Lymphocyte subset in HPV infection

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Summary: The outcome of HPV infection and its progression to neoplastic disease may be conditioned by the immune status of patients. In the present study we observed the systemic lymphocyte subset, with particular regard to NK cells and NK activity in 7 patients with HPV infection.

The lymphocyte phenotypes in peripheral blood were studied using a panel of monoclonal antibodies, while NK activity was evaluated as percentage of lysis of K 562 target cells.

We noticed a significant decrease of basal NK activity especially in patients with HPV 16 - 18 infection ($p<0.001$), without any difference in the absolute number of these cells.

Key words: HPV infection; NK activity, Lymphocyte subset; Cervical cancer.

INTRODUCTION

Human Papillomavirus (HPV) plays an important role in the pathogenesis of Cervical Intraephitelial Neoplasia (CIN) and invasive cancer ([1, 2]); specific papillomavirus DNA sequences, particularly of HPV 16-18, have been present in the vast majority of premalignant and malignant cervical tumors analysed ([3]).

This role of HPV was first suggested by zur Hausen ([4]). It is possible that HPV may act as an "initiator" of malignancy, or as a "promotor" following initiation by carcinogenic agents ([5]).

The immune system is important in the prevention of malignant disease as evidenced by the high incidence of lower genital neoplasia in immunosuppressed women: 10 fold increase in the relative risk of cervical cancer ([6]).

So it is becoming increasingly evident that the immune status of the patient may be an important element in the outcome of the HPV infection and the progress to neoplastic change.

Recent studies of in situ lymphocyte subsets have suggested a possible local immune deficiency state in women with cervical neoplasia and HPV infection ([7]).

In the present study we evaluated the systemic lymphocyte subsets and the NK activity in 7 patients with HPV infection. The NK cells in fact, are considered among the most important natural cells against tumor and viral disease.

SUBJECTS AND METHODS

Subjects:

Study was done on 7 HPV infection patients treated on the colposcopy service. The patients' age at the time of diagnosis ranged from 18 to 42. The mean age was 31.2 years.

All patients were examined in the colposcopy service where targeted biopsies were taken

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from the suspicious lesions and prepared for histology and in situ ibridization.

Three women had the HPV 31-35-51 positivity with a histological pattern of acanthosis or fibroephitelial papillomatosis; 4 women had the HPV 16-18 positivity with an histologic pattern of CIN I, CIN II, CIN III and chronic inflammation. Seven women with normal cervical smear and normal colposcopy pattern were randomly selected as normal control.

Methods:

Peripheral blood mononuclear cells (PBMC) were fractioned on Ficcol-Paque and separated by density gradient centrifugation (400 rev's, 30'). Cells from the interface of the gradients were washed twice with PBS (Ca^{++} and Mg^{++} free), counted and suspended in RPMI 1640 (Gibcop +10% fetal calf serum) containing penicillin (100 U/ml) and streptomycin (100 microgr/ml) (complete medium, CM). Viability was always greater than 98% as determined by Trypan blue exclusion.

The K 562 tumor cell line, a myeloid cell line derived from a splenal effusion from a patient with a chronic myelocyte leukemia in blast crisis, was used as the target cell in NK cell assay. The K 562 were labelled with 100 uCi of Cr 51 Amersham by incubation at 37° C in 5% CO_2 for 30 min.

The labelled target cells were washed twice with PBS and resuspended in C Mat a concentration of 1x10 5/ml.

1x10 4 Cr-51-labelled K 562 cells were incubated with effector cells in triplicate in round bottomed 96 well plastic microtiter plates (Nunc) in a total volume of 200 ul.

Target cells were also placed as controls in walls that contained medium only, to determine the spontaneous release of Cr 51. The plate incubated for 4h at 37° C in 5% CO_2.

The radioactivity of supernatants was counted by a gamma counter. The specific lysis of K 562 target cells was calculated as follows:

$$\frac{\text{Test Cr release - Spontaneous Cr release}}{\text{Total Cr release - Spontaneous Cr release}} \times 100$$

Leu 4, Leu 3, Leu 2, Leu 7, and Leu 11 monoclonal antibodies were purchased from Beckton Dickinson. For cell staining 20 ul of each MoAb was added to 100 ul of effector cells (10/ml). After incubation in cold ice for 30' in the dark, the cells were washed with PBS and 0.1% formaldehyde was added. Before the counting at the Epics/flow cytometer (Coulter), 0.6 ml of Isoton were added to each sample.

