Variations of lymphocyte sub-populations in vulvar condylomata during therapy with ß-interferon

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Summary: Several experiences induced us to consider genital HPV infection as an expression of a local immunodeficiency. The aim of our study was to research the effect of immunotherapy on the lymphocyte subpopulations and Langerhans cells in vulvar condyloma. Twenty women with persistent vulvar condylomata, treated with 2,000,000 IU/die of β-interferon for 15 days, were submitted to vulvar biopsy before and 2-5 months after medical treatment. The frozen sections obtained were assayed with the following monoclonal antibodies: OKT 4 (T helper lymphocytes), OKT 8 (T suppressor lymphocytes), OKB 7 (B lymphocytes) and S-100 protein (Langerhans cells). Using a morphometric evaluation, the average number of both intraepithelial and stromal lymphocyte subsets and of the intraepithelial Langerhans cells was assessed.

In all the biopsies preceeding the medical treatment we found a low number of T helper lymphocytes both in the epithelium and stroma, with inversion of T4/T8 lymphocyte ratio and rare presence of Langerhans cells. In patients with a good therapeutic response (50-100% of condyloma reduction) we observed an increase in intraepithelial T4 lymphocytes and a decrease in both intraepithelial and stromal T8 lymphocytes. In cases with persistent disease after therapy, the histological pattern was similar to that observed in the first biopsy, with the exception of a significant increase in the average number of Langerhans cells. Our data correlate the clinical response to the immunotherapy with the histology of lymphocyte subsets in the vulvar condylomata. The increase in Langerhans cells observed in patients with negative response may be interpreted with a probable inability of these cells to promote the immune reaction.

Kel words: Vulvar condylomata; Lymphocyte sub-populations; β-interferon.

INTRODUCTION

On the basis of data from literature and clinical observation, genital HPV-infection seems to have a special predilection for patients with immunodepressive conditions: pregnancy, transplantation or drugs. Moreover, since the viral diffusion is actually more extensive than the clinical or subclinical evidence, we can hypothesize that genital HPV infection may be the expression of an immunodeficiency status, as in other viral and parassitic diseases. Even the spontaneous regression of condylomata may be the sign of the reconstruction of a valid immunity. Therefore, the medical treatment of HPV infection is performed with immunostimulant drugs.

In medical literature, few studies illustrate the local expression of immunity before and after medical treatment. This kind of study is extremely useful in order

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to know the correlation between the immune system and the HPV infection as well as the role of drug interaction.

MATERIAL AND METHODS

Our study concerned 20 women affected by florid vulvar condylomata for a minimum of 6 months (persistent disease). The mean age was 28.8 years (range 19-40). 18 women were nulliparous and 2 had had one pregnancy. All patients were treated daily with 2,000,000 IU of β-interferon i.m. for 15 days, and re-evaluated after 1-5 months (mean 2,4 months). Eleven women showed a persistent disease or a minimal reduction of condylomata, while in nine women we observed consistent reduction (>50%) or disappearance of vulvar disease. In each patient a first vulvar biopsy had been performed before treatment and a second biopsy at time of reevaluation.

The surgical specimen was divided in two fragments. One fragment was fixed in neutral formalin, embedded in paraffin and stained with Hematoxylin-Eosine according to common histological techniques. Frozen sections of the second fragment were incubated with the following monoclonal antibodies.

- OKT 4 (Ortho-DS predilution) for T-helper lymphocytes;
- OKT 8 (Ortho-DS predilution) for T-suppressor lymphocytes;
- OKB 7 (Ortho-DS predilution) for B-lymphocytes;
- S-100 protein (Ortho-DS predilution) for intraepithelial Langerhans cells (LC).

The obtained reactions were revealed by PAP-immunoperoxidase according to Sternberger techniques.

A morphometric evaluation of cells positive to OKT 4, OKT 8 and OKB 7 was performed separately in the epithelium and in the stroma of condyloma, while the S-100 positive cells (LC) were evaluated only in the epithelium. For this calculation the slide was projected on a computer screen ("microconf"), with an original magnification of 400x. A microscopic field of 200x150 micra (surface of field mm^2 0,03) was obtained. For each slide 10 microscopic fields were randomly selected in the epithelium or in the stroma and the positive cells were calculated. A mean value of elements for field was then obtained.

RESULTS

Histologically, the vulvar lesions both before and after medical treatment were represented by typical condilomata acuminata, without cytologic dysplasia. Cases with clinical remission after therapy showed normal epidermis with minimum focal koilocytic atypia (subclinic disease).

Table 1. – *Comparison between cases and controls after therapy.*

	Mean (±S.D.)	
	Cases	Controls (after therapy)
OKT4 epithelium	9.38 (0.896)	15.44 (5.69)
OKT4 stroma	47.97 (1.53)	47.14 (2)
OKT8 epithelium	27.35 (2.36)	19.22 (6.43)
OKT8 stroma	46.53 (4.52)	34.27 (16.78)
OKB7 epithelium	0.225 (0.129)	0.195 (0.188)
OKB7 stroma	36.36 (1.96)	31.08 (6.52)
S-100	2.02 (0.57)	2.93 (0.9)

The number of immunocompetent cells revealed by monoclonal antibodies was compared in the surgical specimens obtained before and after medical treatment (Tab. 1). We observed a mean number of intraepithelial T4 lymphocytes lower than that of T8 lymphocytes (9.38±.896 and 27.35±2.36). After therapy we found an increase in the mean number of intraepithelial T4 lymphocytes and a decrease in T8 lymphocytes, although the latter were still more representative (15.44±5.69 and 19.22±6.43). In addition, we noted a decrease in stromal T8 and B lymphocytes and an increase in LC.

