cytokine serum concentration and human papillomavirus (HPV) infection.

MATERIAL AND METHODS

Tissue specimens

We evaluated 109 exocervix samples spanning the spectrum from normal cervical tissue to high-grade squamous intraepithelial lesions. Samples were obtained from women who visited the gynecological out-patient clinic of The Manuel Noriega-Trigo Hospital, National Health Clinic "La Victoria" and private practice. Patients were informed about the study procedures and their consents were obtained before enrollment in the investigation, following the ethical committee guidelines of each hospital, the Committee of Bioethical and Biosecurity of FONACIT (Caracas, Venezuela) and the Committee of Bioethical of Medical School (Universidad del Zulia, Maracaibo, Venezuela). Each patient had a personal interview including gynecological and obstetric history and underwent a gynecological examination, Pap smear and a colposcopic examination of exocervix. Sexually active, no pregnant patients with cervix lesions and without immunosuppressant treatment or treatment for HPV were included in this study. Colposcopic examinations were performed under a standard protocol included conventional visual assessment, application of 5% acetic acid, identification of the squamocolumnar junction and transformation zone and recognition of suspected CIN lesions for direct cervical biopsies. A direct biopsy was taken when a colposcopic abnormality was found (n = 50). Biopsies were also taken in normal cervix at least 1cm from the colposcopic lesion (n = 50). Samples from 9 healthy individuals with normal cervix were taken as controls. All specimens were fixed in 10% buffered formalin, embedded in paraffin for 6 μm sections, stained with hematoxilin-eosin and analyzed following Richart's terminoly (1). Colposcopic cervical samples were also included in OCT compound (Tissue Tek, Miles Inc. Diagnostics Division, Kankakee, IL, USA), frozen in dried ice and acetone and kept at –70ºC until use.

Immunofluorescence

The frozen sections (4 μm) from control and patients tissues were fixed with cold acetone and then reacted with a mouse monoclonal anti- human IL-2 receptor or with a rat monoclonal anti-human IL-10 or rat monoclonal anti-human IL-2 antibodies (Pharmigen, Torreyana Rd. San Diego, CA, USA). Mouse and rat immunoglobulins were detected on tissues with FITC-conjugated goat anti-mouse IgG and FITC-conjugated rabbit anti-rat IgG antibodies (Sigma Chemical Co. (St. Louis, MO, USA). Controls included sections subjected to monoclonal antibodies with the same isotype, but against non-relevant antigens as a primary antibodies. Samples were examined using an epifluorescence microscope (Zeiss, Axioskop, Göttingen, Germany).

Detection of HPV DNA in cervical specimens

Exfoliated epithelial cells from all individuals were obtained by cervical brushing and placed in transport medium, supplied with a specimen collection kit of PVHfast 2.0 (Pharma Gen S.A. Madrid, Spain) for the detection of HPV DNA. Cervical specimens are collected to test for HPV DNA through the use of a previously described PCR method that amplified a highly conserved segment (450 bp) of the *L1* gene (4, 5). Restriction enzymes analysis was used to determine the HPV types. This kit detects 34 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).

Detection of serum cytokines

All of the participants in the study were asked to provide a blood sample. Whole blood was obtained using a Vacutainer system and an empty vacuum-sealed blood tube without preservative. Serum was separated by centrifugation, aliquoted and stored at –70°C until use. Human IL-2, IL-10 and soluble IL-2 receptors were quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis MN, USA), according to the manufacture's protocol. The amount of cytokines was expressed as pg per milliliter.

Calculations and statistical analysis

Positive cells were counted in tissue areas observed at X 400. Results in the groups are shown as mean ± SEM. Comparisons between groups were done using the Mann-Whitney test and ANOVA. For correlations between two variables, Pearson's correlation was used. Two tailed $p < 0.05$ was interpreted as being statistically significant.

RESULTS

Cytokine positive cells in tissues

Histological analysis showed the following pathological categories: CIN 1 (n = 36), CIN 2 (n = 6), CIN 3 (n = 8). In general, increased numbers of IL-2, IL-10 or IL-2R positive cells were found in tissues from patients with CIN when compared to adjacent normal tissues and normal controls. This increment was observed in the different grades of CIN, however, in some instances non significant differences were observed (Figs. 1, 2 and 3). Cytokine-posi-

