Interaction of Human Papillomavirus DNA with Factor XIIIa-positive Dermal Dendrocytes in Vulvar Lesions

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Sir,

Human papillomaviruses (HPVs) are double-stranded DNA virus with over 100 different identified types. The virus mainly infects the cutaneous and mucosal squamous stratified epithelia of the anogenital region. HPV replication is linked to the differentiation and maturation of squamous cells, followed by terminal differentiation, with hyperplasia associated with the formation of local lesions (1–3).

Some factors minimize or prevent the exposure of HPV to the immune system. The immune escape mechanisms are related to the absence of viral lyses in keratinocytes, or the absence of infection and replication of antigen-presenting cells (APC) in epithelium (1).

Langerhans' cells (LC) are dendritic cells that engulf and process antigen, and migrate through the dermis to draining lymph nodes (4).

Factor XIIIa+ dermal dendrocytes (FXIIIa+ DD) constitute another skin dendritic cell population. They are able to differentiate and migrate to epidermis, indicating an interaction between dermis and epidermis in order to develop an immune response (5, 6). Although recent studies have shown FXIIIa+ DD involvement in other infectious process (7, 8), their interaction with HPV has been poorly explored (9).

It has been demonstrated that in HPV infection of the lower genital tract, the virus has a direct effect on the local immune response, which may result in a malignant transformation. As shown previously, HPV alone decreases the number of cervical LC and induces changes in the pattern of cytokines released by these cells (10, 11).

The role of LC in the immune response to HPV infection has been well studied in uterine cervix lesions. There are, however, other aspects to be clarified concerning the role of dendritic cell populations in HPV lesions. The aim of this study was to improve our knowledge of HPV infection of the vulva, focusing on the role of FXIIIa+ DD in such lesions.

MATERIALS AND METHODS

Twenty-nine biopsies of vulvar HPV lesions were studied between 2002 and 2004. Ten specimens from normal skin constituted the control group.

An immunohistochemistry technique was used to detect the presence of HPV, using an anti-HPV antibody (B580 Dako Corporation, Carpinteria, CA, USA). Briefly, the rabbit-derived polyclonal antibody anti-HPV was applied at 1:100 dilution,

with an overnight incubation at 4°C, followed by incubation with a biotinylated anti-rabbit immunoglobulin for 30 min, then by streptavidin-peroxidase LSAB System (K0690 Dako) for 30 min, both incubated at 37°C. 3,3-diaminobenzidine tetrahydroxychloride (D5637 Sigma Chemical Co, St Louis, MO, USA) was used as chromogen and the slides were counterstained with Mayer haematoxylin. A similar technique was employed using an anti-factor XIIIa antibody (PU337-UP BioGenex, San Ramon, CA, USA) at a 1:200 dilution to study a possible role of FXIIIa+ DD in such lesions.

Quantification of FXIIIa+ DD was performed by covering all the tissue sections with a ×10 ocular lens with a square grid and a ×40 objective. Statistical analysis was performed using the Mann-Whitney non-parametric test with level of significance set at 95% (GraphPad Software, San Diego, CA, USA).

In order to verify the possible interaction of HPV and FXIIIa+ DD, a combined HPV *in situ* hybridization (ISH) and immunohistochemistry technique with anti-factor XIIIa antibody was performed.

Following deparaffinization in xylene, and hydration in ethanol, antigen recovery was performed with a 0.01M citric acid solution at pH 6.0. The probe, a mixture of HPV types 6, 11, 16, 18, 31, 33, 35, 45, 51 and 52 (wide-spectrum HPV biotinylated DNA probe Y1404, Dako) was applied and incubated at 95°C for 5 min, followed by an overnight incubation at 37°C. Stringent wash solution was used at a 1:50 dilution for 30 min at 56°C. The peroxidase streptavidin-biotin complex (CSA, Dako) was applied at a dilution of 1:70 for 15 min and the amplification reagent was used for 15 min, both at room temperature. Streptavidin-peroxidase was applied for 15 min at the same temperature. 3,3-diaminobenzidine tetrahydroxychloride (Sigma) with nickel chloride (Sigma) was applied as chromogen.

The next step was the immunohistochemistry technique, which consisted of the application of the rabbit-derived polyclonal antibody anti-factor XIIIa (BioGenex) at a 1:200 dilution, with an overnight incubation at 4°C. LSAB 2 System (alkaline phosphatase – K0676 Dako) was used, applying biotinylated anti-rabbit immunoglobulin for 30 min and streptavidin-alkaline phosphatase conjugated for 30 min, both incubated at 37°C. Fuchsin was used as chromogen and the slides were counterstained with Mayer haematoxylin. The reaction was considered positive when cells immunostained in red were co-localized with black material within their cytoplasm. The reaction was performed using positive controls to HPV (vulvar lesions with positive immunohistochemistry reaction to HPV) and positive controls to FXIIIa+ (skin biopsies with dermatofibroma).

