

Human cervical epithelial cells that express HLA-DR associated with viral infection and activated mononuclear cell infiltrate

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Abstract

The association between the expression of HLA-DR antigens on cervical epithelium and the local immune state of activation in colposcopically obtained biopsy specimens from patients with histologically documented wart virus infection was investigated. In normal cervical epithelium no HLA-DR staining was detected. No or few IL-2R positive cells were found in the contiguous sections. HLA-DR was expressed by epithelial cells in six out of the 14 samples of wart virus infection. The pattern of fluorescence was focal, but strong and diffuse, to the whole epithelial layer. In the six samples with HLA-DR positive epithelium the numbers of IL-2R positive cells in the lamina propria were strongly increased, ranging between 75 and 90%. HLA-DR expression by cervical epithelium was observed in only two of 12 samples from patients with mixed epithelial non-virus related abnormalities. No increase in the numbers of IL-2R positive cells was observed in this group of patients. Additionally, no significant differences in terms of T lymphocyte infiltrate were found among the three groups. The results indicate that wart virus infection is associated with enhanced HLA-DR epithelial expression and they lend support to the concept that in the human cervix the epithelium actively participates in the local immune response.

epithelial expression of HLA-DR antigens has been shown *in vivo* to depend on activation of the local immune system⁶ and the formation of autologous T cell clones.²¹ It is therefore conceivable that in human cervical epithelium the expression of HLA-DR antigens is related to the state of activation of the intraepithelial lymphocytes.

The purpose of this study was to investigate the association between the expression of HLA-DR antigens on cervical epithelium and the local immune state of activation in colposcopically obtained biopsy specimens from patients with histologically documented wart virus infection and from controls.

Methods

Tissue samples were obtained from 36 subjects undergoing colposcopy. Cytological and histological analyses of punch biopsy specimens were performed. There were 10 subjects with normal colposcopic, cytological, and histological appearances, 12 with evidence of mixed epithelial non-viral disease (MENVD), and 14 with wart virus infection.

Diagnosis of wart virus infection was based on the evidence of flat condyloma. Flat condyloma appeared at colposcopy after treatment of the cervix with acetic acid as raised areas (outside the transformation zone) of shiny, white epithelium with a rough surface and with a punctation or mosaic-like vascular pattern. The diagnosis was confirmed in all 14 cases by histology and cytology, showing koilocytic cells and dyskeratotic cells without evidence of dysplasia.²²⁻²⁵

Tissue samples were oriented, embedded in Tissue Tek OCT compound (Miles Scientific, USA), snap frozen in liquid nitrogen, and stored at -80°C until used.

At least six frozen sections, 5 μm thick, for each specimen were cut on a cryostat, air dried, and fixed in absolute ethanol for 10 minutes at 4°C . Frozen sections were then stained by an indirect immunofluorescence technique, as previously described.⁶ Briefly, contiguous sections for each specimen were stained for HLA-DR and IL-2R and incubated with the optimal dilutions of each of the monoclonal antibodies listed below for one hour at 4°C . Additional sections were incubated with the OKT3 monoclonal antibody. Sections were then washed twice with PBS and incubated with a fluorescein isothiocyanate-conjugated goat-anti-mouse antibody for 30 minutes at room temperature. Sections were then washed three

There is now convincing evidence that enhanced HLA-DR antigen expression on epithelial cells is a feature of immune mediated inflammatory reactions.¹⁻¹⁰ In health and disease this expression plays a crucial part in the immunological homeostasis of different organs and tissues.¹¹⁻¹⁵ HLA-DR is also associated with neoplastic transformation in different organs.^{9,10} In the human cervical squamous epithelium HLA-DR antigens are present during viral infection and carcinoma but absent in health.¹⁶ In contrast, HLA-DR antigens are present in normal human endometrial epithelium; they can also be induced *in vitro* by exposure to interferon gamma.^{17,18}

The most potent inducer of HLA-DR antigens on cells of different lineages is interferon gamma,¹⁹ mostly produced by activated T lymphocytes.²⁰ In other disease states the

