Evaluation of Langerhans' cells in human papillomavirus-associated squamous intraepithelial lesions of the uterine cervix

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Summary

Current research has been evaluating morphological modifications and density of Langerhans' cells in women with histopathological HPV lesions of the uterine cervix. Fourteen women with subclinical HPV infections underwent clinical, colpocytological colposcopy examinations, and paired biopsies of the uterine cervix. Histopathological, HPV hybrid capture and S-100 immunohistochemical examinations were performed in biopsy specimens. Groupings of viral lesions and normal tissue were analyzed by the Wilcoxon rank-sum test. Langerhans' cells in the specimens were frequently located in the intermediate and basal layers of the epithelium. A significant reduction of cytoplasmic profiles occurred in viral lesions (144.08 profiles/mm^2) when compared to normal tissue (256.27 profiles/mm^2) of the epithelium. An inverse modulation occurred in the cytoplasmatic profiles/nuclei ratio with 2.80 in viral lesions and 4.89 in normal tissue of the stroma. A local immunodeficiency based on cytoplasmic changes of Langerhans' cells has been postulated as a mechanism by which HPV could be involved in the genesis of neoplasia.

Key words: Langerhans' cells; Human papillomavirus; Immunity; Uterine cervix.

Introduction

Recent studies about the cancer vaccine program indicate a measurable response of lymphocyte B to HPV-16- and 18-VLP particles in 56% and 39% of patients with uterine cervix cancer [1]. These facts suggest that somehow HPV may inhibit the rejection ability of the immunological system. T-CD4 and T-CD8 lymphocytes, Langerhans' cells (LC), macrophages and histiocytes are involved in the immunological defense of the uterine cervix transformation zone [2, 3]. S100-positive cells form one-third of Langerhans' cells of the uterine cervix and it is this fraction that undergoes pronounced depression in the presence of HPV [4].

Current research aims at studying the cell-mediated immunological system in patients with subclinical HPV infection and at analyzing the histological and histometric LC modifications in uterine cervix tissue with and without viral lesions.

Material and Methods

Fourteen women, aged between 16 and 49 years, who presented low-grade squamous intraepithelial lesions (LSIL) on cytology or minimal cervical abnormal area on colposcopy and a positive HPV DNA test in cervical biopsy specimens were selected for the study. The presence of HPV DNA was confirmed by Hybrid Capture® (Digene Diagnostics Inc.) using a mixture of RNA probes for 18 HPV types commonly found in anogenital infection (types 6, 11, 16, 18, 31,33, 35,39,42, 43, 44, 45, 51, 52, 56, 58, 59, 68).

The interviewed women were previously instructed about the research aims. A written consent was then obtained by signing the Information Document and Terms of Consent. Research was also approved by the Hospital Ethics Committee of UNIFESP/São Paulo Hospital, Brazil.

After colposcopic examination, paired cervical biopsy specimens were taken from equidistant areas of the external cervical orifice with two different biopsy forceps. Specimen A was removed from an area with abnormal colposcopic aspects and the other, specimen B, was taken from a colposcopically normal cervical zone. Specimens A and B were subdivided into two parts: one was sent for histopathological and immunohistochemical examination; the second, frozen at -200°C, was later sent for HPV Hybrid Capture® test.

The biopsy specimens were divided into two groups according to the histopathological diagnosis: viral lesion (VL) specimens, consisting of tissue of the uterine cervix with low-degree intraepithelial neoplasm; normal (control) specimens without viral lesion, consisting of tissue of the uterine cervix without histological alterations due to HPV infection or neoplasm.

For immunohistochemistry, 3- to 4-μm tissue sections, placed on two silanized slides, were deparaffinized and rehydrated. Endogenous peroxidase was blocked by 3% H_2O_2 and the slides washed with distilled water and buffered saline solution (PBS). The sections were incubated with 1/5000 solution of S100 protein antibody (DAKO), for 16 hours at 4°C and then with anti-rabbit antibody (Vector Laboratories) for 30 minutes, at 37°C. The bound antibodies were localized by the streptavidin-biotin-peroxidase complex method using a chromogenic substrate prepared with 0.6% diaminobenzidine (Sigma, USA),

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0.06% hydrogen peroxide and 1% dimethyl sulfoxide in PBS. The sections were finally counterstained with Harris' hematoxylin, and evaluated by light microscopy (x 400 magnification) coupled to a microcamera and digitized by Cytoviewer software. The cell counts were expressed as numbers of positive nuclei/mm^2 or cytoplasmic profiles/mm^2 sectional area (measured with a graduated Neubauer camera). S100-positive Langerhans' cells were identified by the brown color of their cytoplasm. According to the axis of the histological sections they presented considerable morphologic variation such as dots, commas, lines and trees defined as cytoplasmic profiles. The cytoplasmic profiles and LC nuclei were counted separately according to their localization in the epithelial or in the stroma layers, in agreement with the histological division of the squamous epithelium. The epithelium was thus divided into superficial, intermediary and basal layers; only the subepithelial region was considered stroma.

