Transformation of Hamster Embryo Fibroblasts by Herpes Simplex Viruses Type 1 and Type 2

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Summary

Hamster embryo fibroblast cells can be transformed after infection by ultraviolet light-irradiated herpes simplex viruses type 1 (HSV-1) or type 2 (HSV-2). Two of 12 strains of HSV-1 and 7 of 15 strains of HSV-2 induced cell transformation. Transformed cells from two strains of HSV-2 and one strain of HSV-1 have been tested for oncogenicity in newborn Syrian hamsters. One cell line transformed by HSV-2 strain 333 was found to be oncogenic. The remaining two cell lines were not oncogenic. Herpesvirus antigens were found in the cytoplasm and on the surface of the transformed cells. Neutralizing antibodies were produced in hamsters developing tumors after the injection of the HSV-2-transformed cells. Weanling hamsters that were preimmunized by injection of HSV-1 or HSV-2 did not develop transplantation immunity to the HSV-2-transformed cells as a result of this preimmunization. HSV-2-transformed cells metastasized after injection into hamsters. The number of metastases was not inhibited by HSV-1 or HSV-2 immunization but was actually enhanced. Immunization of hamsters with simian virus 40 inhibited metastases but did not reduce the rate of primary tumor development.

Introduction

Four of the major groups of viruses containing DNA induce in vitro transformation and in vivo induction of tumors (2, 9). The most likely candidates for viruses oncogenic in humans appear to be members of the herpesvirus group. Several lines of evidence have been documented that link these viruses to cancer in both lower animals and man. The best documented case of the expression of oncogenic potential by a herpesvirus is found in a disease of chickens, Marek’s disease (4, 22). The infection of a young chicken with a herpesvirus induces a highly proliferative lymphosarcoma that ultimately results in the death of the animal. In addition to the direct demonstration by virus injection that a herpesvirus is responsible for this disease, further evidence that Marek’s disease is caused by a herpesvirus comes from the observation that the disease can be prevented by the injection of a similar attenuated virus that does not induce the neoplasia (23).

More recently a herpesvirus has been implicated in lymphomas that can be induced in monkeys (16-19, 29). This virus, Herpesvirus saimiri, can be isolated from squirrel monkeys (Saimiri sciureus) without a tumor; however, it induces lymphomas or lymphocytic leukemia in marmosets (Saguinus oedipus, Saguinus fuscicolis, and Saguinus nigricollis), owl monkeys (Aotus albifrons), and cinnamon rington monkeys (Cebus albifrons). In addition to these demonstrations of herpesvirus oncogenicity in animals, the Epstein-Barr virus of humans has been associated with Burkitt’s lymphoma. Cells derived from Burkitt lymphomas contain virus antigens that can be detected by immunofluorescence and herpes-like particles that can be observed with the electron microscope (10, 13). The virus that can be isolated is not highly infectious but has been shown to induce the in vitro transformation of leukocytes (15). A very similar, and probably identical, virus has been shown to be associated with infectious mononucleosis and is now thought to be the causative agent of that disease (14).

A 2nd human herpesvirus which has been associated with human neoplasia is HSV-2 (3). The original observation that implicated this virus in cancer was the result of seroepidemiological studies. It was demonstrated that women with cervical carcinoma have circulating antibodies directed against the HSV-2 more often than women without the disease (21, 24). HSV-2 has also been isolated from cervical carcinoma cells and HSV-2 antigens can be demonstrated in similar cells by immunofluorescent techniques (1, 27). However, HSV’s when injected into experimental animals rarely, if ever, induce tumors (20, 25). Recently, in our laboratory we have demonstrated that HEF cells can be transformed by UV-irradiated HSV-2 and that these cells are oncogenic when injected into newborn hamsters (8, 9).

Transformation of Hamster Embryo Cells

HSV-1 and HSV-2 are extremely cytopathic in tissue culture cells that have been infected by the virus. Any potential oncogenic transformation would therefore be masked by the cell death that results from HSV infection.
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The elimination of cell death induced by HSV-1 and HSV-2 was accomplished by UV irradiation (42 ergs/sec/sq cm) of the virus before infection of the host cell. Inactivated HSV-1 and HSV-2 were absorbed onto HEF cells in vitro while the cells and virus were in suspension. Three to 4 weeks after the HSV infection, transformed cells appeared in the culture monolayer. These cells could then be isolated from the monolayer and grown in culture into cell lines. Under the conditions used, both HSV-1 and HSV-2 have transformed HEF in vitro (Table 1). Seven of 15 HSV-2 strains induced HEF cellular transformation and 2 of 12 HSV-1 strains also transformed HEF cells. The morphology of the transformed hamster embryo cells has been constant depending upon the type of HSV used to transform the cells. Cells transformed by HSV-2 were usually of mixed morphology (Fig. 1). Three types of cellular morphology were generally seen: (a) a fibroblastic cell, (b) a giant cell, and (c) a rather primitive mesenchymal-like cell. In contrast to this type of transformation induced by HSV-2, HSV-1-transformed cells usually exhibited an epithelial-like morphology (Fig. 2). In addition to the epithelial cells, many giant cells were also observed in HSV-1-transformed HEF cells.