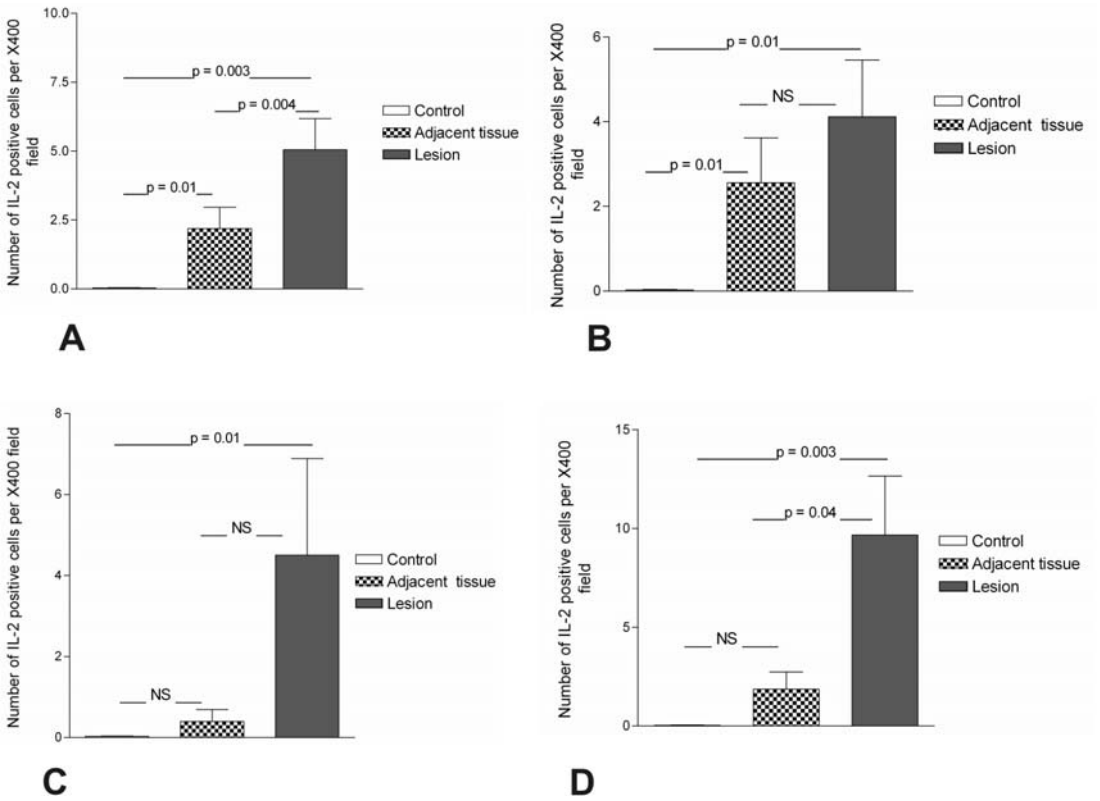

Fig. 1. Number of IL-2-positive cells in controls and patients with premalignant lesions. Data represents mean ± SEM of normal controls, normal adjacent tissues and lesions from patients with CIN. A) General data. B) CIN 1. C) CIN 2. D) CIN 3.

