The Effect of an Antireceptor Serum on Mammalian Cell Lines

(Accepted 24 January 1974)

SUMMARY

The treatment of a suspension of living HeLa cells, JJH strain, with anti-polio receptor serum resulted in the specific inhibition of these receptors. Inhibition was maintained for at least 48 h at 37 °C provided the cells remained in suspension. Allowing treated cells to adhere to a glass surface resulted in the rapid disappearance of this inhibition. No inhibition of receptors was observed when similar experiments were performed at 37 °C with HeLa (S3) cells, a strain routinely cultured in suspension. Inhibition was demonstrated only when these cells were treated and tested at 15 °C, and was rapidly lost when the cells were incubated at 37 °C. These results suggest that the state of the cell influences the persistence of antibody on the cell surface.

Conflicting observations have been reported concerning the inhibition of surface receptors by anticellular serum. The observation that anticellular serum inhibited attachment of enteroviruses to suspended cells (Axler & Crowell, 1968; Much & Zajac, 1973) while attachment of enteroviruses to cell monolayers was not inhibited (Timbury, 1962, 1963, 1969a, b) suggested that variations in the state of the cells may explain this inconsistency. In the present experiments we determined if the suspended or monolayer status of cells had an effect on the persistence of antireceptor antibody on the cell surface.

Poliovirus Tt (Mahoney) was propagated in cultures of HeLa (JJH) cells. The methods of virus preparation and plaque assay have been described (Zajac & Crowell, 1965). Cells designated HeLa (JJH) were cultured as monolayers in growth media composed of Eagle’s BME supplemented with 10% calf serum (Zajac & Crowell, 1965). Cultures of cells of the human heteroploid lines, AV-3 and HEp-2, were propagated as described for HeLa (JJH) cells. HeLa (S3) cells were cultured in suspension in Eagle’s BME media free of calcium and magnesium and supplemented with 10% calf serum.

To determine virus attachment, virus was added to a known number of suspended cells to give an input multiplicity of less than 1 p.f.u./cell. The virus–cell mixture was incubated at 37 °C for 1 h in a water bath with intermittent shaking. At various intervals, a 0.1 ml sample of the suspension was added to 10 ml of phosphate-buffered saline supplemented with 5% calf serum to stop virus attachment, and the diluted cell suspension centrifuged to remove the cells. For virus plaque assay, prepared monolayers were inoculated in triplicate with 0.1 ml of each virus dilution from each time interval. The inoculated cultures were incubated for 1 h at room temperature, overlaid with growth media containing 5% calf serum and 0.6% Difco agar, and incubated at 37 °C for 40 to 48 h. After plaque development, the overlay media was removed and the monolayer stained with crystal violet. Virus concentrations were recorded as the number of p.f.u./ml of original suspension (Crowell & Syverton, 1961; Zajac & Crowell, 1965).

The preparation of anticellular serum (ACS) to HeLa cells has been described (Much & Zajac, 1973). Specific antireceptor serum (ARS) was prepared by adsorbing ACS with HeLa (JJH) cells exposed to 56 °C for 30 min to inactivate the poliovirus receptor (Zajac & Crowell, 1969; Much & Zajac, 1973).
To determine antireceptor serum (ARS) activity, washed HeLa (JJH) cells were suspended in a 1:10 dilution of ARS to give a final concentration of $2 \times 10^6$ cells/ml. Following treatment for 1 h at 37 °C, the cells were washed and resuspended in Hank’s balanced salt solution (BSS) containing 3 % calf serum (BSS-3CaS) to give a concentration of $1 \times 10^7$ cells/ml. Poliovirus T1 or Coxsackievirus B3 was added at a multiplicity of 0.1 p.f.u./cell. Following incubation for 1 h at 37 °C, samples were withdrawn and the amount of attached virus determined. Untreated cells or cells treated with 1:10 normal rabbit sera served as controls. We have shown that treatment of HeLa (JJH) cells with ARS completely inhibits the attachment of poliovirus T1, while the attachment of Coxsackievirus B3 is unaffected (Much & Zajac, 1973).

In order to establish the duration of inhibition of poliovirus attachment to cells maintained in suspension or monolayer, HeLa (JJH) cells at a concentration of $2 \times 10^6$ cells/ml were treated with a 1:10 dilution of ARS for 1 h at 37 °C. Following treatment the cells were washed by sedimentation and resuspended in growth media supplemented with 3 % calf serum (GM-3CaS). A sample of the suspended cells was assayed for the content of attached poliovirus. One portion of the remaining suspension was maintained at 37 °C in suspension ($1 \times 10^7$ cells/ml), and another portion allowed to settle on to the glass surface of a 32 oz prescription bottle ($1 \times 10^7$ cells/bottle). At various intervals, poliovirus T1 was added to samples of suspended cells at a multiplicity of 0.1 p.f.u./cell. Following incubation at 37 °C for 1 h samples were withdrawn and the amount of attached virus was determined. Also, at various intervals, cells maintained in monolayer were removed from the glass surface with 0.02 % EDTA in phosphate-buffered saline, pH 8.0. The cells were washed, resuspended in BSS-3CaS and counted in a haemacytometer to determine that there was no increase in cell number. Poliovirus T1 was added at a multiplicity of 0.1 p.f.u./cell. Following incubation at 37 °C for 1 h, samples were withdrawn and the amount of attached virus determined. Untreated cells in suspension or monolayer were tested as controls.

The data presented in Table 1 clearly show that HeLa (JJH) cells maintained in suspension at 37 °C did not regain their ability to attach poliovirus for 48 h. In contrast, the same cells allowed to settle on to the glass surface rapidly regained their ability to attach the virus.