Antigenic Characteristics of HSV-transformed Cells

Cells that have been transformed by either DNA or RNA oncogenic viruses usually contain virus-specific antigens. For example, the transformation of a cell by the SV40 usually results in the induction of the SV40 T or tumor antigen in the nucleus and in the induction of the SV40 S antigen on the surface of the cell (3, 24, 28). These antigens are usually demonstrated by the use of immunofluorescence techniques. Cells that have been transformed after infection by HSV-1 or HSV-2 were tested by indirect immunofluorescence techniques for the presence of HSV-specific antigens. When anti-HSV serum that had been prepared in weanling hamsters was absorbed to acetone-fixed HSV-transformed cells, a cytoplasmic fluorescence was detected within these cells (Fig. 3). The diffuse fluorescence observed throughout the cytoplasm was seen in 5 to 25% of the transformed HEF cells. The cytoplasmic antigen reacted with both anti-HSV-1 and anti-HSV-2 sera.

The HSV-2 transformed cells were also examined for the presence of herpesvirus-specific antigens on the surface by utilizing indirect immunofluorescence techniques and unfixed cells. As shown in Table 2, significant numbers of these transformed cells demonstrated fluorescence on their surface after treatment with anti-HSV serum. Normal cells reacted in very low numbers and normal weanling hamster serum stained only a small percentage of the transformed cells exposed to this reagent.

Characteristics of HSV-transformed Cells in Vivo

The transformed cells (2 x 10⁴ cells/hamster) were injected into weanling and newborn Syrian hamsters. No tumors were induced in weanling hamsters by any of the 7 cell lines tested. All hamster cell lines transformed by the HSV-2 strain 333 were oncogenic after injection into newborn hamsters. However, cell lines that had been established following transformation by this strain of HSV-2 differed with respect to their relative oncogenic potential. As shown in Table 3, 1 cell line (333-8-9) was of relatively low oncogenic potential after injection into newborn hamsters. However, the remaining 4 cell lines that were transformed by strain 333 (333-2-20, 333-2-21, 333-2-26, and 333-2-29) induced tumors with greater frequency and with shorter latent periods than did the 333-8-9 cells. In contrast to the lines transformed by strain 333, cells that were transformed by HSV-2 strain 332 and HSV-1 strain KOS were not oncogenic when injected into newborn hamsters. From these results we have now classified HSV-transformed cells into 3 types with regard to their oncogenic potential: high, low, and nononcogenic.

Sera from tumor-bearing hamsters were tested for antibodies against HSV's. It was found that a significant number of the tumor-bearing hamsters developed neutralizing antibodies directed against HSV-2. Sera that neutralized HSV-2 did not neutralize HSV-1 as efficiently in most cases (Chart 1). It was also found that the development of neutralizing antibodies was inversely proportional to the latent period of tumor development in the host hamster. The cell lines transformed by HSV-2 strain 333 that had a shorter latent period in the newborn hamsters did not induce

### Table 1

<table>
<thead>
<tr>
<th>Virus type</th>
<th>No. of isolates tested</th>
<th>No. of isolates with detectable transformation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>HSV-2</td>
<td>15</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Serum</th>
<th>Cell type</th>
<th>% of cells reacting at surface</th>
</tr>
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<tbody>
<tr>
<td>Anti HSV-1</td>
<td>333-8-9</td>
<td>60.9</td>
</tr>
<tr>
<td></td>
<td>HEF</td>
<td>12.2</td>
</tr>
<tr>
<td>Anti HSV-2</td>
<td>333-8-9</td>
<td>60.3</td>
</tr>
<tr>
<td></td>
<td>HEF</td>
<td>9.3</td>
</tr>
<tr>
<td>Weaning hamster</td>
<td>333-8-9</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>HEF</td>
<td>14.7</td>
</tr>
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</table>

