Absence of Adenovirus-specific Repressor in Adenovirus Tumour Cells

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SUMMARY

Sendai virus mediated fusion of adenovirus type 12 (Ad. 12) transformed cells (HTC) and permissive cells (HEp-2) was used to (i) rescue Ad. 12, (ii) determine if a repressor is present in the HTC cells. No evidence of virus rescue was observed. The presence of repressor in the transformed cells was tested by superinfecting heterokaryocytes of HTC and HEp-2 cells, with Ad. 12. Inclusion bodies and virus antigens were observed in both types of nuclei of heterokaryocytes demonstrating the absence of Ad. 12 specific repressor in the HTC cells.

Adenovirus type 12 (Ad. 12) tumour or transformed cells have so far proven resistant to virus rescue (Burns & Black, 1969). Experience with the rescue of SV40 from SV40-transformed cells has shown that rescuability may vary greatly between transformed lines of different origin (Dubbs & Kit, 1968, 1970). It is not known whether a repressor-like mechanism is responsible for the maintenance of the virus genome in the integrated form in the transformed cell. The present communication deals with this problem. In order to test for the presence of repressor two types of experiments were performed: Ad. 12 tumour cells (HTC) were co-cultivated or fused with permissive cells; secondly, tumour cell-permissive cell heterokaryocytes were superinfected with Ad. 12. Virus replication, or the lack of it, would strongly argue against or for the repressor hypothesis, respectively.

The methods of cell culture and virus growth and assay, and cell fusion techniques have been described previously (Weber & Stich, 1969 b; Weber, 1972). The HTC cells were derived from an Ad. 12 induced Syrian hamster tumour (Weber, 1973a, b). HTC cells pre-labelled with [3H]-thymidine were used in most experiments to facilitate subsequent identification of heterokaryocytes by autoradiography. In the first set of experiments HTC cells and HEp-2 cells, which are permissive for adenovirus replication, were co-cultivated and samples were taken at 2, 4, 6, 8, 14 and 27 days. Crude extracts were prepared by freeze-thawing and treatment in a sonifier and tested for infectious virus by plaque formation (p.f.u.) on KB cells and inclusion body formation (ICU, Weber, 1972) on HEp-2 cells. Infectious virus was not recovered by either of these techniques (Table I).

Treatment with antimetabolites and Sendai virus mediated cell fusion have been successfully used for the rescue of latent virus from transformed cells (Watkins, 1970). In one set of experiments HTC cells were fused with HEp-2 cells, by means of Sendai virus, and cultured for 2, 4, 8 or 14 days. Samples taken during these times were either prepared for in situ tests for virus antigens by immunofluorescence (Weber, 1973b) or virus inclusion bodies by cytological staining with aceto-orcein (Weber, 1972), or extracts were made and tested for infectious virus by the ICU and p.f.u. methods (Table I, line 2). Some samples were embedded for electron microscopy (Weber & Stich, 1969 a). All the in situ tests were routinely followed by autoradiography to identify the heterokaryocytes.

In a second set of experiments the HTC cells were exposed to various antimetabolites 24 h prior to cell fusion. Table 1 shows the drug concentrations and the time of exposure.
### Table 1. Summary of virus recovery experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Drug</th>
<th>µg/ml</th>
<th>Exposure 5 (h)</th>
<th>Days after mixing or fusing of HTC and indicator cells</th>
<th>Tests for recovery of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Virus antigen (%)*</td>
</tr>
<tr>
<td>Extracts of mixed cultures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ICU/0.025 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p.f.u./0.1 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2, 4, 6, 8, 14</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Fused cultures and extracts of fused cultures†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8-azaguanine</td>
<td>100</td>
<td>24</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p-fluorophenylalanine</td>
<td>100</td>
<td>24</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2-thiouracil</td>
<td>100</td>
<td>24</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Actinomycin D</td>
<td>0.5</td>
<td>2‡</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mitomycin C</td>
<td>4</td>
<td>4‡</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Puromycin</td>
<td>50</td>
<td>4‡</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5-iodo-2-deoxyuridine</td>
<td>100</td>
<td>24</td>
<td>2, 4, 8</td>
<td>0</td>
</tr>
</tbody>
</table>

* Based on 3000 or more cells examined per sample.
† This represents two separate set of expts.: in one set of expts, the fused cells were examined for the presence of virus antigens and IB; in the second set of expts, extracts were prepared from the fused cell cultures and examined for infectious virus by the ICU and the p.f.u. methods.
‡ Cells were treated 24 h prior to fusion.
§ Not tested.
Subsequent to cell fusion, the samples taken at 2, 4 and 8 days later, were examined for evidence of virus rescue in the same way as described above.

No evidence of virus recovery was observed in any of these experiments (Table 1) with one possible exception. In some of the fused-cell samples examined in the electron microscope, occasional particles with a morphology and size characteristic of adenovirus were observed in the nuclei. Such particles were never seen in either the HTC or the HEp-2 cells. These particles may represent a low level of recovery of non-infectious virus particles in the heterokaryocytes. It is of interest in this regard that the adventitious presence in some Ad. 12 tumour cells of incomplete particles has been reported previously (Smith & Melnick, 1964).