Similar experiments were performed with AV-3 and HEp-2 cells to determine whether the above observation could be extended to other cell lines. These cells were treated with ARS for 1 h at 37 °C, washed and resuspended in GM-3CaS. The cells were maintained for 24 h in suspension and monolayer and the persistence of blockade by ARS determined by their ability to attach poliovirus at 37 °C.
Short communications

Table 2. The persistence of antireceptor serum on AV-3 and HEp-2 cells incubated for 24 h in suspension or monolayer

<table>
<thead>
<tr>
<th></th>
<th>AV-3</th>
<th>HEp-2</th>
<th>AV-3</th>
<th>HEp-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>1, 1</td>
<td>21, 23</td>
<td>80, 80</td>
<td>90, 81</td>
</tr>
<tr>
<td>Untreated</td>
<td>50, 65</td>
<td>80, 73</td>
<td>85, 74</td>
<td>89, 97</td>
</tr>
</tbody>
</table>

Table 3. The effect of antireceptor serum on the attachment of poliovirus T1 to HeLa (S3) cells

<table>
<thead>
<tr>
<th>Conditions of treatment</th>
<th>Temperature of attachment assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 °C</td>
</tr>
<tr>
<td></td>
<td>37 °C</td>
</tr>
<tr>
<td>With ARS</td>
<td>Per cent of initial virus</td>
</tr>
<tr>
<td></td>
<td>attached in 60 min</td>
</tr>
<tr>
<td>10 °C</td>
<td>3, 4, 6</td>
</tr>
<tr>
<td>37 °C</td>
<td>—</td>
</tr>
<tr>
<td>Control, with GM-3CaS</td>
<td>10 °C</td>
</tr>
<tr>
<td></td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>37 °C</td>
</tr>
</tbody>
</table>

The results presented in Table 2 demonstrate that ARS-treated AV-3 or HEp-2 cells retained the specific antibody only when the cells were maintained in suspension. Allowing the cells to settle on to the glass surface resulted in the loss of the blocking effect.

In order to determine whether the above observations could be applied to a cell line normally maintained in suspension, HeLa (S3) cells were used. The S3 cells were treated with a 1:10 dilution of ARS at 37 °C for 1 h at a concentration of 2 x 10^6 cells/ml. Following treatment, the cells were washed by sedimentation, resuspended in GM-3CaS, and then tested for the presence of receptor blocking antibodies. The results from these experiments failed to demonstrate any inhibition of attachment since the cells attached poliovirus T1 whether maintained in suspension or monolayer. Since the treatment of cells with ARS was carried out at 37 °C, it was reasoned that the S3 cells rapidly removed the blocking antibody. Therefore an experiment of similar design was performed except that the sensitization of S3 cells with ARS and subsequent attachment of virus was carried out in the cold to prevent removal of the specific antibody from the cell surface.

HeLa (S3) cells at a concentration of 2 x 10^6 cells/ml were treated with a 10-fold dilution of ARS for 5 h at 10 °C. Following treatment, the cells were washed by sedimentation and resuspended in GM-3CaS. The persistence of ARS on the cell surface was assayed by the ability of poliovirus T1 to attach to treated cells for 60 min at 37 °C or 15 °C. Cells treated with GM-3CaS served as a control.

The data presented in Table 3 demonstrated that at 15 °C, antireceptor antibody remained at the cell surface, effectively blocking the attachment of poliovirus T1. These results indicate that receptors for poliovirus T1 on HeLa (S3) cells could be inhibited with ARS only when these cells were treated and maintained at temperatures below 15 °C. However, when the cells were suspended at 37 °C, the blockade of receptors was rapidly eliminated. This is in direct contrast to the results with HeLa (JJH) cells, in which the inhibition of receptors with specific sera occurred only when these cells were maintained in suspension. Permitting HeLa (JJH) cells to settle on to the glass surface resulted in a loss of the receptor blockade.
Axler & Crowell (1968) reported that antiserum to HeLa cells in suspension inhibited virus attachment. In contrast, Timbury (1969b) observed that antibody to HEp-2 cells in monolayer did not inhibit virus attachment. An explanation of these conflicting results, offered by Timbury, is that the agglutination of suspended HeLa cells by antibody results in the masking of sites for virus attachment.

Our present study suggests still another possibility to explain the inhibition of enterovirus attachment. When HeLa (JJH) cells are treated with antireceptor serum (ARS), the specific attachment of poliovirus T1 is inhibited. This inhibition persists for up to 48 h if the cells are maintained in suspension. However, if these cells are allowed to form a monolayer they regain their ability to attach poliovirus T1, as do HEp-2 and AV-3 cells. The attachment of the cells to the glass may permit the surface membrane to stretch and expose additional sites for virus attachment. Another possibility is that cells in monolayer may phagocytose adsorbed antibody, as suggested by Oda & Puck (1961) following their demonstration that somatic cells treated with specific antibody lose their sensitization to complement within 1 h at 37 °C. Our experiments with S3 cells, a strain of HeLa cells normally maintained in suspension, also support this hypothesis. There was no inhibition of poliovirus attachment when S3 cells were treated in suspension with ARS at 37 °C and assayed for virus attachment at 37 °C or 15 °C. However, when S3 cells were treated with ARS at 10 °C, no attachment of poliovirus was observed at 15 °C, indicating that removal of specific antibody by the cell was prevented in the cold. We suggest that the monolayer or suspension status of cells influences the persistence of antibody on the cell surface, and that these observations may explain previous conflicting reports on the effects of anticellular sera on virus infections.

Department of Microbiology
Jefferson Medical College of Thomas Jefferson University
Philadelphia, Pennsylvania, U.S.A.

REFERENCES

* Present address: Department of Biology, Princeton University, Princeton, New Jersey 08540.
† Present address: Smith Kline and French Laboratories, L-33, 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101.

(Received 12 November 1973)