### Table 3

<table>
<thead>
<tr>
<th>Cell line</th>
<th>% of hamsters with tumors</th>
<th>Latent period (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>333-8-9</td>
<td>36</td>
<td>10-16</td>
</tr>
<tr>
<td>333-2-20</td>
<td>100</td>
<td>2-4</td>
</tr>
<tr>
<td>333-2-21</td>
<td>100</td>
<td>2-4</td>
</tr>
<tr>
<td>333-2-26</td>
<td>100</td>
<td>2-4</td>
</tr>
<tr>
<td>333-2-29</td>
<td>98</td>
<td>2-7</td>
</tr>
</tbody>
</table>
Chart 1. Neutralization of HSV-2 and HSV-1 by sera obtained from hamsters with tumors induced by an s.c. injection of HSV-2-transformed cells (333-8-9, LSH-1). All hamsters were given injections of the transformed cells at 3 to 4 weeks of age. The results are expressed as the percentage of virus survivors after incubation of 1000 plaque-forming units of HSV with 1 ml of a 1/10 serum dilution.

neutralizing antibodies with as great a frequency as did the cell line with the longer latent period (Table 4). The location of the antigens that induced the neutralizing antibody response in the tumor-bearing hamsters is not clear at the present time. The surface or cytoplasmic antigens which have been observed may be the antigens that induce this immune response. However, in a few of the tumor cells immature HSV-like particles have been observed and this low-level production of presumably noninfectious HSV may result in the induction of HSV-2-neutralizing antibodies (11).

Table 4
Neutralization of HSV-1 and HSV-2 by sera from hamsters bearing tumors induced by HSV-2-transformed cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Anti-HSV-1</th>
<th></th>
<th>Anti-HSV-2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>333-8-9</td>
<td>3/5</td>
<td>60</td>
<td>5/5</td>
<td>100</td>
</tr>
<tr>
<td>333-2-20</td>
<td>9/14</td>
<td>64.3</td>
<td>5/14</td>
<td>35.7</td>
</tr>
<tr>
<td>333-2-21</td>
<td>2/16</td>
<td>12.5</td>
<td>10/16</td>
<td>62.5</td>
</tr>
<tr>
<td>333-2-26</td>
<td>7/17</td>
<td>41.2</td>
<td>12/17</td>
<td>70.6</td>
</tr>
<tr>
<td>333-2-29</td>
<td>4/29</td>
<td>13.8</td>
<td>22/29</td>
<td>75.9</td>
</tr>
</tbody>
</table>

* Positive sera are defined as the sera that neutralized at least 50% of 1000 plaque-forming units of HSV-2 at a 1/10 serum dilution.
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Chart 2. The rate of tumor induction in weanling Syrian hamsters after the injection of HSV-2 transformed hamster cells. Hamsters received s.c. injections of 333-8-9 cells (○) or 333-2-26 (O). TPD/50, 50% tumor-producing dose.

Since it was possible that the attenuation of HSV-1 strain 35 destroyed its potential for the induction of tumor immunity, nonattenuated strains of HSV-1 and HSV-2 were also injected after UV irradiation to destroy virulence. However, the results using UV-inactivated HSV-1 and HSV-2 were the same as the results obtained using the HSV-1 strain 35. No detectable inhibition of the primary tumors was observed using UV-irradiated HSV-1 or HSV-2.

If extremely low levels of transplantation immunity were induced by injections of HSV-1 or HSV-2 in weanling hamsters, this low level of immunity might be detected by inhibition of metastases rather than by inhibition of the primary tumor. To answer this question, animals were immunized by HSV-1 strain 35, SV40, UV-irradiated 333-8-9 cells, and UV-irradiated HEF cells. The immunization of weanling hamsters by HSV-1 resulted in an enhancement of metastases in these animals (Table 5). The immunization of weanling hamsters by UV-irradiated 333-8-9 cells and HEF cells resulted in a significant decrease in the number of metastases. SV40 completely inhibited metastases by the HSV-transformed cells when animals were immunized with 3 injections of this virus. Since it has been previously shown that SV40-transformed cells contain new surface embryonic antigens (5, 7), it can be postulated that the inhibition of metastases might be a result of the stimulation of an immune response against hamster embryonic antigens. The enhancement of metastases after the injection of HSV-1 is possibly due to the presence of blocking antibodies that have been previously described in other tumor systems (12). These results that demonstrate the enhancement and inhibition of metastases after an s.c. injection of 333-8-9 cells have been repeated utilizing a 2nd HSV-2-transformed cell line, the 333-2-26 line (Table 5). Although the frequency of metastases was somewhat less with the 333-2-26 line, the pattern of metastases with regard to the inhibition by SV40 and the enhancement by HSV-1 was essentially the same.

