Induction of Interferon in Chick Cells by Polyoma Virus

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SUMMARY

Polyoma virus induces high levels of interferon in non-permissive chick embryo fibroblast cultures, and certain features of this induction have been examined. In non-pre-treated cultures interferon first appears at around 30 h after infection and reaches maximum levels at around 70 h. An input multiplicity of at least 0.5 p.f.u./cell is required for induction. In cultures pre-treated with interferon (primed), interferon induction is enhanced, with interferon first appearing in the medium at around 20 h and reaching 4- to 8-fold higher levels than in non-primed cultures. In this case interferon is induced by an input multiplicity of as low as 0.1 p.f.u./cell. ‘Full’ or intact polyoma particles induce, but ‘empty’ shells do not. Interferon can also be induced by polyoma on continuously passaged lines of chick embryo fibroblasts.

Polyoma virus induces interferon in mouse cells (Allison, 1961; Friedman, Rabson & Kirkham, 1963), and in hamster embryo cells (Talas, Weiszfeiler & Batkai, 1968; Gotlieb-Stematsky & Vansover, 1970). In mouse cells interferon is produced late after infection, the titres are extremely low, and it has been suggested that less oncogenic variants of polyoma induce higher levels of interferon (Friedman et al. 1963). In hamster cells, interferon is again produced late after infection, the reported levels of interferon differ, and it appears that in this case more oncogenic variants produce higher levels (Gotlieb-Stematsky & Vansover, 1970). Polyoma is a small, simple virus, containing a circular genome of mol. wt. 3·0 x 10^6 (Crawford, 1962), sufficient genetic information to code for between five and eight polypeptides, and in theory it should be a useful simple model for analysing the mechanism by which a DNA virus induces interferon in cells. As a pre-requisite to using it as a model, it is, however, necessary to have an efficient, consistent system for induction of interferon by polyoma. To this end we tested chick embryo fibroblasts (CEF), which produce good levels of interferon after human adenovirus infection (Beladi & Pusztai, 1967; Ustacelebi & Williams, 1972). This communication reports the results of experiments which show that polyoma induces good levels of interferon in CEF cells.

Small plaque variants of the Toronto strain (McCulloch et al. 1959) of polyoma were used. One variant originated in Glasgow (Crawford, 1962) and the other in Toronto (Stanners, Till & Siminovitch, 1963). Both were grown and titrated by plaque assay on either primary or secondary mouse embryo fibroblasts (MEF), prepared from 15- to 17-day-old embryos by a standard procedure (pregnant mice of the Pifbright strain were obtained from the Animal Virus Research Institute, Pirbright), and cultivated in Eagle's medium (Glasgow modification) supplemented with 10% calf serum. Polyoma virus was extracted from infected cells by a modification of the procedure described by Crawford (Crawford, 1962) and was purified by centrifuging in caesium chloride (Crawford, Crawford & Watson, 1962). Vaccinia virus stocks were grown on BHK 21/C 13 cells and were titrated on these and on secondary chick embryo fibroblasts (CEF). CEF were prepared from 10-day-old embryos (eggs from birds of the Thornbers' 909 strain were obtained from the Poultry
Research Association, Edinburgh) by a standard procedure, and were grown in Eagle's medium supplemented with 10% calf serum and 10% tryptose phosphate broth. The procedures used for interferon induction and assay were those previously described (Ustacelebi & Williams, 1972b). Interferon was assayed by measuring the inhibition of vaccinia virus plaque formation on CEF and the titre is expressed as the PDD_{50}, the reciprocal of the dilution causing a 50% depression of the control plaque count. One ml of this dilution contains 1 unit of interferon.

In initial experiments, using both the Glasgow and the Toronto variants of polyoma, CEF cells were infected with non-purified virus at an input multiplicity of 10 p.f.u./cell, and interferon levels of 128 to 256 PDD_{50}/ml were reproducibly detected in the culture fluid at 3 days post-infection. Polyoma purified by banding on CsCl gradients induced similar levels of interferon. Extracts of non-infected MEF cells prepared in identical fashion to the polyoma failed to induce interferon. The kinetics of interferon synthesis were examined in primary CEF infected at an input multiplicity of 10 p.f.u./cell. Interferon first appeared in the culture fluid around 30 h after infection, and continued to increase for about the next 40 h (see Fig. 1), attaining a maximum level at 70 h and remaining at that level. The kinetics of production were also examined in cells pre-treated with 20 units of interferon for 15 to 18 h before infection and earlier, and enhanced production of interferon was obtained. As shown in Fig. 1, in pre-treated cultures interferon appears in the medium as early as 12 h post-infection, and attains levels 4- to 8-fold higher than in non-treated cultures. We previously reported a priming action of interferon for adenoviruses (Ustacelebi & Williams, 1972b), and it is known to occur in induction with certain other viruses (Isaacs & Burke, 1958; Lockart, 1963; Stewart, Gosser & Lockart, 1971). The effect of the priming dose on the yield of interferon is shown in Fig. 2. At least 10 units of interferon are required for maximum effect.

