Demonstration of a Receptor for IgG in Syrian Hamster Cells transformed with Herpes Simplex Virus

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SUMMARY

Hamster embryo fibroblasts transformed by herpes simplex virus types 1 and 2 have a receptor for immunoglobulin on their surface similar to that on the surface of cells lytically infected by these viruses.

Sheep erythrocytes sensitized with rabbit anti-sheep erythrocyte antibody bind strongly to the surface of cells lytically infected with herpes simplex virus (Watkins, 1964, 1965). Studies with rabbit IgG labelled with $^{125}$I suggest that this phenomenon is due to the appearance on the infected cells of a receptor for the Fc fragment of IgG (Westmoreland & Watkins, 1974); these studies favour the view that the receptor is coded by the herpes simplex genome. Rapp and his colleagues have shown that hamster embryo fibroblasts can be transformed by herpes simplex virus types 1 and 2, when virus infectivity has been abolished by u.v. irradiation. The transformed cells are highly malignant, and antibodies against virus proteins appear in the serum of animals bearing tumours. Cytoplasmic fluorescence can be demonstrated in some of the cells, using fluorescent anti-herpes serum (Duff & Rapp, 1971a, b, 1973).

We have examined several of these herpes-transformed hamster cell lines for the presence of the IgG receptor. Two methods were used. In one, confluent cultures of transformed cells on coverslips were sensitized by incubation at 37 °C for 1 h in rabbit anti-sheep erythrocyte serum (diluted 1/50 in phosphate-buffered saline) (Yasuda & Milgrom, 1968). The cells were washed in saline and re-incubated at 37 °C for 30 min in a 0.5% suspension of sheep erythrocytes in saline. After adsorption of erythrocytes, the coverslips were washed, fixed in 1-25% (v/v) glutaraldehyde and stained by Giemsa stain for microscopic examination. In the second method the binding of IgG by herpes transformed cells was directly measured using $^{125}$I-labelled IgG (Westmoreland & Watkins, 1974). IgG purified from whole rabbit serum was iodinated by an adaptation (Jensenius & Williams, 1974) of the technique described by Byrt & Ada (1969). 10 μl of sodium $^{125}$I (sp. act. 14 mCi/μg) in NaOH, pH 8 to 11, was mixed with 10 μg of IgG and 10 μl of Chloramine T solution (2 mg/ml) at pH 7.3. After 2 min incubation at room temperature the reaction was stopped by the addition of excess tyrosine, and the reaction mixture fractionated on a Sephadex G-50 column. The iodinated IgG had a sp. act. of approx. $2 \times 10^{-6}$ ct/min/mol. Ten μl of the labelled IgG, diluted to contain about $5 \times 10^5$ ct/min were added to unfixed confluent cultures on coverslips 11 mm in diam. After incubation in a humid atmosphere at 37 °C for 30 min the coverslips were repeatedly washed in phosphate-buffered saline, dried in air, and placed in 5 ml of scintillation fluid (2,5-diphenyloxazole + p-bis-(O-methylstyrlyl)-benzene in toluene) for estimation of their radioactivity in a Nuclear Chicago Scintillation Counter Model 724.

Three types of result were obtained in the haemadsorption experiments. Some lines showed no adsorption of erythrocytes, some showed adsorption to every cell (Fig. 1), and some showed adsorption to occasional cells only. In this third group the positive cells were often much larger than the negative cells, frequently with more than one nucleus, or a nucleus of
Fig. 1. Cells of a herpes-transformed hamster cell line showing binding of sheep erythrocytes to every cell: stained Giemsa.

Fig. 2. Cells of a herpes-transformed hamster cell line showing binding of sheep erythrocytes to occasional 'giant' cells: stained Giemsa.
Table 1. Antibody mediated binding of sheep erythrocytes and binding of radioiodinated IgG to hamster fibroblasts transformed with herpes simplex virus

<table>
<thead>
<tr>
<th>Cells</th>
<th>Code*</th>
<th>Transforming virus</th>
<th>Number of passages in vitro</th>
<th>Oncogenicity in hamsters†</th>
<th>IgG bound (ct/min)‡</th>
<th>Haemadsorption§</th>
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<tr>
<td>Hamster embryo fibroblasts</td>
<td>14-012-8-1</td>
<td>HSV-1</td>
<td>144</td>
<td>+</td>
<td>7355</td>
<td>0-1</td>
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<td>nt</td>
<td>7858</td>
<td>0</td>
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<td>nt</td>
<td>8790</td>
<td>1</td>
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<td>HSV-1</td>
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<td>nt</td>
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<td>9531</td>
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<td>+</td>
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<tr>
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<td>—</td>
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<td>—</td>
<td>3085</td>
<td>0</td>
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<tr>
<td>BHK-21 clone 13</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>—</td>
<td>3085</td>
<td>0</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>4447</td>
<td>0</td>
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</table>
| Hamster embryo fibroblasts
16 h post-infection with
HSV-1(input virus 5TCID₅₀/ cell) | —      | —                 | —                           | —                        | 39621              | 2               |

* Code numbers used by Dr R. Duff and Dr F. Rapp.
† Oncogenicity was tested in newborn Syrian hamsters as described by Duff & Rapp (1971 a). nt = Not tested.
‡ Mean of three estimations.
§ 0 = No haemadsorption; 1 = patchy haemadsorption, as shown in Fig. 2; 2 = generalized haemadsorption, as shown in Fig. 1.

an irregular shape (Fig. 2). No nuclear changes characteristic of herpes simplex lytic infection were seen in these cells.

The results with [¹²⁵I]-labelled IgG correlated well with the results of the haemadsorption experiments. All the transformed cell lines bound more IgG than either primary hamster embryo cells or those of the continuous hamster cell line, BHK-21 (Macpherson & Stoker, 1962). None of the transformed lines bound as much IgG as did primary hamster cells infected with herpes simplex virus type 1.

In view of the observation that haemadsorption and binding of [¹²⁵I]-labelled IgG have been demonstrated in a variety of cells which are lytically infected with herpes simplex virus, but not in normal cells, the results reported here are consistent with the view that herpes virus functions persist in the transformed cells. However, it has not yet been formally proved that the receptors for IgG on lytically infected and transformed cells are identical. The fact that one of the lines was negative indicates that the presence of the receptor is not essential for transformation. The association of the receptor with altered morphology of occasional cells, as seen in the third group described above, is of great interest, since it raises the possibility that the information for the appearance of the receptor is normally suppressed in these lines, and that the expression of the information may be spontaneously induced.

In hamster cell lines transformed in vitro by herpes simplex virus and shown to be malignant, the receptor, or the information for the receptor clearly persists. It has been suggested that certain human tumours, for example, carcinoma of the cervix (Nahmias, Naib & Josey, 1972) and of the prostate (Centifanto et al. 1973) may be caused by herpes simplex virus. In addition, Sabin & Tarro (1973) have reported the presence of antibodies to non-virus particle antigens of herpes simplex virus in many patients with malignant tumours. The results we report here suggest that the presence or absence of the receptor for IgG may be of crucial
importance in demonstrating that a given human tumour is caused by herpes simplex virus.

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REFERENCES


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