The significant difference between the means was assessed by T test.

Table 1.

Patients	HPV	Pan T-cells				
		Leu 4 %	Leu 3 %	Leu 8 %	Leu 7 %	Leu 11 %
1	31, 35, 51	76	59	19	12	16
2	31, 35, 51	70	53	18	17	14
3	31, 35, 51	74	35	23	12	10
4	16, 18	84	45	35	15	14
5	16, 18	79	51	22	16	11
6	16, 18	87	41	35	15	14
7	16, 18	77	39	25	19	8
n.v.		68-82	35-55	19-27	9-20	8-22

RESULTS

In peripheral blood from HPV cervical infection patients, the number of NK cells and other functional subclasses of T-lymphocytes was not significantly different from that in a control group (Table 1).

On the contrary basal NK cytotoxic activity was found significantly decreased in HPV 16-18 infection patients ($p < 0.001$).

A reduction of NK activity was found in patients with HPV 31-35-51 infection too ($p = 0.002$) (Table 2).

Table 2.

Patients	HPV	Histology	NK activity
1	31, 35, 51	acanthosis	22.7
2	31, 35, 51	fibroepithelial papillomatosis	24.1
3	31, 35, 51	fibroepithelial papillomatosis	20.3
4	16, 18	CIN I	5.2
5	16, 18	CIN II	8.7
6	16, 18	CIN III	14.0
7	16, 18	chronic inflammation	3.5
Controls	HPV	normal	30.0
			$p < 0.001$

DISCUSSION

Little is known of the natural history of cervical HPV infections or of the immunological mechanisms which control their progression. If cervical infections persist, progression or regress is likely to be controlled by the balance between the frequency of exposure to HPV on the one hand and host cell mediated immune responce on the other.

Tay pointed out a depletion of intraephitelial lymphocytes, especially to T4 subset with reversal of the ratio of T4 to T8 subsets to less than one in HPV infection and CIN.

Our recent finding of NK activity' reduction in patients with invasive cervical cancer ([8]), suggests a specific immune deficiency state in these women. Since the NK cells are considered among the most important natural cells against tumor and viral disease, we studied NK activity in 7 patients with cervical HPV infection to seek an eventual depletion in these women. The reduction in NK activity in patients with HPV infection proves that there is a depression of natural systemic immunocompetence in HPV infection patients. The cause for such a depression is at present unknown, though the normal phenotype distribution in peripheral blood tends to exclude a lack of NK cells.

This systemic immune deficiency (with the local immune deficiency of Tay) might facilitate a persistence and progression of HPV infection in the cervix so that the virus exerts an oncogenic effect.

REFERENCES

1) Mc Cance D. J., Campion M. J., Clarkson P. K., Chester P. M., Jenkins D., Singer A.: "Prevalence of human papillomavirus type 16 DNA sequence in cervical intraepithelial neoplasia and invasive carcinoma of the cervix". *Br. J. Obst. Gyn., 92,* 1101, 1985.

2) Gissman L., Wolnik L., Ikenberg H., Koldovsky U., Schnurch H. G., zur Hausen H.: "Human papillomavirus type 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancer". *Proc. Natl. Sci. USA, 80,* 560, 1983.

3) Pfister H.: "Human papillomavirus and genital cancer". *Adv. Cancer Res., 48,* 113, 1987.

4) zur Hausen H., Gissman H. L., Stiner W., Dippold W., Dreger I.: "Human papillomavirus and cancer". *Bibl. Haemat., 43,* 569, 1975.

5) zur Hausen H.: "Human genital cancer: synergism between two virus infections or synergism between a virus and initiating events?". *Lancet, II,* 1370, 1982.

6) Porreco R., Penn I., Droegmueller W., Greer B., Makowski E.: "Gynecologic malignancies in immunosuppressed organ homograft recipientes". *Obst. Gyn., 45,* 359, 1975.

7) Tay S. K., Jenkins D., Maddox P., Singer A.: "Lymphocyte phenotypes in cervical intraepithelial neoplasia and human papillomavirus infection". *Br. J. Obst. Gyn., 94,* 16, 1987.

8) Garzetti G. G., Cignitti M., Marchegiani F., Provinciali M., Zaia A. M., Fabris N., Pieri C., Romanini C.: "Improvement of decreased natural killer cell activity in cervical cancer patients by active lipids (AL 721)". *Eur. J. Gyn. Oncol., 2,* 123, 1990.

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