Effect of Herpesvirus Type 2 and Hormonal Imbalance on the Uterine Cervix of the Mouse

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SUMMARY

BALB/c mice were treated with different combinations of hormones (estrogens, progesterone, and Enovid) and herpesvirus type 2 (HSV-2). Characteristic cytological changes of herpesvirus infection were observed in 40% of 140 mice treated with the virus; these mice had previously been immunized with HSV-2 inactivated with ultraviolet light. No significant differences were observed in the frequency of precancerous and cancerous lesions of the cervix and/or vagina between the group treated with estrogens plus HSV-2 (47.2%) and that treated with estrogens alone (41.2%), or between the group treated with Enovid plus HSV-2 (22.6%) and that treated with Enovid alone (31.2%). No carcinomas were observed in either the group treated with progesterone alone or the two control groups, but one carcinoma was found in the group treated with progesterone and HSV-2, and two carcinomas were found in the group treated with HSV-2 alone. These three carcinomas are of special interest since spontaneous cervical carcinoma in mice has not been described, and progesterone has been found to exert a protective effect against chemical carcinogens in the mouse cervix. These results suggest that, at least in these three animals, HSV-2 may have a role in tumor induction.

INTRODUCTION

An association between HSV-2 and cervical carcinoma has been suggested, based on prospective studies showing that women with genital herpetic infection have an increased risk of developing cervical carcinoma (10). In the United States, a higher frequency of HSV-2 antibodies has been observed in women with cervical carcinoma than in control groups (1, 8, 13, 14). However, no differences between cases and controls were found in other geographic areas (11 12).

Data suggesting that this virus plays a role on cellular transformation in vitro have been reported (3). The long-term effects of HSV-2 infection in laboratory animals are not well established, but a few sarcomas in newborn hamsters and atypical changes in the uterine cervix of mice have been reported (9). Since the carcinogenic effect of estrogens on the murine cervix is well established (5, 7, 16), we carried out an experiment to study the combined effect of HSV-2 and hormonal imbalance.

MATERIALS AND METHODS

Nine groups of BALB/c female mice, 2 to 3 months old (Table 1), were studied. A human strain of HSV-2 (Benedfield strain, obtained from Dr. A. Nahmias) was used. The hormones used were estrogens (estradiol), progesterone, and the p.o. contraceptive Enovid (norethynodrel and mestranol). Estrogens and progesterones were administered as s.c. pellets (5 mg) of a hormone-cholesterol mixture. These pellets were replaced every 3 to 4 months. The Enovid was administered daily in a liquid diet (Metrecal) in the same dose used in previous experiments (4). Since herpesvirus is lethal for mice, immunization with UV-inactivated HSV-2 was performed. The viral infection was carried out by introduction of a cotton pellet soaked in a virus solution (titer, $10^4.3$) in the vagina. The virus was administered twice [at the beginning of the experiment (at any stage of the estrous cycle) and then again 10 months after the 1st infection (at the diestrous stage only)].

A month after initiation of treatment, a male mouse was placed in each of the cages containing 10 female mice each. We took vaginal smears 2, 4, 6, and 10 days after virus inoculation to check the level of viral infection and every 2 months thereafter to detect early neoplastic changes. The smears were stained with Papanicolaou. Twenty months after the beginning of the experiment, the survivors were sacrificed and autopsies were performed.

RESULTS

In the vaginal smears of the animals treated with estrogens and virus, progesterone and virus, and virus alone (taken 2, 4, 6, and 10 days after virus inoculation), multinucleated giant cells typical of herpetic infection were observed, but there were no viral inclusions. The frequency of the giant cells was low on the 2nd day, reached a peak at the 6th day, and declined after the 10th day. As shown in Table 1, the percentage of mice with multinucleated giant cells was a little lower after the 1st inoculation, when the virus was given at any stage of the estrous cycle, than it was after the 2nd inoculation, when it was given at diestrous stage. No cytological evidence of viral infection was de-
tected in the group treated with Enovid and HSV-2. Most of the mice in which no cytological evidence of viral infection was detected were in estrous stage. No deaths occurred after the 1st inoculation but 9% of the mice died after the 2nd inoculation. Only 2 tumors were detected cytologically before autopsy. They have been described elsewhere (6).