RESULTS

All vulvar biopsies showed granular and spinous layer koilocytosis, acanthosis, hyperkeratosis and parakeratosis. There was a mild inflammatory infiltrate in the dermis composed of lymphocytes, macrophages and a few plasma cells.

Hypertrophic FXIIIa+ DD, with prominent dendrites were observed mainly in dermal papillae and around the superficial blood vessels (Fig. 1). In the control group FXIIIa+ DD presented the same localization, although they exhibited spindle morphology.

The co-localization of viral DNA in the cytoplasm of FXIIIa+ cells was demonstrated in vulvar biopsies by the combined ISH to HPV and immunolabelling to FXIIIa (Fig. 2). In the control group, the combined technique disclosed only FXIIIa+ DD without HPV DNA material.

There was a statistically significant difference in the number of FXIIIa+ DD in the group with HPV lesions compared with the control group (mean $\pm$ SD $= 47.6 \pm 48.0$ and 19.3 ± 15.1, respectively).

DISCUSSION

We have demonstrated a cross-reaction between polyclonal anti-papillomavirus antibody and FXIIIa+ cells (12) and, for that reason, we standardized the combined HPV-ISH and FXIIIa+ DD demonstration by immunohistochemistry to perform this research. The localization of viral DNA in the cytoplasm of FXIIIa+ DD suggests that these cells might be able to internalize HPV virus. To the best of our knowledge, this is the first evidence of the correlation between FXIIIa+ DD as phagocytes and HPV. This is in accordance with other studies that described the role of FXIIIa+ DD in other diseases (7, 8).

LC are considered the most important APCs in the epithelial surface and, with other dendritic cells, have been explored in strategies for vaccination (13).

The low level of expression of early HPV proteins in basal epithelium could alter proper antigen presentation by these cells. The number of LCs is reduced in HPV-infected lesions and the persistence of HPV seems to be related to this reduction or to LC altered function (14).

In a recent review (1) it was described that HPV does not infect or replicates in APCs located in the epithe-
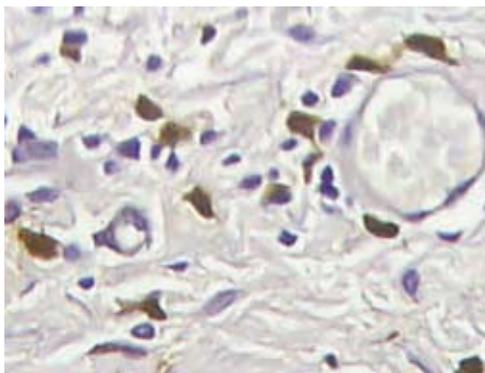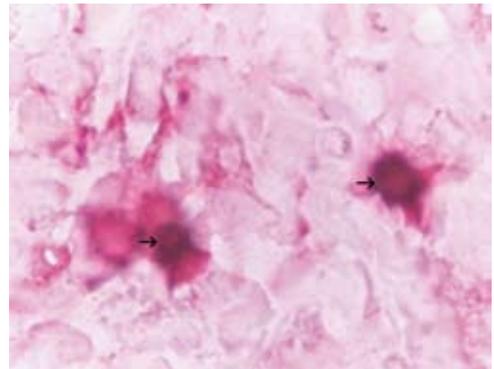

Fig. 2. Vulvar lesion with human papillomavirus. Co-localization of viral DNA in the cytoplasm of FXIIIa+ cells (*in situ* hybridization to human papillomavirus – 3'3' diaminobenzidine with nickel chloride) (*arrow*) and immunohistochemistry to FXIIIa – streptavidin–alkaline phosphatase method with fuchsin (*red*) (×400).

lium, resulting in failure of HPV presentation to the immune system. Another possibility is that LCs, although able to bind and internalize HPV, are not activated by these events and they do not induce a response. This indicates that LC may be a target used by HPV as an immune escape mechanism (15).

The increased number of hypertrophic FXIIIa+ DD in HPV vulvar lesions suggests that this group of cells might play a role in the pathogenesis of HPV infection. Considering that FXIIIa+ DD are capable of antigen presenting, the present study leads us to suggest that FXIIIa+ DD would be able to engulf the viral antigens and to promote their presentation to the immune system.

ACKNOWLEDGEMENTS

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Fig. 1. Vulvar lesion with human papillomavirus. Hypertrophic factor XIIIa+ dermal dendrocytes (FXIIIa+ DD) with prominent dendrites in dermal papillae and around superficial blood vessels – streptavidin–peroxidase method. 3'3' diaminobenzidine (×400).

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