Discussion

The results summarized in this communication provide evidence for the association of HSV-2 as well as HSV-1 with the transformation of normal hamster cells into morphologically altered cells. In some cases this morphological transformation was accompanied by the conversion of the normal cells into cells with oncogenic potential. However, in all cases HSV antigens could be demonstrated in the cytoplasm and on the surface of the cells. Recently, the transformation of human embryonic lung cells has been reported following abortive infection by HSV-2 (6). Neither HSV-1 nor HSV-2 has been conclusively shown to transform human cells in our laboratory. However, experiments now in progress are designed to demonstrate this.

The frequency of oncogenic transformation by HSV-1 or HSV-2 still remains to be completely delineated. Cell lines that have been transformed by 2 strains of HSV-2 and 1 strain of HSV-1 have been tested for oncogenic potential. Only 1 group of 5 transformed cell lines that was transformed by the HSV-2 strain 333 was oncogenic after injection into newborn hamsters. After further extensive testing of many cell lines transformed by several virus strains it can be determined whether this oncogenic conversion is a relatively rare characteristic of HSV-1 and HSV-2 strains or whether it occurs at a relatively high frequency.

The final observation described in this communication is that HSV-2-transformed cells do not contain levels of virus-specific transplantation antigens that are found in most cell lines transformed by other DNA viruses. This apparent lack coupled with failure to stimulate the immune response of the host animal is probably responsible for the high rate of metastases that is found in HSV-2 tumor-bearing animals. The procedure described to control enhancement and repression of these metastases suggest that the HSV-2-transformed cells may represent a model system for the experimental control and characterization of metastases in mammals.
Chart 3. The rate of tumor induction by HSV-2-transformed cells in weanling Syrian hamsters after immunization. Immunizing agents in Chart 3A were HSV-1 (☐), SV40 (●), or Medium 199 control (×). Immunizing agents in Chart 3B were UV-irradiated HEF cells (■), UV-irradiated 333-8-9 cells (☐), and Medium 199 control (×). Hamsters in each immunization group received 3 immunizing injections at 1-week intervals. The animals were challenged with 333-8-9 cells 1 week after the final immunizing injection. TPD/50, 50% tumor-producing dose.

Table 5
Effect of immunization on the development of metastases in weanling hamsters

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Immunizing agent</th>
<th>Total hamsters given injections</th>
<th>Hamsters with lung metastases</th>
<th>% of hamsters with metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>333-8-9</td>
<td>HSV-1*</td>
<td>27</td>
<td>13</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>SV40*</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>333-8-9*</td>
<td>38</td>
<td>3</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>HEF*</td>
<td>40</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Medium* control</td>
<td>34</td>
<td>7</td>
<td>20.6</td>
</tr>
<tr>
<td>333-2-26</td>
<td>HSV-1*</td>
<td>35</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>SV40*</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>333-8-9*</td>
<td>40</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>HEF*</td>
<td>40</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Medium* control</td>
<td>39</td>
<td>4</td>
<td>10.3</td>
</tr>
</tbody>
</table>

*a Hamsters in this group were immunized by 3 injections of 10⁶ plaque-forming units of HSV-1 strain 35.
*b Hamsters in this group were immunized by 3 injections of 10⁶ plaque-forming units of SV40.
*c Hamsters in this group were immunized by 3 injections of 10⁶ 333-8-9 cells that were UV-irradiated for 3 min (42 ergs/sec/sq cm).
*d Hamsters in this group were immunized by 3 injections of 10⁶ HEF cells that were UV-irradiated for 3 min (42 ergs/sec/sq cm).
*e Hamsters in this group were given 3 injections of Medium 199 as a control.
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Acknowledgments

The authors wish to thank Myron Katz and Doreen Lakatosh for their valuable technical assistance.

References

Fig. 1. Photomicrograph of hamster embryo cells transformed by HSV-2 (333-8-9). H & E, × 300.
Fig. 2. Photomicrograph of hamster embryo cells transformed by HSV-1 (KOS-6-9). H & E, × 300.
Fig. 3. Photomicrograph of HSV-2-specific cytoplasmic fluorescence in HSV-2-transformed cells (333-8-9 cells). The indirect immunofluorescence technique was used with anti-HSV-2 serum prepared by the injection of weanling Syrian hamsters. × 300.

Fig. 4. Photomicrograph of a tumor cell metastasis in the lung of an adult hamster. The animal had received injections s.c. as a weanling with HSV-2-transformed hamster cells. × 125.
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