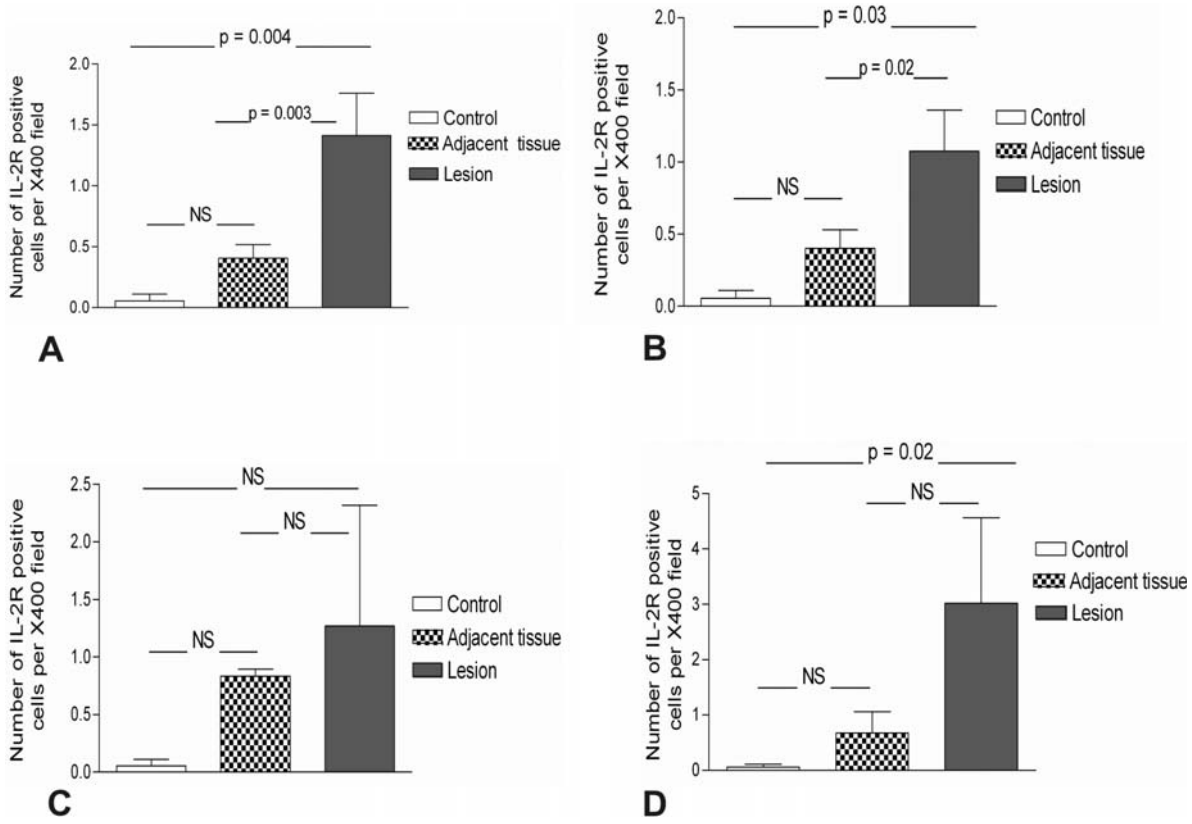

Fig. 2. Number of IL-2 receptor - positive cells in controls and patients with premalignant lesions. Data represents mean ± SEM of normal controls, normal adjacent tissues and lesions from patients with CIN. A) General data. B) CIN 1. C) CIN 2. D) CIN 3.

tive cells were observed in 84% of the patient's lesions and in 10% of controls (Table I). Apparently, normal tissues taken near the site of the lesion showed increased number of cytokine- positive cells when compared to normal control tissues (Figs. 1, 2 and 3). The number of cytokine-positive cells was associated to the severity of the cervical lesion; however, irregular expression was observed in tissues near the lesion (Fig. 4). The expression of IL-2 was correlated with the expression of IL-2R ($p < 0.0001$) and IL-10 ($p < 0.0001$). In addition, the expression of IL-10 was correlated with the expression of IL-2R ($p < 0.0001$) (Table II). Cytokine-positive cells were localized in epithelium and subepithelium and in some instances, clusters of positive cells were observed (Fig. 5).

Cytokine levels in serum

Serum sIL-2R levels were found to be not significantly elevated in patients with CIN compared to normal levels. Levels of IL-2 and IL-10 were undetectable using ELISA (Table III).

HPV infection

Twenty percent of the patients were positive for HPV as shown by the detection of HPV DNA. Twenty one percent of all patients positive for tissue cytokines were also positive for HPV. Ninety percent of HPV-positive patients were positive for tissue cytokine expression (Table I). HPV/cytokine-positive patients were mainly CIN 1 and CIN 3 (Fig. 6).. There were not significant differences when tissue cytokine expression, was compared with positive or

