Herpes Simplex Virus-specific Antigens in Exfoliated Cervical Cells from Women with and without Cervical Anaplasia

Alexander S. Pacsa, Lajos Kummerlander, Bela Pejtsik, Karoly Krommer, and Kalman Pali

SUMMARY
By the indirect immunofluorescence technique the presence of herpes simplex virus-specific antigens was investigated in cervical cells of 530 women with normal cervical epithelium, 175 with bland disorders, 52 with dysplasias, and 38 with invasive cervical carcinomas of the uterine cervix. Antigens were present in 9% of samples from women with normal cervical epithelium; they were present in 41% of the samples from women with bland disorders, 61% of those from dysplasia patients, and 94% of those from invasive carcinoma patients. The testing of 3 consecutive imprints of 68 antigen-positive and 232 antigen-negative women at 6-month intervals revealed that, in cervical cells, herpesvirus-specific antigens persisted throughout the 1-year period of the follow-up.

INTRODUCTION
It has been suggested by epidemiological studies (7, 9, 12) that the genitotrop variant of HSV-2 was 1 of the possible etiological agents in the causation of cervical cancer. Since antibodies specific to HSV-2 could be determined (4, 11), clarification of the nature of the association between herpesvirus genitalis and carcinoma of the uterine cervix seemed feasible by prospective seroepidemiological studies (5, 8). However, any attempt to link HSV-2 to cervical cancer on the basis of antibody determination may be hampered by the presence of the common components in the antigenic structure of HSV-1 and HSV-2 (14, 15). Apart from antibody determination, the role of HSV-2 in the development of cervical neoplasia can be investigated by localizing herpesvirus-specific antigens in cervical cells (1, 13).

Our previous work on the presence of herpesvirus antigens in exfoliated cervical cells revealed that not only anaplastic but also apparently normal cells contained the antigens (10). Therefore, we initiated these investigations to determine the incidence of herpesvirus-specific antigens in normal, dysplastic, and neoplastic cells of women.

MATERIALS AND METHODS

Study Groups (Table 1). Women with normal cervical epithelium were selected by pelvic examination and Papanicolaou test. Women with bland disorders of the cervical epithelium were those that had disorders as previously defined (6); these included ectopium, iodine-negative areas, erosions, inflammations seen by colposcopy and also histological diagnosis like keratos, hyperplasia, metaplasia, and basal activity. Those women having histological diagnosis on their cervical biopsy samples as abnormal epithelium, unque epithelium, and atypical epithelium were considered as patients with cervical dysplasia. Samples from patients with histologically confirmed invasive cervical carcinoma were taken before treatment.

Imprints. Cervical imprints were obtained by the impression of a piece of sponge to the surface of the cervical portion (Group 1), atypical lesion (Groups 2 and 3), and tumor (Group 4). From each person 6 imprints were made on coded slides. They were fixed in cold acetone for 5 min and kept at -20°C until processed.

Immunofluorescence Technique. The presence of HSV-specific antigens in cervical cells was determined by the indirect immunofluorescence method. Imprints were treated with rabbit antiserum to herpesvirus type 2 and then with fluorescein-conjugated sheep anti-rabbit IgG from the Human Sera and Vaccine Institute, Budapest, Hungary. Preparation of anti-HSV-2 rabbit immune serum, details of the technique, and particulars of conjugated IgG were described earlier (10). Slides mounted with 90% glycerol were examined in a Zeiss fluorescence microscope.

RESULTS
Occurrence of HSV Antigens in Cervical Cells. On the basis of immunofluorescence staining, HSV-specific antigens could be detected in the cervical cells. Antigen-positive cells showed intense perinuclear and cytoplasmic fluorescence (Figs. 1 and 2). The test was specific to HSV antigens, for antiserum was prepared by immunizing rabbits with HSV-2 propagated in primary chick embryonic fibroblast cultures, serum used in the test was absorbed with a cell culture derived from human carcinoma of the larynx (H.E.p-2), HEF, and HeLa cells. The serum did not show fluorescence when checked with noninfected H.E.p-2, HEF, HeLa, and cytomegalovirus-infected (strain AD 169)
Herpesvirus Antigens in Cervical Cells

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. tested</th>
<th>Age range (yr)</th>
<th>Mean age (yr)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Women with normal cervical epithelium</td>
<td>530</td>
<td>20-66</td>
<td>37</td>
<td>Apparently normal cervical epithelium by Pap test and pelvic examination</td>
</tr>
<tr>
<td>2. Women with bland disorders of the cervix uteri</td>
<td>175</td>
<td>20-70</td>
<td>35</td>
<td>Diagnosed colposcopically or histologically</td>
</tr>
<tr>
<td>3. Patients with cervical dysplasia</td>
<td>52</td>
<td>20-72</td>
<td>40</td>
<td>Confirmed histologically imprints were taken before treatment</td>
</tr>
<tr>
<td>4. Patients with invasive cervical carcinoma</td>
<td>38</td>
<td>30-65</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

Diagnosis of cervical inflammation.