For the statistical analysis the paired data were analyzed by the Wilcoxon rank-sum test applied to means of Langerhans' cells/mm^2, cytoplasm profiles/mm^2 and ratio of the number of cytoplasmic profiles/number of nuclei was calculated for each VL and histopathologically normal group [5].

Results and Discussion

Twenty-eight paired cervical biopsies were examined for LC nuclei, number of cytoplasmatic profiles and the ratio cytoplasmatic profiles/number of nuclei by S100-immunohistochemistry. Langerhans' cells occurred in the epithelium and in the underlying stroma. Langerhans' cells in the epithelium were more frequent in the basal and intermediary layers, rarely appearing in the superficial layer. Their presence in the stroma was constant in the subepithelial layer.

On analyzing the epithelium, no significant difference in mean densities of LC between both groups was observed (Table 1). According to Caorsi and Figueroa [6], LC dendrites in intraepithelial cervical neoplasms became more branched. On the other hand, Al-Saleh *et al.* [3] found the cells to be more round-shaped in the presence of HPV, with shortening or even absence of dendrite branches. Morphological changes are reported in the present study that quantified the cytoplasmic profiles with filiform, circular and comma-like shapes. The mean of profiles in epithelium specimens with viral lesions was 144.08 profiles/mm^2, while lesion-free tissue presented 256.27 profiles/mm^2 ($p = 0.0210$). Decrease in the density of cytoplasmic profiles in the presence of histopathological lesions due to HPV could mean decline in cell functions. With less cytoplasm they would be less able to process captured antigens. The cytoplasmic profiles/nuclei ratio may reveal the functional state of LC, since it indirectly quantifies the number of dendrites per cell. When compared to normal tissue and to cervicitis [7], this ratio is lower in the presence of HPV. Our analysis showed that ratio tended to be lower in the total epithelium and in the basal layer with 4.96 for the HPV specimen and 6.59 for normal tissue (Table 1).

Table 1. — *Relation between mean and standard deviations of cytoplasmic profiles (/mm^2) and relation of cytoplasmic profiles/nuclei of Langerhans' cells in epithelium and stroma in groups with viral lesions and normal tissue.*

	Cytoplasmic profiles					Cytoplasmic profiles/nuclei				
	Viral lesion		Normal			Viral lesion		Normal		
	Mean	SD	Mean	SD	P*	Mean	SD	Mean	SD	P*
Epithelium										
Superficial	0.4	0.9	4.13	7.0	ns	–	–	10.3	10.0	–
Intermediary	39.3	28.8	110.2	83.9	0.0081	7.7	8.1	7.5	3.9	ns
Basal	99.5	69.4	132.6	111.4	ns	4.6	3.6	6.0	4.1	0.0830
Total	144.0	79.2	256.2	191.0	0.0210	4.9	4.0	6.5	3.2	0.0713
Stroma	30.1	31.1	38.7	30.7	ns	2.8	0.7	6.7	4.8	0.0273

ns = not significant. * significance of difference assessed by Wilcoxon rank-sum test.

Due to their kinetic capacity, LC migration would explain the statistically important difference in the cytoplasmic profiles/nuclei ratio in the subepithelial layer (Table 1). The Langerhans' cells of the stroma are activated cells with phenotypic modifications that include an increase in MHC class I and II expression and also of intercellular adhesion/stimulation molecules [3, 8].

The direct cytotoxic action of HPV on LC or a larger migration of these cells to the lymphoid system would cause a decrease of density in the HPV-infected epithelium. This depression may be due to a lack of maturation or to the chemostatic factor produced by keratinocytes or to an inhibitory factor in the maturation of Langerhans' cells [9]. Experimental studies have confirmed the influence of TNF-α as a stimulator and IL-10 and reduction of E-cadherin as inhibitors of LC functions which results in immunological depression in premalignant cervical lesions [10-12].

Conclusions

Our research points towards significant cytoplasmic changes of LC in tissue with viral lesions. Local intraepithelial immunodeficiency based on cytoplasmic changes of Langerhans' cells has been postulated as a mechanism by which HPV could be involved in the genesis of neoplasia. Further studies are required to investigate Langerhans' cells and immunodepression in order to update programs on uterine cervix cancer prevention.

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