The interferon produced by CEF in response to polyoma infection is stable at pH 2,
Fig. 2. The effect of pre-treatment with increasing levels of interferon on the subsequent yield of interferon produced on CEF cells infected with polyoma virus.

stable to heating at 60 °C for 1 h, not sedimented at 100000g for 2 h, not inactivated by polyoma neutralizing antiserum, sensitive to trypsin treatment and species specific. These are all properties of well-characterized, purified interferons (Wagner, Levy & Smith, 1968).

We find that polyoma does not replicate in CEF cells, so in this respect they are non-permissive for the virus. It should also be mentioned that the virus is not cytotoxic at multiplicities of up to 20 p.f.u./cell, although culture fluid does become acid as a result of infection. Attempts to detect CEF cell transformation by polyoma have met with no success so far. As shown in Fig. 1, the cell-associated infectivity gradually decreases after infection, but some can still be detected even at four days post-infection. We do not know at which stage of infection polyoma is blocked, nor do we know if synthesis of any polyoma-specific products, for instance DNA, takes place. However, the treatment of polyoma-infected CEF with 40μg/ml of cytosine arabinoside, known to inhibit virus DNA synthesis, has no effect on interferon production, showing that virus DNA synthesis is not involved. This has recently also been shown to be the case for induction of interferon in CEF cells by human adenovirus type 12 (Bakay & Burke, 1972), and by both CELO virus and human adenovirus type 7 (Markovits & Coppey, 1972).

In the experiments described above, the input multiplicity was relatively high. To determine the optimum multiplicity for interferon induction by polyoma in both primed and non-primed CEF, multiplicities ranging from 0.05 to 20 p.f.u./cell were used. In non-primed CEF cells very little interferon is induced at input multiplicities of less than 1 p.f.u./cell, and the optimum level is around 10 (Fig. 3). In this respect polyoma is somewhat similar to adenovirus type 1, and different from types 5 and 12 (Ustacelebi & Williams, 1972b). In primed cultures significant levels of interferon are produced at 10-fold lower multiplicities. Even at an input multiplicity of less than 0.1 p.f.u./cell a detectable level of interferon is found.

Clearly the polyoma-CEF culture system provides a simple, efficient reproducible model system for studying interferon induction by a DNA virus, and in these experiments, interferon was produced considerably earlier and at higher levels in CEF cells than it appears to have been produced previously in mouse or hamster cells (Allison, 1961; Friedman et al. 1963; Talas et al. 1968; Gotlieb-Stematsky & Vansover, 1970).
We have found that SV 40, a papovavirus similar to polyoma in some respects, fails to induce interferon on CEF under conditions where polyoma does so. The reason for this is not known.

Crude polyoma virus preparations consist of a mixture of ‘full’ particles, containing DNA and possessing high infectivity, and ‘empty’ particles which are non-infectious (Crawford et al. 1962). The polyoma preparations used in the present work had total particle/p.f.u. ratios of around 30 to 50 and contained many ‘empty’ particles. In order to determine if the ‘empty’ particles possess the capacity to induce interferon, polyoma was purified by banding twice in caesium chloride. ‘Full’ and ‘empty’ bands were isolated, and particle counts were carried out on the electron microscope. ‘Full’ bands consisted of 99.5% ‘full’ particles, and ‘empty’ bands were made up of 99.75% ‘empty’ particles. These were used on both primed and non-primed CEF at particle multiplicities of 700 and 1400/cell. The complete results of one such experiment are shown in Tables 1a and b. ‘Full’ particles induce high levels of interferon in both primed and non-primed cells, while equivalent quantities of ‘empty’ particles fail to do so, even in primed cells.