Sections of cervix and vagina were taken at 6 to 8 different levels. Three types of lesions were considered. (a) Hyperplastic lesions were characterized by moderate extension of epithelial processes into the stroma of the cervix or vagina. The frequency varied from 10 to 15% among the estrogen- and Enovid-treated groups and was around 5% in groups treated with progesterone or HSV-2, and in control groups (Fig. 1). (b) Dyskeratotic lesions had deeper extensions of epithelial downgrowth into the stroma. Intraepithelial atypia consistent with dysplasia was observed in only 1 mouse. These lesions were interpreted as precancerous (Fig. 2). (c) Well-differentiated squamous cell carcinomas with keratin pearl formation comprised the 3rd type of lesion. All but 2 of these carcinomas were small cancers, only detected microscopically at autopsy (Fig. 3). The 1st of these 2 tumors was a cervical carcinoma detected cytologically 7 months after the 1st viral inoculation in the group treated with estrogens and HSV-2 (Fig. 4). The 2nd was a carcinoma of the vagina diagnosed cytologically 9 months after the 1st viral inoculation in the group treated with HSV-2 alone. These 2 carcinomas have been transplanted s.c. into BALB/c mice (Fig. 4), and epithelial cell lines have also been established in tissue culture. Some of the structural and behavioral characteristics of these 2 tumors have been described elsewhere (6). Virological and immunological studies to detect herpesvirus antigens in these tumors and corresponding antibodies in the serum of mice bearing transplanted tumors are being carried out.

The number of mice at risk was computed at the 10th month after the beginning of the experiment, when the 1st carcinoma was detected macroscopically. Most of the early deaths that occurred in the groups fed Metrecal were due to intolerance of diet, and the late deaths were caused by chronic endocarditis and thrombosis of the auricles. Table 2 shows the frequency of the precancerous and cancerous lesions in the different groups. In our study, the frequency of the lesions in the groups treated with estrogens and Enovid was higher than has been reported by others (5, 7). This result could be related to the number of sections of the cervix studied. The protective effect of the progesterone is in agreement with previous observations (16).

We observed no significant differences between the fre-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial no. of mice</th>
<th>Treatment</th>
<th>After 1st inoculation</th>
<th>After 2nd inoculation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>Estrogens + HSV-2</td>
<td>30</td>
<td>40</td>
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<td>Estrogens</td>
<td></td>
<td></td>
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<td>42</td>
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</tr>
<tr>
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<td>Enovid + HSV-2</td>
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</tr>
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<td>20</td>
<td>Enovid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>HSV-2</td>
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<td>50</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>Control (liquid diet)</td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>Control (solid diet)</td>
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</tr>
</tbody>
</table>

* Detected by vaginal cytology.

* Twenty mice were given injections of the virus 1 week after hormonal treatment and the other 20 mice received injections 3 weeks after hormonal treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>No. of mice at risk*</th>
<th>Mean age at death (mo.)</th>
<th>Precancer</th>
<th>Cancer</th>
<th>Total</th>
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<td>10</td>
<td>27.8</td>
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<td>2</td>
<td>11.8</td>
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<tr>
<td>3</td>
<td>Progestrone + HSV-2</td>
<td>39</td>
<td>17.6</td>
<td>1</td>
<td>2.6</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Progestrone</td>
<td>20</td>
<td>19.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Enovid + HSV-2</td>
<td>31</td>
<td>17.2</td>
<td>1</td>
<td>3.2</td>
<td>6</td>
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<tr>
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<td>18.7</td>
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<td>17.8</td>
<td>1</td>
<td>5.2</td>
<td>2</td>
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<tr>
<td>8</td>
<td>Control (liquid diet)</td>
<td>15</td>
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<td>1</td>
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<td>Control (solid diet)</td>
<td>16</td>
<td>19.5</td>
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</tr>
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</table>

* Number of mice alive 10 months after the beginning of the experiment.
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Frequency of precancerous and cancerous lesions of the uterine cervix and/or the vagina in groups treated with the different hormones alone and that in groups treated with hormones plus HSV-2. However, 2 carcinomas were observed in the group treated with HSV-2 alone, and no carcinomas were observed in the 2 control groups. Although this difference is in the borderline of statistical significance \(\chi^2 = 3.4\) on 1 d.f.: \(0.10 < p > 0.05\), it is biologically interesting. Spontaneous carcinoma of the uterine cervix is very rare in all animals, domestic, laboratory, and wild (2). Only 1 “glandular” carcinoma (which may have arisen in the cervix or the vagina) was found among 39,000 mice (15).

Although the role of the virus in the causation of these tumors is still unknown, these observations justify repetition on a larger scale. The finding of 2 cervical carcinomas in mice treated with HSV-2 alone and 1 carcinoma in the group treated with progesterone and HSV-2 suggests that the virus may have played an important role in the induction of these tumors, and virological studies to demonstrate the presence of HSV-2 in the tumor cells are in progress.

ACKNOWLEDGMENTS

I am deeply grateful to Dr. G. de Thé and Dr. A. Rabson for their advice, to C. Picoli for her technical assistance, and to B. Montagnon for the inactivation of the HSV-2.

REFERENCES

Fig. 1. Hyperplastic lesion, formed by epithelial downgrowths into the stroma of the uterine cervix. H & E, x 100.

Fig. 2. Dyskeratotic lesion interpreted as precancerous. H & E, x 100.
Fig. 3. Small squamous cell carcinoma of the uterine cervix with infiltration of the stroma. Note the formation of keratin pearls. H & E, × 95.

Fig. 4. Invasive, very-well-differentiated squamous cell carcinoma of the uterine cervix. H & E, × 95.
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