The failure to rescue Ad. 12 from the tumour cells could be due to (i) a defective virus genome in the tumour cell, or (ii) the presence, in the tumour cells, of a repressor or repressor-like mechanism preventing the expression of late virus genes. The technique of cell hybridization provides a powerful tool to test the second possibility. HTC cells, pre-labelled with [3H]-thymidine were fused with the permissive HEp-2 cells and the resulting mixture of fused cells, which contained a large proportion of heterokaryocytes, were superinfected 12 h later with 0.1, 1 or 10 p.f.u./nucleus of Ad. 12. Three days later the coverslip cultures were appropriately fixed and either stained with aceto-orcein or with fluorescein conjugated
Table 2. Presence of virus inclusions in heterokaryocytes of HEp-2 and HTC cells super-infected with Ad. 12

<table>
<thead>
<tr>
<th>Experiment*</th>
<th>HEp-2</th>
<th>HTC</th>
<th>Number of infected heterokaryocytes examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>181/182</td>
<td>139/133</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>648/668</td>
<td>192/204</td>
<td>76</td>
</tr>
</tbody>
</table>

Three days post-infection the cells were fixed with acetic-alcohol (1:3), stained with aceto-orcein and submitted to autoradiography.
* Expt. 1 was carried out with a Sendai stock producing small fused cells, while expt. 2 with a stock producing large fused cells, nearly all being heterokaryocytes.
† The number of inclusion body containing nuclei over the total number of nuclei in the heterokaryocytes.

type specific virus antiserum. Autoradiography was performed either immediately or, as in the latter case, after initial examination and characterization of the fluorescence pattern. A typical autoradiograph of a heterokaryocyte infected at a multiplicity of 1 is shown in Fig. 1. Of the 15 HEp-2 nuclei, 10 contain inclusion bodies, while of the three labelled HTC nuclei two contain inclusion bodies. At the highest multiplicity of infection usually all the nuclei of both types of cells contained inclusion bodies. The results of two experiments using the highest multiplicity of infection is shown in Table 2. Expt. 1 was carried out with a Sendai stock producing small fused cells, while expt. 2 with a stock producing large fused cells, nearly all being heterokaryocytes. By this expedient it was possible to compare the effect of small or large numbers of nuclei of either type on the formation of virus inclusion bodies in heterokaryocytes. The results show that the incidence of inclusions is close to 100% for the HEp-2 and HTC nuclei for both experiments. The percentage ratio of inclusion body containing HEp-2/HTC heterokaryocyte nuclei remained close to unity when the input multiplicities of 0·1 or 1 p.f.u./nucleus were used. The relative number of either type of nuclei within a heterokaryocyte did not appear to have any effect on the ratio of inclusion bodies. Furthermore, among the uninfected cells there were both types of heterokaryocytes; cells with an excess of HEp-2 nuclei, or cells with an excess of HTC nuclei. The equal response of both types of nuclei within the heterokaryocytes rules out the possibility that the putative repressor substance was overcome by either an excess of human material or by an excess of infecting virus particles. Since the formation of virus inclusion bodies is evidence for virus replication (Martinez-Palomo, LeBuis & Bernhard, 1967; Weber, 1972), these experiments show that not only is the heterokaryocyte susceptible to superinfection by Ad. 12, but virus replication may take place with equal efficiency in both types of nuclei. The dominance of the permissive response to adenovirus infection has been observed previously with BHK21-HEp-2 heterokaryocytes (Weber & Stich, 1969b; Weber & Mak, 1970).

The synthesis of virus antigens was examined by immunofluorescence. The heterokaryocytes were identified by relocalization of fluorescent polykaryocytes on autoradiographs (Weber & Mak, 1972). In the present experimental conditions nearly all the fused cells were heterokaryocytes, and within the limits of observation, either all or none of the nuclei were fluorescent. It appears therefore that once a heterokaryocyte is infected, the virus proteins are transported to all the nuclei within the heterokaryocyte.
The permissive response of the heterokaryocytes to superinfection by Ad. 12 in these experiments, argues against the hypothesis of the presence of a repressor or repressor-like mechanism in this Ad. 12 tumour cell. It is of interest to note that the same conclusion was reached for SV 40 transformed cells using similar experimental criteria (Jensen & Koprowski, 1969). By contrast, Champe, Strohl & Schlesinger (1972), demonstrated a specific inhibitory factor in extracts from their Ad. 12 tumour cell line. The two cell lines, however, differ in their capacity to support superinfection by Ad. 2; while the HTC line is superinfectible (J. Weber, unpublished observations) the HT2 line used by Champe and co-workers were not significantly superinfectible (Strohl et al. 1970). The superinfectibility of Ad. 5 transformed hamster cells by Ad. 5 has led Williams (1973) to suggest the absence of effective repressor or inhibitor in these cells. The discrepancies in these observations may possibly be accounted for by differences in the transformed cells. As Pettersson & Sambrook (1973) have shown, although the majority of the DNA sequences of the Ad. 2 genome are present in transformed rat cells, the absence of a different small segment in independently isolated transformed cells cannot be ruled out.

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A preliminary report of this investigation has been presented at the annual meeting of the American Society for Microbiology, 1973, p. 207.

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REFERENCES


Short communications


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