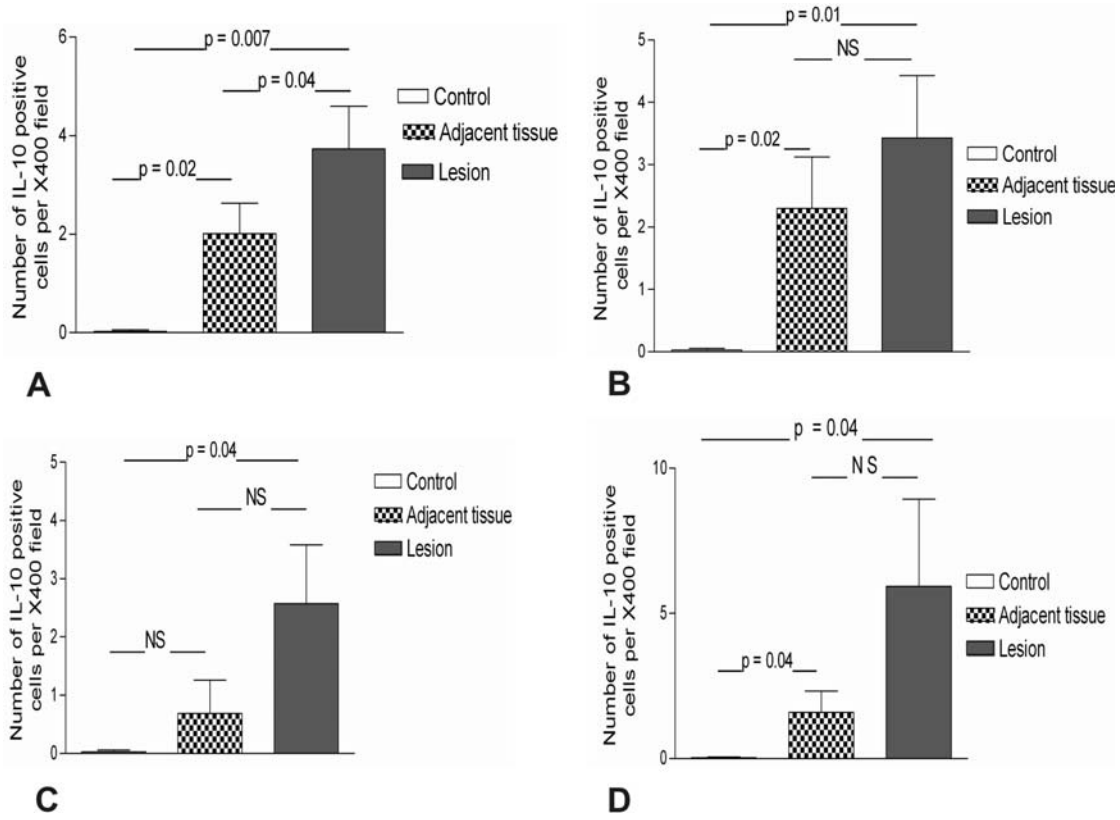

Fig. 3. Number of IL-10 - positive cells in controls and patients with premalignant lesions. Data represents mean ± SEM of normal controls, normal adjacent tissues and lesions from patients with CIN. A) General data. B) CIN 1. C) CIN 2. D) CIN 3.

TABLE I
TISSUE CYTOKINES AND HPV INFECTION IN SAMPLES FROM PATIENTS AND NORMAL CONTROLS

Positive	Controls		Patients	
	Relation	%	Relation	%
Cytokine/total*	1/9	11	42/50	84
HPV/total*	3/9	33	10/50	20
Cytokine/total HPV	1/3	33	9/10	90
HPV/total cytokine	1/1	100	9/42	21

* Total individual in controls or patients.

negative HPV-infection status (Fig. 7). In controls 33 % were HPV positive. Seven out of 10 samples from HPV-infected patients and 2 out of 3 from infected controls, were tested for HPV types. High-risk type infection was detected in patients (16.31) and low-risk type infection (6, 11) was detected in controls (Table IV).

DISCUSSION

Cytokine production is a reliable measure of the quality of cell-mediated immunity. Host factors are critical in regulating tumor growth and cytokines that modulate immunologic control may be of particular importance (3). The Th 1 cytokine inter-

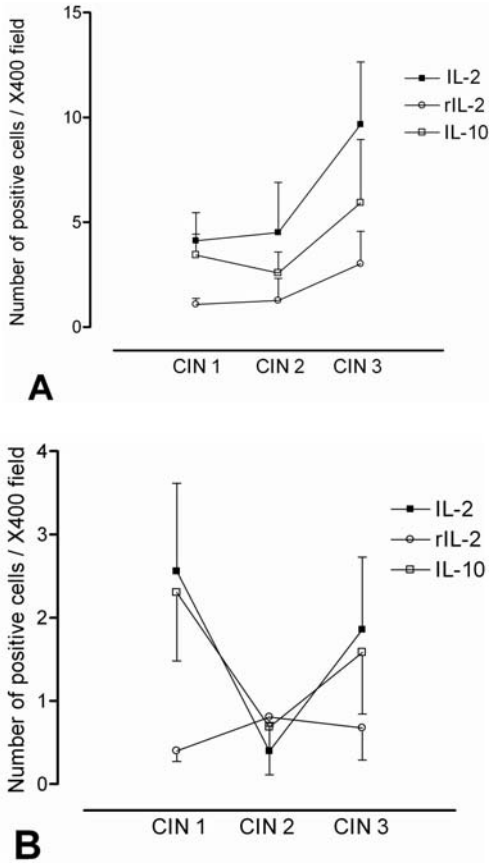

Fig. 4. Expression of IL-2, IL-2R and IL-10 in the different grades of CIN. A) Lesion. B) Adjacent apparently normal tissue.

leukin 2 is immunostimulatory and it is thus capable of limiting tumor growth (6). The Th 2 cytokine interleukin 10 is immunoinhibitory and it is capable of stimulating tumor growth (6). In the present study an increased number of cells positive for IL-2, IL-2R and IL-10 was showed in cervical samples from patients with different grades of CIN. The presence of those cells was associated with the severity of the cervical lesion, suggesting a role for IL-2 and IL-10 in the evolution of the dysplasia. A previous report has demonstrated a low density of IL2-positive cells in high-grade squamous intraepithelial lesions compared to normal tissues (7); conversely, increased number of IL-2-positive cells was found in CIN 3. This finding could be related to the increased expression of IL-10, a cytokine with IL-2-stimulatory effect (8). The presence of both cytokines in the dysplasia and the high correlation found between them are consistent with that explanation.

The presence of IL-2 and IL-2R positive cells in the cervical microenvironment suggests the interaction of IL-2 with its receptor. This could promote nonspecific tumor killing by activated macrophages, lymphokine-activated killer cells (9) and tumor-infiltrating lymphocytes (10). Because of its stimulatory activity on a variety of immune cell types, IL-2 has been studied extensively in the augmentation of the innate immune response to malignant disease (11, 12). In addition, signal transduction pathways triggered in IL-2R-expressing solid tumors could be mediated by an alternative mechanism. Stem Cell Factor -activated c-Kit induces phosphorylation of the IL-2R beta chain in the absence of IL-2 in HPV-associated cervical cancer cells (13). Thus, the presence of increased numbers of IL-2R positive cells in cervical tissues during CIN could represent an important target for IL-2 and different stimuli produced in the uterine microenvironment.

