Induction of cervical neoplasia in the mouse by an extract of cells infected by varicella-zoster virus

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Since several human herpesviruses, including varicella-zoster virus (VZV), have been demonstrated to transform mammalian cells in vitro, VZV was tested in a mouse model of virus-induced cervical neoplasia to determine whether it is oncogenic in vivo. Herpes simplex viruses types 1 and 2 and cytomegalovirus have been previously shown to induce cervical neoplasia in this mouse model. VZV was propagated in WI-38 cell cultures and inactivated by ultraviolet irradiation. Control material was prepared in an identical manner from uninfected cell cultures. Cotton tampons, saturated with inactivated virus or control material, were inserted into the vaginas of C57BL mice three times a week for 60 weeks. Cervical dysplasia was detected in 40% and invasive carcinoma in 34% of virus-exposed mice by histological examination. No lesions were detected in control animals. These observations indicate that VZV, or some product of virus-infected cells, is oncogenic in vivo for the mouse cervix.

Varicella-zoster virus (VZV) is the cause of chickenpox, an infection contracted by 95% of people during childhood or adolescence (Gershon et al., 1976). Although herpes zoster is a frequent complication in patients with impairment of the immune system secondary to malignant disease or its treatment, there is no epidemiological evidence to suggest that VZV is an oncogenic virus. However, it belongs to the human herpesvirus group, of which four members, herpes simplex viruses types 1 and 2 (HSV-1, HSV-2), cytomegalovirus (CMV) and Epstein-Barr virus (EBV), have been shown to have oncogenic properties (zur Hausen, 1980; Macnab, 1987). All four of these viruses induce transformation of cells in culture (Henle, 1967; Duff & Rapp, 1971, 1973; Rapp & Albrecht, 1973; Geder et al., 1976) and three (HSV-2, CMV and EBV) have been reported in many, but not all, studies to have seroepidemiological associations with human malignancies (zur Hausen, 1980; Rapp & Jenkins, 1981; Vonka et al., 1984; Dale et al., 1988). In a mouse model of cervical carcinogenesis, a progression from dysplasia to microinvasive to invasive carcinoma has been induced by repeated exposure of the cervix to inactivated HSV-1, HSV-2 or CMV (Wentz et al., 1975, 1981, 1983; Heggie et al., 1986). Transfection is thought to be the mechanism by which neoplasia is initiated in this model (Anthonv et al., 1989). As VZV is a human herpesvirus that has also been shown to induce transformation of mammalian cells in vitro (Gelb et al., 1980; Gelb & Dohner, 1984), it was tested in this mouse model to determine whether, under appropriate conditions, VZV can induce oncogenesis in vivo.

VZV (VR-586) and WI-38 human diploid lung cells (line CCL-75) were obtained from the American Type Culture Collection. For propagation of VZV, monolayer cultures of WI-38 cells, grown in Eagle's MEM containing 10% (v/v) foetal calf serum, were inoculated with trypsin-EDTA-dispersed cells from VZV-infected WI-38 cell cultures in a ratio of one infected cell to six uninfected cells (Schmidt & Lennette, 1976). After incubation of cultures for 48 h at 36 °C in 5% carbon dioxide, VZV was harvested by dispersal of infected cell monolayers into the culture fluids by shaking with glass beads. The virus titre of the harvested cultures was determined by immediate inoculation of serial 10-fold dilutions of the suspension of infected cells into fresh WI-38 cell cultures. The harvested cell suspensions were then centrifuged at 1000 g at 4 °C for 15 min. The supernatant fluid was stored at 4 °C; the pellet of cells was resuspended in culture fluid, frozen and thawed three times, and sonicated for 30 s (Fisher Model 300 Sonic Dismembrator, 50% power, cup tip adapter on probe). The sonicated cells were pelleted, the pellet was washed three times with aliquots of culture fluid and the washes were added to the stored culture fluids. For inactivation of infectivity, 90 ml of the extract of VZV-infected cells

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was dispensed into 22 x 34 cm sterile glass dishes and exposed to an ultraviolet light source consisting of two G15T8 germicidal lamps (General Electric Company) at a distance of 20 cm for 12 min. Extracts contained approximately $10^5$ TCID$_{50}$ of virus per ml before inactivation. No infectious virus was detected after irradiation, except that WI-38 cell cultures were not inoculated with VZV. Preparations of inactivated VZV and control fluid were stored at -70 °C until used for exposure of mice.

C57BL virgin female mice were used in this study. Mice, approximately 6 weeks old at the time of receipt, were assessed for pre-existing abnormalities by examination of cytological preparations of vaginal aspirates obtained weekly for 4 weeks. After completion of this assessment, cotton tampons, saturated with approximately 0.1 ml of a VZV preparation that contained $10^5$ TCID$_{50}$ of virus before inactivation, or with the same volume of control fluid, were inserted into the vaginas of mice three times a week for 60 weeks. Sixty mice were exposed to inactivated VZV and control fluid were stored at -70 °C until used for exposure of mice.

Table 1. Cytological diagnoses in C57 mice in relation to duration of exposure in VZV-exposed and control mice

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>VZV-exposed mice</th>
<th>Control mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>52</td>
<td>40</td>
</tr>
<tr>
<td>Microinvasion</td>
<td>31</td>
<td>40</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>13</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 2. Frequencies of histopathologically confirmed cervical lesions in VZV-exposed and control mice*

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>VZV-exposed mice</th>
<th>Control mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cervix</td>
<td>13 (26-0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dysplasia</td>
<td>20 (40-0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Invasive cancer</td>
<td>17 (34-0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* Differences in frequencies, both of dysplasia and invasive carcinoma, between VZV-exposed and control mice are statistically significant when tested separately by the Chi-square test ($P < 0.001$).
The relationship of VZV-associated cervical neoplasia in this mouse model to the development of disease in humans is unclear. Although VZV-infected lesions occur in the genital mucous membranes during varicella, the ubiquity of this disease makes it unlikely that VZV is a direct cause of human cervical cancer. However, prolonged latent infection with VZV is a frequent occurrence and under appropriate conditions the virus might function as a cofactor with some other carcinogen. Further studies using this mouse model may contribute to the understanding of the molecular mechanisms by which neoplasia is induced by the human herpesviruses.

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