In the group of women with poor response to medical treatment (Tab. 2), the distribution of lymphocyte subpopulation in the second biopsy resulted very similar to that in the first biopsy, with the exception of a minimal decrease in intraepithe-

Table 2. – *Comparison between first and second biopsy in the group of patients with poor response (negative and ≤30%) to therapy (1° Group - 11 cases).*

	Mean (±S.D.)		
	Cases	Controls significance (after therapy)	
OKT4 epithelium	9.41 (0.78)	10.76 (2.63)	
OKTA4 stroma	48.15 (1.84)	47.8 (1.34)	
OKT8 epithelium	26.74 (2.88)	24.67 (2.1)	$p<0.01$
OKT8 stroma	48.87 (1.69)	47.34 (2.85)	
OKB7 epithelium	0.22 (0.16)	0.23 (0.15)	
OKB7 stroma	36.59 (1.89)	33.6 (6.04)	
S-100	2.11 (0.42)	3.26 (0.88)	$p<0.001$

lial T8 lymphocytes (26.74±2.88 vs. 24.67±2.1) and of a significant increase in LC (2.11±4.25 vs. 3.26±.882).

In the group of patients with a good response to administration of interferon (Tab. 3) we observed a significant increase in intraepithelial T4 lymphocytes (9.35± 1.024 vs. 21.17±1.149) and a decrease in T8 lymphocytes (28.1±1.321 vs. 12.57±1.418) with inversion of the T4-T8 ratio. Even the stromal T8 and B lymphocytes were decreased. The LC showed an increase, although not significant.

DISCUSSION

Several reports in literature correlate the HPV infection with trouble in the immunological status; vulvar condylomata are frequently observed during pregnancy (Gardner & Kaufman 1981) and in women treated with immunosuppressive drugs for transplantation or systemic chronic disease (Coskey, 1976; Shokri-Tabibsadeh *et al.*, 1981; Schneider *et al.*, 1983; Halpert & Fruchter, 1986).

In the serum of patients, the production of IgG and IgM against the viral capsid and the tests of lymphocyte activations has been documented and correlated with the clinical course of the disease (Pyrrhonen & Penttinen, 1972; Matthews & Shirodario 1973; Shirodario & Matthews, 1975; Pyrrhonen & Johansson, 1975; Ivanyi & Morison, 1976; Lee & Eisinger, 1976).

Studies on the lymphocyte subpopulation in the stroma of condyloma are very rare.

Chardonnet *et al.* (1986) examined 55 patients with cutaneous warts, including 7 with ano-genital condylomata. These Authors noted a rare presence of LC and a high number of warts with T8 lymphocytes.

In the cervical HPV infection Syrjanen (1983) found a considerable number of T lymphocytes. Tay *et al.* (1987) stressed a

Table 3. – *Comparison between first and second biopsy in the group of patients with good response (from 50% to 100%) to therapy (2° Group - 9 cases).*

	(Means (±S.D.)		
	Cases	Controls significance (after therapy)	
OKT4 epithelium	9.35 (1.02)	21.17 (1.14)	$p<0.001$
OKT4 stroma	47.74 (1.1)	46.33 (2.43)	
OKT8 epithelium	28.1 (1.32)	12.57 (1.41)	$p<0.001$
OKT8 stroma	43.68 (5.32)	18.63 (12.61)	$p<0.001$
OKB7 epithelium	0.22 (0.08)	0.14 (0.21)	
OKB7 stroma	36.11 (2.12)	28.03 (5.97)	$p<0.001$
S-100	1.91 (0.72)	2.52 (0.79)	

decrease of T4 hymphocytes in cervical condylomata and CIN with respect to the normal cervix, with an inversion of T4/T8 ratio.

The decrease of LC in cervical condylomata has been documented with different methods by several Authors (Morris *et al.,* 1983 (b); Varynen *et al.,* 1984; Mc Ardle & Muller, 1986; Warhol & Gee, 1989; Alberico *et al.,* 1989).

Our observations confirmed the low number of LC in the epithelium of vulvar HPV infection and the increase in the number of T8 suppressor lymphocytes with an inversion of T4/T8 ratio. This datum indicates an evident depression of immunity, as observed in the serum of patients (Seski *et al.,* 1978; von Krogh 1979; Schneider *et al.,* 1983).

Moreover, the clinical regression of the disease seems to be correlated with a modification of cell-mediated immunity, consistent with a decrease in intraepithelial and stromal T-suppressor lymphocytes, with an increase in T-helper lymphocytes and with a normalization of T4/T8 ratio. These data are in agreement with the observations carried out on the serum of the healed patients in which the activity of T lymphocytes (Morison, 1976; Viac *et al.,* 1977) and the production of lymphokine (Tyring *et al.,* 1987; Tyring *et al.,* 1988) were higher with respect to patients who did not respond to radical treatment.

The significant increase in LC among our patients with persistent disease after interferon therapy may suggest two hypotheses: first, we may suppose an insufficient immunological stimulation, with a consequent response limited to LC and not to lymphocytes; second, we can hypothesize in these patients a functional deficiency of macrophages and LC, which, although stimulated by the interferon therapy, are still unable to promote a valid cell-mediated immune response (Gross & Pfister, 1988).

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