Interleukin-10 is widely known as an immunosuppressive cytokine by virtue of its

TABLE II
CORRELATION BETWEEN IL-2, IL-2R AND IL-10 EXPRESSIONS IN UTERINE TISSUES

	Pearson r	P values
IL-2 / IL-2R	0.713	$P < 0.0001$
IL-2 / IL-10	0.742	$P < 0.0001$
IL-10/ IL-2R	0.709	$P < 0.0001$

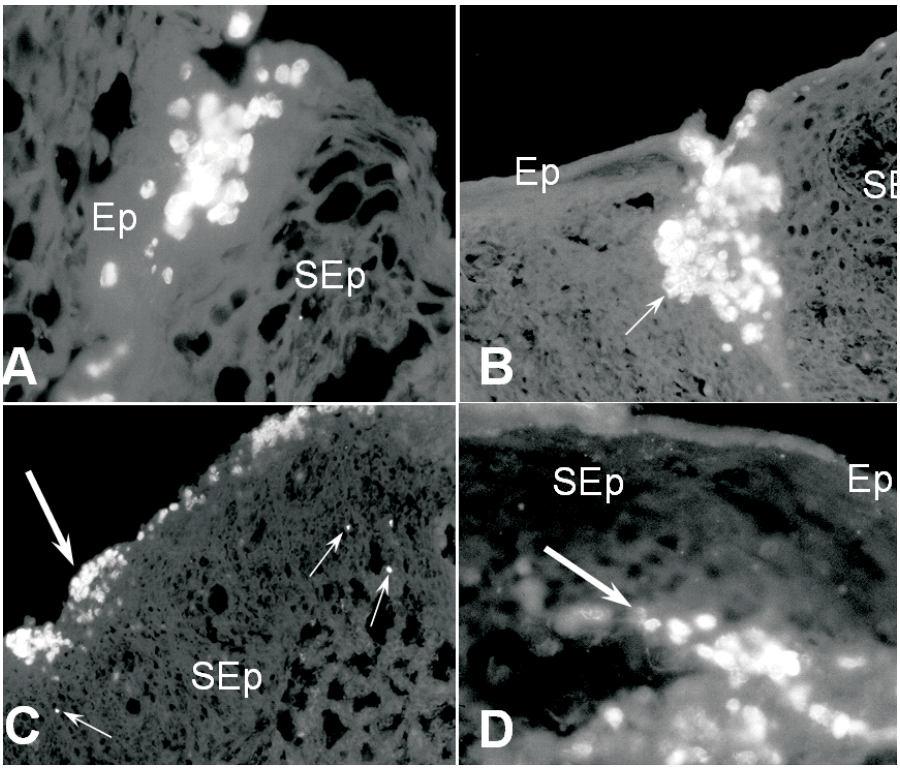

Fig. 5. Indirect immunofluorescence staining for IL-2, IL-2R and IL-10. A) Cluster of IL-2-positive cells in the uterine epithelium. B) Cluster of IL-2R positive cells in the epithelium and in the adjacent subepithelium (arrow). C) Epithelial disposition of IL-10-positive cells (thick arrow). Observe some positive cells in the subendothelium (small arrows). D) Subepithelial localization of IL-10-positive cells (arrow). Ep: epithelium; SEp: subepithelium. A -C: CIN 2; D: CIN 1. Magnification: X 400.

TABLE III
CYTOKINE SERUM LEVELS IN NORMAL AND PATIENTS WITH PREMALIGNANT LESIONS OF UTERINE CERVIX

	Controls	Patients
IL-2	ND	ND
IL-2R	99.23 ± 43.86*	160.37 ± 34.01
IL-10	ND	ND

* pg/mL; ND: not detectable.

ability to inhibit macrophage-dependent antigen presentation, T-cell proliferation, and Th1 cytokine secretion (6). The increased expression of IL-10 in the different grades of CIN found in this study, could contribute to the immunodeviation, that could participate in the immunoescape of preneoplastic cervical keratinocytes, and represent an antagonistic activity compared to the effect of IL-2. Consistent with our results, it has been reported that the expression of IL-10 increased with the severity of the lesion to a maximal level in high-grade cervical squamous intraepithelial lesions (14). However, several studies have challenged the perception of IL-10 solely as an immuno-

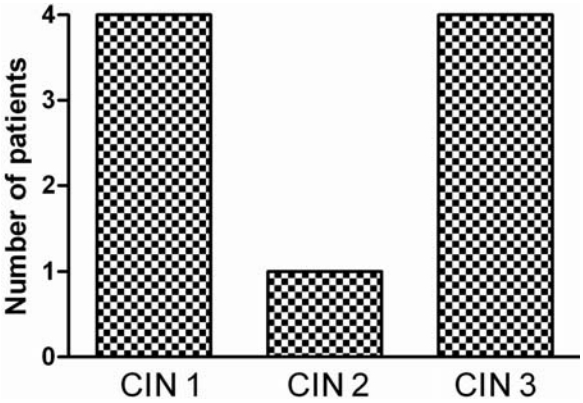

Fig. 6. Distribution of HPV cytokine-positive patients according to the different grades of CIN.

