Sensitivity to Inactivation by Ultraviolet Light of Certain Functions of Polyoma Virus: Cell Surface Antigen

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SUMMARY

Ultraviolet irradiation of polyoma virus inactivates different functions of the virus genome. This inactivation is related to the radiation dose.

It has been shown that the ultraviolet radiation target size of the nuclear T antigen is one-half that of the ability to form plaques, and that the radiation target size of the homograft rejection antigen is one-sixth that of the latter. In the present experiments, ultraviolet irradiation has been applied in a similar manner in order to study the cell surface antigen. Quantitative analysis shows that the radiation target size for this antigen is one-half that of the ability to form plaques (infectivity).

The difference in responses of the cell surface antigen and in the homograft rejection antigen observed after irradiation of the virus can be due either to differences in sensitivities of the techniques used or to a difference in antigenicity. Experiments with defective polyoma strains favour a difference in antigenicity.

INTRODUCTION

In a preceding paper (Meyer, Lhérisson & Bonneau, 1969) we reported the ability of ultraviolet-irradiated polyoma virus to induce the nuclear T antigen and the homograft rejection antigen. The loss of these two antigens was compared with the inactivation of the infectivity and it was shown that about half the virus genome necessary for replication was required for induction of the T antigen, while only one-sixth of the virus genome was needed for induction of the homograft rejection antigen.

Another antigen, the cell surface antigen (Irlin, 1967; Lhérisson, Meyer & Bonneau, 1967; Malmgren, Takemoto & Carney, 1968), has been detected by several authors in BHK 21 cells or mouse embryo cells infected at high multiplicity with polyoma virus. In the present experiments, ultraviolet irradiation of the virus was used similarly to follow the inactivation of this cell surface antigen. This was done in order to compare the cell surface antigen with the homograft rejection antigen: in the case of SV 40, Tevethia et al. (1968) showed a lack of relationship between these two antigens.

METHODS

Virus. The small plaque TORONTO strain of virus was used. Virus was produced in mouse embryo cells harvested after treatment with neuraminidase (Crawford, 1962). The infectivity obtained was 10^9 p.f.u. (plaque forming units), as measured either by plaque formation (Dulbecco & Freeman, 1959) on mouse embryo cells or by haemagglutination on guinea-pig red cells (Deinhardt, Henle & Marks, 1960).
Ultraviolet irradiation of virus was as previously described (Meyer, Lhérison & Bonneau, 1969).

Cells. The cells used were baby hamster kidney cells of the BHK 21/13 cell line (Macpherson & Stoker, 1961). Stationary monolayers of BHK 21 cells were infected with 200 p.f.u./cell. Three hours after infection the unadsorbed virus was discarded and replaced by Eagle's medium. BHK 21 sublines transformed by Rous virus (RB 12; Montagnier, Meyer & Vigier, 1969) were used as controls.

The cells were grown in Eagle's medium with a doubled concentration of amino acids and vitamins supplemented with 10% bactotryptose phosphate (Difco) and 10% filtered and heat-inactivated calf serum.

Antisera were obtained from the blood of hamsters which had rejected polyoma-induced tumours. Sera from hamsters which had rejected Rous-induced tumours were used as controls.

Anti-hamster globulin fluorescent antisera were obtained commercially (BD Merieux) and adsorbed on mouse liver powder and on normal BHK 21 cells; they were used at one-third dilution. It was always necessary to test their own fluorescence on hamster cells.

Cell surface immunofluorescence. Cell surface antigen was revealed by a modification of Möller's technique (1961) for living cells: cell suspensions were prepared for fluorescent examination by treatment of the cells on glass for 5 min. with 0.125% trypsin in phosphate-buffered saline (PBS) at pH 7.4. Cells were then centrifuged at 1000 g for 10 min.

The supernatant fluid was discarded and replaced by 1 ml. Eagle's medium. Cells were then centrifuged again and resuspended in Eagle's medium with 10% calf serum, and washed twice in this medium by centrifugation and resuspension. Eagle's medium supplemented with 10% calf serum was added (2 ml.) to the last cell pellet which was carefully homogenized. The cells were kept in suspension for 2 hr by means of a slow magnet stirrer. This stirring in medium was necessary because treatment with trypsin reduced the number of fluorescent cells.

After 2 hr or more, cells were removed from the magnetic stirrer, pipetted into a centrifuge tube, and centrifuged. The supernatant fluid was discarded and the cells were suspended in 0.05 ml. of hamster antiserum. The mixture was incubated at 37° for 30 min. and homogenized occasionally.

Cells were then washed three times in PBS, pH 7.4, the supernatant fluid was removed and the pellet resuspended in 0.1 ml. of rabbit anti-hamster gamma-globulin labelled with fluorescein isothiocyanate. The mixture was allowed to incubate for 30 min. at 37° in the dark with frequent mixing by pipette.

Cells were then washed 3 times with PBS and suspended in a drop of buffered glycerine for mounting beneath a coverslip. Cell preparations were finally examined with an ultraviolet microscope.