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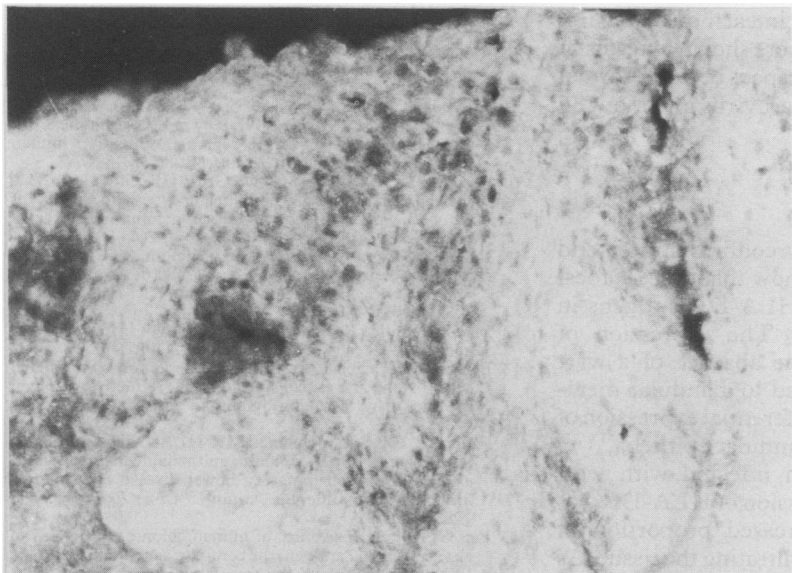


Figure 1 Cryostat section of cervical epithelium infected with wart virus showing strong immunofluorescence staining for HLA-DR.

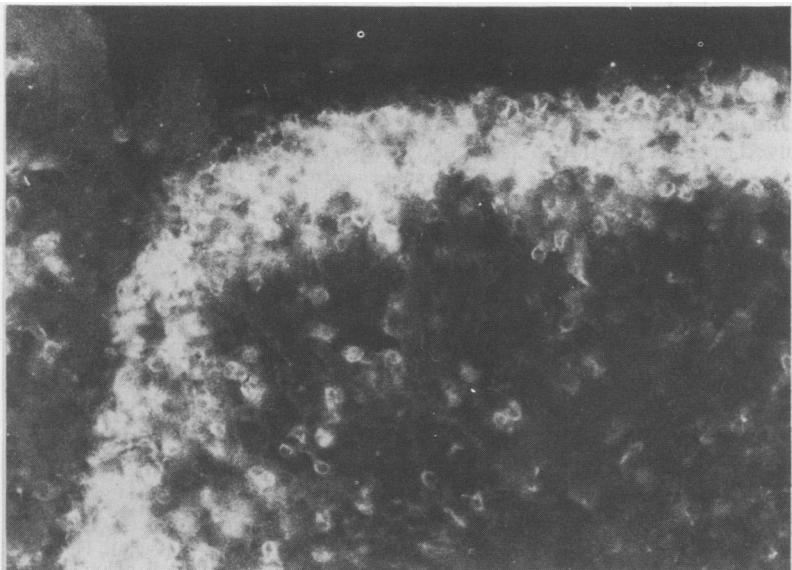


Figure 2 Cryostat section of cervical epithelium infected with wart virus showing an impressive number of mononuclear cells stained for IL-2R.

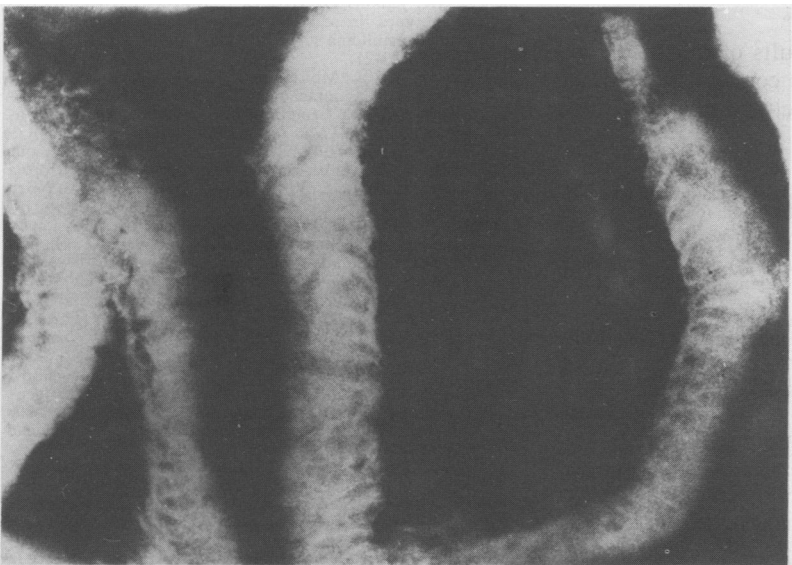


Figure 3 Cryostat section of an area of glandular metaplasia from a non-virally infected cervical epithelium showing intensive diffuse staining of glandular cells for HLA-DR.

times in PBS and the slides examined using a Leitz Laborlux 12 fluorescence microscope.