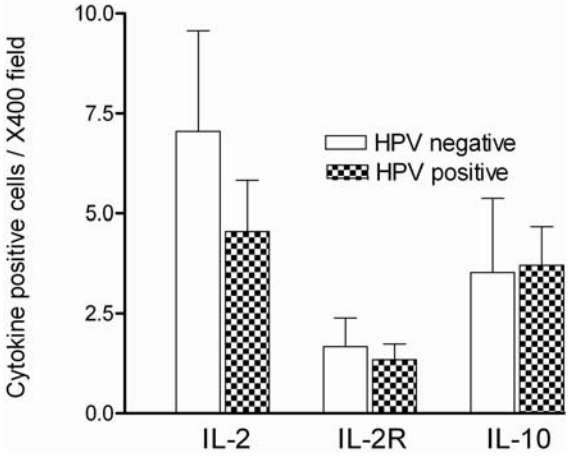

Fig. 7. Cytokine profiles in HPV-positive and negative patients.

TABLE IV
HPV TYPES AND PREMALIGNANT LESION DISTRIBUTION IN SAMPLES OF UTERINE CERVIX FROM NORMAL CONTROLS AND PATIENTS

	Controls	Patients
HPV/total individuals	3/9	10/50
Typification/total HPV	2/3*	7/10**
HPV types	6, 11	CIN 1 (n=2: 16, 31) CIN 2 (n=1: 16/11) CIN 3 (n=4: 16,16, 16, 16/31)

* All samples were negative for tissue cytokine expression. ** All samples were positive for cytokine expression.

suppressive cytokine. In this regard, it has been reported that IL-10 in combination with IL-2 was able to consistently increase the cytotoxic activity of human papillomavirus E7-specific CD8(+) cytotoxic T lymphocytes. In addition, IL-10 in combination with IL-2 after antigen stimulation, increased the intracellular expression of Th1 cytokines (i.e., IFN-gamma and IL-2) in CD8 positive T cells (8). IL-2 can also induce the production of IL-10 by human T cells (15). In addition, IL-10 increases the natural killer cell anticancer activity by enhancing the levels of cell activation/cytotoxicity-related genes (eg secretogranin, TIA-1, HMG-1, interferon-inducible genes) and the expression of cell migration-related genes (e.g. L-selectin, vascular endothelium growth factor receptor-1, plasminogen activator, tissue; formyl peptide receptor, lipoxin A4 receptor) (16). IL-10 has also been shown to induce IL-2Rα expression in inflammatory processes and viral infections (17). Taking together these data, it is possible to speculate that the increased levels of IL-2-positive cells, IL-2R positive cells and IL-10-positive cells and the high correlation between those cells in CIN could represent an IL-2/IL-2R – inducing effect of IL-10 and a mechanism to escape from an invasive cancer. The association of IL-2/IL-10 found in this study, probably is not going to be preserved in invasive carcinoma, since, low levels of IL-10 in invasive carcinoma compared with premalignant biopsies have been reported

(18). An increased number of cytokine-positive cells in normal tissues adjacent to the lesions was also found, suggesting that the cytokine expression is a phenomenon not limited to the lesion, but it may be generalized in the cervical tissues. This could represent a chemotactic effect from the CIN on dendritic cells and leukocytes capable of producing these cytokine profiles (19-21).

Lymphocytes are the main IL-2 producers, however in normal tissues, and several tumours, including stomach, renal and spinocellular cancer and squamous cell carcinomas of the head and neck, IL-2 seems to be present and stimulate cell proliferation (22, 23). Thus, the presence of some cytokine positive cells observed in this study could represent epithelial cells or other types of cells.

Human papillomavirus type 16 is a major factor in cervical carcinogenesis (24). Previously it has been reported an increased production of IL-10 and decreased production of IL-2 by peripheral blood mononuclear cells in women with CIN, associated with localized or extensively spread of HPV infection, and suggesting that a pronounced shift from Th 1 to Th 2 cytokine production is associated with extensive HPV infection (25). In general, a shift from a Th1 (IL-2) and gamma interferon (IFN-) to a Th2 (IL-4 and IL-10) cytokine profile is associated with poor prognosis for patients with HPV-associated cervical lesions (25). This study showed that patients were infected mainly by HPV type 16, however, the Th1 to Th2 shift at the tissue level was not found. This could be related to the fast that only 20% of individuals were positive for HPV and extensive HPV infection was not observed. This percentage agrees with prevalence studies showing that between 5% and 20% of the general population has HPV DNA detectable in cervical samples (26) and that in most cases, infection with HPV is transient and may or may not be associated with cervical abnormalities of low squamous intraepithelial lesions (27). The low frequency (20%) observed in the patients in our population agree with other studies which reported 9.9 to 12.5 % in the occidental Venezuelan population (28, 29). High frequency of HPV infection in controls (33%) was observed when compared to patients (20%), however, only low risk virus types were found, suggesting low risk to have CIN. The persistence of IL-2 positive cells found in these patients could have a beneficial effect limiting HPV-induced tumor growth. In this regard, local use of IL-2 has been successful in cervical condylomatosis therapy (30). IL-10 positive cells could also play a beneficial role in the progression of premalignant lesions during HPV infection, since elevated levels of IL-10 have been associated with the absence of cervical lesions among HPV-infected women (31). The cytokine profile and cytokine values were similar in HPV- infected and noninfected patients suggesting that virus infection does not alter the local cytokine production during the course of uterine premalignant lesions.