While these experiments show that when polyoma virus particles are used, complete particles, though not necessarily infectious particles, are required for interferon induction in CEF, they do not allow us to deduce which virus components are involved in induction. The findings support the view that either the nucleic acid or an internal protein of the virus is involved in induction although the important experiment of testing the inducing capacity of the virus DNA remains to be done. The DNA could act directly as an inducer, or alternatively it may be necessary for the synthesis of some early virus products in CEF, which are in turn required for interferon induction. In this context, induction of interferon in mammalian cells by T4 coliphage is relevant (Kleinschmidt, Douthart & Murphy, 1970).
Table 1a. Particle composition and infectivity of ‘full’ and ‘empty’ bands

<table>
<thead>
<tr>
<th></th>
<th>‘Full’ band</th>
<th>‘Empty’ band</th>
<th>Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus particles/ml</td>
<td>7.0 x 10^11</td>
<td>3.5 x 10^9</td>
<td>2.0 x 10^10</td>
</tr>
<tr>
<td>‘Full’ band</td>
<td>1.5 x 10^8</td>
<td>6.0 x 10^7</td>
<td>2.0 x 10^6</td>
</tr>
<tr>
<td>‘Empty’ band</td>
<td>1.5 x 10^8</td>
<td>6.0 x 10^7</td>
<td>2.0 x 10^6</td>
</tr>
</tbody>
</table>

Table 1b. Interferon-inducing capacity of ‘full’ and ‘empty’ polyoma particles

<table>
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<th>Input multiplicity</th>
<th>Interferon titre*</th>
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<tbody>
<tr>
<td>Virus</td>
<td>p.f.u./cell</td>
<td>Primed CEF</td>
</tr>
<tr>
<td>‘Full’ band</td>
<td>20</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1400</td>
</tr>
<tr>
<td>‘Empty’ band</td>
<td>0.002</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0.004</td>
<td>2</td>
</tr>
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</table>

* PDD_{50}/ml (medium harvested 3 days post-infection).

Table 2. Interferon induction by polyoma virus on cultures of the continuous line CEF-B

<table>
<thead>
<tr>
<th>Passage number</th>
<th>Interferon* titre</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td></td>
<td>256</td>
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Cells were infected as described in the text, at an input multiplicity of 10 p.f.u./cell. Medium was collected at 3 days post-infection, and assayed for interferon on secondary cultures of CEF.

* PDD_{50}/ml.

Complete T4 particles induce, but neither empty ghosts nor T4 DNA do so. These authors interpret their data as indicating that the virus DNA is the inducer, but that it only induces when in the correct configuration within the particle. Our findings suggest that a capsid component of the virus particle is not directly involved in induction, since ‘empties’ fail to induce. However, one cannot completely exclude the possibility that a coat component is involved, since the empty coat might differ in composition or configuration from that of the full, or the inducing component might be unable to act because the empty particles fail to adsorb to or to enter the cells.

During the course of this work, two separate lines of normal, non-infected CEF designated CEF-A and CEF-B were initiated and passaged weekly for up to 20 consecutive passages. They grew successfully during this period, but degenerated at or around the 20th passage and failed to grow further. Another line, derived from an early passage of CEF-A but infected previously with an input multiplicity of 10 p.f.u./cell of polyoma, was also established, but this line also degenerated after around 20 passages in vitro. These three lines have been tested for their capacity to produce interferon after infection with polyoma virus and in particular CEF-B was tested at various passages after initiation. Relatively high levels of interferon were produced by line B even after 20 passages (Table 2) and late passage cultures of lines A and PYE were also found to produce interferon after polyoma infection. None of these lines spontaneously produced interferon in the absence of added polyoma. Adeno-virus type 5 also induced good levels of interferon on these cell lines. These facts, coupled with the facility with which the cells can be stored frozen in liquid nitrogen and recovered, suggest that these and similarly derived lines might in future be useful for chick interferon studies as an alternative to using freshly prepared primary CEF. It remains to be shown that a wider variety of viruses including RNA viruses can induce interferon on them.
At present we do not know how DNA viruses induce interferon in animal cells. Evidence from the adenovirus type 5-CEF system, using ts mutants (Ustacelei & Williams, 1972a) suggests that a specific virus function(s) might be required for induction, but as yet this function(s) has not been identified. It is feasible to consider that ts mutants of polyoma (Eckhart, 1969) may provide useful probes in the system reported here.

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REFERENCES


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