RESULTS

Inactivation of cell surface antigen

Previous experiments on the development of cell surface antigens (Meyer, Birg & Bonneau, 1969) showed that the maximum percentage (75%) of BHK 21/13 cells was fluorescent at 19 hr after infection at high multiplicity with polyoma virus (100 or 200 p.f.u./cell). Thus, all determinations of the percentages of fluorescent cells were made at this time after infection. The virus received various doses of ultraviolet irradiation ranging from 3000 to 10,000 ergs/mm². Table 1 shows results for such an experiment. The percentage of cells infected with unirradiated virus and carrying cell surface antigen was taken as 100%. The
Table 1. Deletion of cell surface antigen in BHK 21/13 cells after infection with u.v.-irradiated polyoma virus

<table>
<thead>
<tr>
<th>U.v. irradiation dose (ergs/mm.²)</th>
<th>Cells with cell surface antigen (%)</th>
<th>Cells with surface antigen (cells infected with unirradiated virus = 100 %) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>3 000</td>
<td>44</td>
<td>51.7</td>
</tr>
<tr>
<td>6 000</td>
<td>21</td>
<td>24.7</td>
</tr>
<tr>
<td>9 000</td>
<td>10</td>
<td>11.8</td>
</tr>
<tr>
<td>10 000</td>
<td>7</td>
<td>8.3</td>
</tr>
</tbody>
</table>

fraction of cells showing fluorescence fell exponentially with irradiation dose (Fig. 1). Fig. 1 also shows the loss of infectivity (p.f.u.): the slope of the line for loss of cell surface antigen is half that for the loss of infectivity.

Comparison of cell surface and homograft rejection antigens

The inactivation of the homograft rejection antigen by ultraviolet irradiation of virus has been studied in a similar manner (Meyer, Lhérisson & Bonneau, 1969), and Fig. 1 shows the exponential declines of cell surface antigen and homograft rejection antigen.

DISCUSSION

Specification of the cell surface antigen by the virus genome

It is clear that the virus genome is involved, either directly or by a derepression pathway, in the specification of the surface antigen, since ultraviolet irradiation of the virus decreases the number of fluorescent cells. This is not due to a loss of adsorption as shown by electron microscope studies of SV 40-irradiated virus (Ferreira-Saldago, 1968). Furthermore, the cell surface antigen can be detected not only in abortive transformations, but also in transformed cells following the complete cycle of transformation; these transformed cells presumably contain the virus genome in an integrated state (Benjamin, 1966; Westphal & Dulbecco, 1968; Dulbecco, 1968).

The part of the virus genome involved in the coding for cell surface antigen

Fig. 1 shows the inactivation of infectivity (Latarjet, Cramer & Montagnier, 1967) and of the activity of the cell surface antigen. The relative decay slopes suggest that about half the virus genome necessary for replication is involved in direct or indirect coding of the cell surface antigen: this recalls a similar result for inactivation of the induced complement-fixing antigen.

In this determination one cannot reject the possibility of cellular repair of irradiation damage and of complementation between irradiated viruses. Cellular repair, for example, may be completed by the intervention of an excision enzyme and a polynucleotide ligase.

Comparison of cell surface antigen and homograft rejection antigen

Fig. 1 shows the decline of cell surface antigen and of homograft rejection antigen. The slopes of the lines are different, one being three times the other. Thus it is unlikely that these two antigens are coded by the same part of the virus genome, but it is possible that the
homograft rejection antigen is an incomplete or partial cell surface antigen. However, the comparison of these two antigens was not performed in the same cellular system. The immunofluorescent surface antigen was studied in an abortive cycle of BHK 21 cells while the homograft rejection antigen was studied in vivo with oncogenic grafted cells. Presumably the sensitivity of the in vitro technique was greater than that of the in vivo technique, but the difference in the results was too great to be due to this.

Fig. 1. Influence of exposure to ultraviolet light on infectivity of polyoma virus and on induction of cell surface and homograft rejection antigens. Δ—Δ, inactivation of cell surface antigen in BHK 21 cells after infection with u.v.-irradiated polyoma virus. The percentage of fluorescent cells is shown as a function of irradiation dose with confidence limits. Cells infected with unirradiated virus are taken to show 100% fluorescence. ○—○, virus infectivity as p.f.u. with confidence limits. Infectivity of unirradiated virus is 100%. □—□, inactivation of homograft rejection antigen with confidence limits. Activity is expressed as the mean reciprocal of the time of tumour appearance in immunized animals: the control at 100% represents protected animals without tumours following administration of unirradiated polyoma virus. For details of statistical data see Meyer, Lhérisson & Bonneau (1969).

In order to specify the relationship between cell surface and homograft rejection antigens, we studied a polyoma strain defective in homograft rejection antigen (Ipd strain as described by Hare, 1967). The observation that the Ipd strain, which does not induce the
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homograft rejection antigen, is able to induce the cell surface antigen is in favour of the non-identity of these antigens. In these experiments the difference in the sensitivities of detection must be emphasized. Experiments are in progress to test the homograft rejection antigen by more sensitive techniques.

In conclusion, there are two arguments in favour of the difference between homograft rejection antigen and immunofluorescent surface antigen: difference in sensitivity to ultraviolet irradiation in induction of these two antigens and inability of defective strains to induce one of these antigens.

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REFERENCES


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