The tissue cytokine expression was not reflected at the serum level. IL-2 and IL-10 were not detectable in the serum using enzyme-linked immunosorbant assays. Only soluble receptor for IL-2 was detected, but no significant differences were observed when compared to the normal controls. This suggests that increased expression of cytokines in CIN was a local phenomenon and it is consistent with the association of excessive circulating levels of cytokines with invasive cancer (32). Thus, the serum cytokines values found in this study most likely are related to the conditions of the patients where noninvasive neoplasia is detected.

In conclusion, it has been reported the notion that the progression of cancer of the cervix is associated with a preferential con-

straint on the development of a Th 1 cellular mediated response, which is necessary to efficiently eliminate (pre)neoplastic cells. In our study premalignant lesions did not show a preferential Th1 or Th2 cytokine profile and elevated number of cells Th1 and Th2 positives were observed in the different grades of CIN. The presence of these cells were not associated either with increased serum profile of cytokines or HPV infection. This could represent a transitory stage with further locally reduced cellular Th1 immunity and invasive cervical carcinoma or, represent a cytokine combination with a potential anti-tumor effect. These data reinforce the need to perform further studies to determine changes on the local IL-2/IL10 profile in invasive cancer.

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REFERENCES

1. **Richart RM**. Cervical intraepithelial neoplasia. En: Sommers, Ed. Pathology Annual S.C. New York, USA; 1997. P 301-328.
2. **Apgar BS, Brotzman G.** Management of cervical cytologic abnormalities. Am Fam Physician 2004; 70:1905-1916.
3. **Moss RB, Moll T, El-Kalay M, Kohne C, Soo Hoo W, Encinas J, Carlo DJ.** Th1/Th2 cells in inflammatory disease states: therapeutic implications. Expert Opin Biol Ther 2004; 4:1887-1896.
4. **Bauer H M, Ting Y, Greer CE, Chambers JC, Tashiro C J, Chimera J, Reingold A, Manos MM.** Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 1991; 265:472-477.
5. **Hildesheim A, Schiffman MH, Gravitt P E, Glass A G, Greer C E, Zhang T, Scott DR, Rush BB, Lawler P, Sherman M E, Kurman R J, Manos MM.** Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994; 169:235-240.
6. **Mocellin S, Wang E, Marincola FM.** Cytokines and Immune Response in the Tumor Microenvironment. J Immunother 2001; 24:392-407.
7. **Al-Saleh W, Giannini SL, Jacobs N, Moutschen M, Doyen J, Boniver J, Delvenne P.** Correlation of T-helper secretory differentiation and types of antigen-presenting cells in squamous intraepithelial lesions of the uterine cervix. J Pathol 1998; 184:283-290.
8. **Santin AD, Hermonat PL, Ravaggi A, Bellone S, Pecorelli S, Roman JJ, Parham GP, Cannon MJ.** Interleukin-10 increases Th1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8(+) cytotoxic T lymphocytes. J Virol 2000; 74:4729-4737.
9. **Grimm E, Mazumder A, Zhang H, Rosenberg S.** Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med 1982; 155:1823-1841.
10. **Rosenberg SA, Spiess P, Lafreniere R**. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 1986; 233:1318-1321.
11. **Atkins M, Lotze M, Dutcher J, Fisher R, Weiss G, Margolin K.** High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol 1999; 17:2105-2116.
12. **Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC.** Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol 1995; 13:688-696.
13. **Rocha-Zavaleta L, Huitron C, Caceres-Cortes JR, Alvarado-Moreno JA, Valle-**

Mendiola A, Soto-Cruz I, Weiss- Steider B, Rangel-Corona R. Interleukin-2 (IL-2) receptor-betagamma signalling is activated by c-Kit in the absence of IL-2, or by exogenous IL-2 via JAK3/STAT5 in human papillomavirus-associated cervical cancer. Cell Signal 2004; 16:1239-1247.