Antisera comprised the following: monoclonal antibodies directed against non-polymorphic determinants of the HLA-DR molecules included OKIa1 (Ortho-mune, USA) (diluted 1 in 10) and the anti-DR (clone L243, Becton-Dickinson, USA) (diluted 1 in 10); anti-IL-2 receptor including anti-IL-2R (Becton-Dickinson, USA) (undiluted) and the OKT26a (anti-Tac, Ortho-mune, USA) (undiluted); a pan-T (CD3) monoclonal antibody (OKT3, Ortho-mune, USA).

A FITC-conjugated goat-antimouse immunoglobulin (Becton-Dickinson, USA) (diluted 1 in 30) was used as a second layer antibody.

The IL-2R and the OKT3 positive cells per high power field were quantitated in contiguous sections stained with haematoxylin and eosin of each case by counting the percentage of positive cells in at least 100 mononuclear cells in a minimum of 10 high power fields.

The Wilcoxon rank sum test was used for the comparison of results in the different groups. Results were expressed as mean (SEM) and range.

Results

NORMAL CERVICAL EPITHELIUM (NCE)

No HLA-DR staining was detected in normal cervical squamous epithelium with both the antibodies used. No or few IL-2R (mean (SEM): 0.8 (0.6); range: 0-2) positive cells were found in the contiguous sections. The percentage of OKT3 positive cells was 86 (9), range: 76-89%.

WART VIRUS INFECTION

HLA-DR expression in epithelial cells was seen in six out of the 14 samples of wart virus infection. The pattern of fluorescence was focal, but strong and diffuse, to the whole epithelial layer (fig 1). In the six samples with an HLA-DR positive epithelium the numbers of IL-2R positive cells in the lamina propria were increased ranging between 75 and 90% (84 (4)) ($p < 0.001$, with respect to normal controls, cases of mixed epithelial non-viral disease, and wart virus infection without epithelial HLA-DR expression) (fig 2). In the eight cases of wart virus infection negative for HLA-DR the percentage of IL-2R positive cells did not differ from that of the control group (1.1 (0.7), range 0-2).

In all the cases of wart virus infection the percentage of the OKT3 positive cells did not differ from that of the control group (88 (6), range 80-92).

MIXED EPITHELIAL NON VIRAL DISEASE (MENVD)

HLA-DR expression in cervical epithelium was observed in two out of 12 patients with MENVD and the positivity was observed only when glandular metaplasia was present (fig 3). Only a few or no IL-2R positive cells were observed in this group of patients (0.9 (0.6), range 0-2) and in two cases with glandular metaplastic epithelium positive for HLA-DR

(one IL-2R positive cells in each case). Again, no statistical differences were shown in terms of OKT3 positive cells in respect of both normal controls and those with wart virus infection (85 (7), range 78–90).

Discussion

The results of this study confirm and extend previous reports which show that there is focal epithelial expression of HLA-DR antigens in wart virus infection.^{16,26} The expression of HLA-DR antigens in the absence of a wart virus infection was limited to glandular metaplasia, confirming the preferential expression of HLA-DR antigens by glandular epithelia.^{3,6,7,17}

In cervical epithelium infected with wart virus the enhanced expression of HLA-DR was related to a highly increased proportion of activated lymphocytes infiltrating the tissue. As shown by others,²⁷ we found that in both normal and infected cervix intraepithelial lymphocytes were predominantly T lymphocytes. These findings add support to the hypothesis that epithelial cells bearing HLA-DR are antigen presenting cells which interact with T lymphocytes as targets.²¹ Activated T cells are able to synthesise in vivo and release interferon gamma; they are also involved in the regulation of HLA-DR expression on cells.^{19,28–32} Thus in the cervical epithelium of patients infected with wart virus activated T cells may regulate, via the release of interferon gamma, the expression of HLA-DR antigens on epithelial cells. In turn, HLA-DR positive epithelial cells may operate as classic antigen-presenting cells locally activating lymphocytes and inducing specific T cell clones.

We have shown that the enhanced epithelial HLA-DR expression was restricted to the cervix infected with wart virus. As DNA and RNA viruses modulate major histocompatibility complex (MHC) molecule expression in various virus-transformed cell lines, inappropriate HLA-DR expression on the cervical epithelium of these patients may be associated with wart virus, as suggested for other viruses in other disease states.^{33–36}

In conclusion, the results of this investigation lend support to the concept that human cervical epithelium actively participates in the local immune response.

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