Fig. 1. Fluorescent staining of cervical imprint containing HSV antigens. Intense specific fluorescence is seen in nuclear membrane and in cytoplasm of the cells with antigens.

Fig. 2. Fluorescent staining of H.E.p-2 cells infected with herpesvirus type 2.

HEF cells. For an inquiry into the presence of HSV-specific antigens in the cervical cells, the following staining pattern was used. Two impressions of cervical cells were treated with HSV-2 immune serum; 2 others were treated with normal rabbit serum, and the remaining 2 were treated 0.1 M phosphate-buffered saline (pH 7.2; 0.07 M NaCl). Samples were considered positive for HSV antigens when cervical cells had shown characteristic fluorescence with HSV-2 immune serum only. Evaluation was done by a technologist not aware of the code.

In samples from women with apparently normal cervical epithelium, HSV antigens could be detected rather frequently (Table 2). The results of testing more than 500 samples have established that not only neoplastic but also normal cervical cells may harbor the antigens. Comparison of the prevalence of antigens by groups reveals a steady increase in antigen positivity from normal to cancerous stage.

Follow-up of Women for the Presence of Antigens. After interviewing women of Groups 1, 2, and 3 (Table 2), HSV antigen-negative and -positive cases were selected on the ground of comparable marital status and history of pregnancies. At the time of interview women were examined colposcopically and cytologically. Those persons showing signs of herpetic cervicitis were excluded from the study. The 3 groups of women (Table 3) were monitored for 1 year. At 6-month intervals after examining the 1st series of imprints, 2 further series were taken and tested for the presence of HSV antigens. By these 3 successive tests, 68 imprints were proven to be antigen positive. In 5 imprints (4 in Group 1 and 1 in Group 2) antigens were detected only at the 2nd and the 3rd tests. Apart from these 5 samples, none of the other negative imprints became antigen positive during the 1-year period of monitoring. On the other hand, positive imprints contained the HSV antigens throughout the year.

Of the 30 antigen-positive patients with dysplasia, 15 have received treatment (conisation or electrocoagulation) after the 1st test. Despite treatment, antigens were detectable by the subsequent 2 tests.

These results may indicate that antigen carrying is lasting and not influenced by treatments.

DISCUSSION

Based on our examination of hundreds of cervical imprints for the presence of herpesvirus antigens, we find that...
the indirect immunofluorescence technique provides a useful approach to clarifying the association of HSV-2 with cervical neoplasia. It is not disturbed by the antigenic similarity of HSV-1 and HSV-2 (which is always a hindrance in HSV-2 antibody determination), because herpetic cervical infections are attributed almost entirely to HSV-2 (4).

Contrary to the finding reported by Aurelian (2), in these experiments we have demonstrated that HSV antigens were present not only in neoplastic but also in cervical cells from women with normal cervical epithelium. The 9% positivity rate seems characteristic for healthy women in this country. Since noncytopathogenic, cell-associated virus is capable of transforming cells in vitro (3), we suppose that healthy women with herpesvirus antigens in their apparently normal cervical cells are at risk of developing cervical cancer. This assumption is strengthened by the gradual increase of the prevalence of antigens from normal to cancerous stage. As expected, a somewhat higher rate of antigen positivity characterizes the so-called bland disorder group of women. It may be due to the fact that cases of acute cervicitis caused by HSV-2 fall in this category.

The antigen carrying revealed by follow-up of selected groups of women indicates that herpesvirus antigens, when once acquired, remain in the cervical cells for at least 1 year. Since women with acute cervicitis were not included in this part of the study, it is very unlikely that the continuous antigen positivity was caused by repeated reinfections. If reinfection played a role in antigen positivity, it would have an influence on antigen-negative cases too. Whether cervical dysplasias or tumors originate from antigen-carrying cells is a question still to be answered. Nevertheless, the results presented here support this possibility. In order to provide further evidence either for or against the herpesvirus hypothesis of cervical neoplasia, histological investigations on biopsy samples from antigen-negative and -positive cases as well as studies on cell-transforming capacity of viral antigens present in apparently normal cervical cells are in progress.

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REFERENCES

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