14. **Giannini SL, Al-Saleh W, Piron H, Jacobs N, Doyen J, Boniver J, Delvenne P.** Cytokine expression in squamous intraepithelial lesions of the uterine cervix: implications for the generation of local immunosuppression. Clin Exp Immuno 1998; 113:183-189.
15. **Rafiq KL, Charitidou DMA, Bullens A A, Kasran K, Lorre J L, Ceuppens J, Van Gool S W.** Regulation of the IL-10 production by human T cells. Scand J Immunol 2001; 53:139-147.
16. **Mocellin S, Panelli M, Wang E, Rossi CR, Pilati P, Nitti D, Lise M, Marincola FM.** IL-10 stimulatory effects on human NK cells explored by gene profile analysis. Genes Immun 2004; 5:621-630.
17. **Graffen SL, Lai SY, Xu W, Gouilleux F, Groner B, Goldsmith MA, Greene WC.** Signalling through the interleukin-2 receptor β chain activates a stat-5-like DNA-binding activity. Proc Natl Acad Sci USA 1995; 92:7192-7196.
18. **de Gruijl TD, Bontkes HJ, van den Muysenberg AJ, van Oostveen JW, Stukart MJ, Verheijen RH, van der Vange N, Snijders PJ, Meijer CJ, Walboomers JM, Scheper RJ.** Differences in cytokine mRNA profiles between premalignant and malignant lesions of the uterine cervix. Eur J Cancer 1999; 35:490-497.
19. **Hubert P, van den Brule F, Giannini SL, Franzen-Detrooz E, Boniver J, Delvenne P.** Colonization of in vitro-formed cervical human papillomavirus- associated (pre) neoplastic lesions with dendritic cells: role of granulocyte/macrophage colony-stimulating factor. Am J Pathol 1999; 154: 775-784.
20. **Inoue T, Iwasaki K, Chihara T, Torisu M.** Cervical carcinoma and eosinophil I. Eosinophil chemotactic factor derived from invasive cervical carcinoma with eosinophil infiltration. Clin Immunol Immunopathol 1981; 21:320-331.
21. **Megiovanni AM, Sanchez F, Gluckman JC, Rosenzwajg M.** Double-stranded RNA stimulation or CD40 ligation of monocyte-derived dendritic cells as models to study their activation and maturation process. Eur Cytokine Netw 2004; 15:126-134.
22. **Azzarone B, Pottin-Clemenceasu C, Krief P, Rubinstein E, Jasmin C, Scudeletti M, Indiveri F.** Are interleukin-2 and interleukin-15 tumor promoting factors for human non-hematopoietic cells? Eur Cytokine Netw 1996; 7:27-36.
23. **Reichert T, Watkins S, Stanson J, Johnson JT, Whiteside TL.** Endogenous IL-2 in cancer cells: a marker of cellular proliferation. J Histochem Cytochem 1998; 46: 603-611.
24. **El-Sherif AM, Seth R, Tighe PJ, Jenkins D.** Quantitative analysis of IL-10 and IFN-gamma mRNA levels in normal cervix and human papillomavirus type 16 associated cervical precancer. Am J Pathol 2001; 195:179-185.
25. **Clerici M, Merola M, Ferrario E, Trabattoni D, Villa ML, Stefanon B, Venzon DJ, Shearer GM, De Palo G, Clerici E.** Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection. J Natl Cancer Inst 1997; 89:245-250.
26. **Critchlow CW, Koutsky, LA.** Epidemiology of human papillomavirus infection. En: Mindel A, Ed. Genital warts: human papillomavirus infection. London: Edward Arnold; 1995. P 53-81.
27. **von Knebel Doeberitz M.** The role of papillomaviruses in the etiology of cervix cancer. Geburtshilfe Frauenheilkd 1990; 50:511-517.
28. **Muñoz M, Mendoza JA, Téllez L, Noguera ME, Moret O, López M, Toro M.** Detección de VPH-16 y 18 en muestras de cérvix de mujeres que acuden a centros asistenciales de la ciudad de Mérida, Venezuela. Rev Biomed 2003; 14:61-68.
29. **Mendoza JA, Muñoz M, Vielma S, Noguera ME, López M, Toro M.** Infección cervical por el virus del papiloma humano: diag-

nóstico por citología y por captura de híbridos del ADN viral. Rev Obstet Ginecol Venez 2000; 60:103-107.

30. **Zarcone R, Bellini P, Cardone G, Barletta E, Vicinanza G.** Association of interleukin 2 and interferon alpha in the management of cervical condylomatosis. Minerva Ginecol 1996; 48:111-113.
31. **Nakagawa M, Stites DP, Patel S, Farhat S, Scott M, Hills NK.** Persistence of human papillomavirus type 16 infection is associated with lack of cytotoxic T lymphocyte response to the E6 antigens. J Infect Dis 2000; 182:595-598.
32. **Ung A, Kramer TR, Schiffman M, Herrero R, Bratti MC, Burk RD, Swanson CA, Sherman ME, Hutchinson ML, Alfaro M, Morales J, Balmaceda I, Hildesheim A.** Soluble Interleukin 2 Receptor Levels and Cervical Neoplasia. Results from a Population-based Case-Control Study in Costa Rica. Cancer Epidemiol Biomark Prev 1999; 8:249-253.