Effect of Measles Virus Antibodies on a Measles SSPE Virus Persistently Infected C6 Rat Glioma Cell Line

By P. NOEL BARRETT,† KLAUS KOSCHEL, MICHAEL CARTER‡ AND VOLKER TER MEULEN*

Institute of Virology and Immunobiology, University of Würzburg, Versbacher Strasse 7, D-8700 Würzburg, F.R.G.

(Accepted 15 March 1985)

SUMMARY

Maintenance of measles (SSPE-Lec) virus persistently infected C6 rat glioma cells in medium containing polyclonal measles antiserum resulted in the loss of detectable expression of all measles virus proteins. Removal of these cells from antiserum, however, led to a re-expression of virus proteins and the production of infectious virus. Cloning of antibody-modulated non-expressing cells in the presence of antiserum showed that re-expression of virus proteins was not due to an incomplete curing process following the addition of antiserum, as a large number of non-expressing cell clones developed the capacity to express measles virus antigen at different periods after removal of antiserum. Irradiation of persistently infected cells to give a non-growing culture showed that modulation was not mediated by a selection and outgrowth of a small percentage of non-expressing cells originally present in the culture. Antibody directed against C6 membrane proteins did not lead to modulation and it was also shown that only monoclonal antibodies with neutralizing activity could affect intracellular antigen expression. Immunoglobulin Fab fragments with neutralizing activity also had modulating activity. Although all modulated cell clones were more susceptible to homologous virus infection than control C6 cells, it was not possible to rescue any defective measles virus which may have been maintained in the culture.

INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a late complication of acute measles and occurs on average 8 to 10 years after the acute infection (ter Meulen et al., 1983). The basis for this slow virus disease is a persistent measles virus infection in brain cells which is thought to be established during the acute infection in conjunction with the immune response to measles virus. However, no information about the virus–brain cell interaction is available which would explain the long incubation period between acute measles and the onset of SSPE. Therefore, studies on persistent measles virus infections in neural tissue culture cells have recently been undertaken to evaluate the mechanisms of persistence, the effect of measles antibodies on these cultures and the impact of the virus infection on cellular functions (Halbach & Koschel, 1979; Koschel & Münzel, 1980; Miller & Carrigan, 1982; Münzel & Koschel, 1982; Barrett & Koschel, 1983; Yoshikawa & Yamanouchi, 1984).

It has been suggested by Joseph & Oldstone (1975) that antibodies directed against the virus proteins expressed on the cell surface could lead to a persistent state of the infection in which virus membrane antigens are not expressed and no infectious virus is released. Rustigian (1966) first demonstrated that a productive measles virus persistent infection of HeLa cells could be converted into a non-yielder state following cultivation of these cells in antiserum-containing medium. Despite the lack of infectious virus the majority of cells still contained intracellular

† Present address: Immuno AG, Uferstrasse 15, A-2304 Orth/Donau, Austria.
‡ Present address: University of Newcastle, Department of Virology, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP, U.K.
virus antigen. It has also been reported that antibodies directed to measles virus can strip virus-specific antigens from the cell surface, thus rendering infected cells resistant to killing by the cytotoxic immune response (Joseph & Oldstone, 1975). In addition, such antibodies can alter the expression of specific measles virus proteins inside the cell, and the virus matrix protein (M) was found to be particularly sensitive to this effect (Fujinami & Oldstone, 1979, 1980). It was suggested that alterations in the synthesis of these polypeptides might lead to the aberrations in measles virus matrix protein synthesis and consequently in particle maturation, features characteristic of persistent measles virus infection associated with SSPE (Hall & Choppin, 1979). Moreover, we reported recently that persistent measles (SSPE) virus infection of a central nervous system (CNS)-derived cell line (C6 rat glioma cells) resulted in impairment of signal transmission mediated by hormone receptors. This impairment, however, could be fully restored by antibody-induced modulation of viral antigens present in the cell membrane (Barrett & Koschel, 1983).

In the present study, we have examined the effect of anti-measles serum and monoclonal antibodies on a C6 rat glioma cell line persistently infected with an SSPE virus to determine whether antiserum could modify the course of infection in a neural cell line in culture. We describe here a sequence of events leading to a state of infection in which there is no detectable production of virus proteins or infectious virus. This was shown not to be due to antibody-induced selection of cured or non-producing cells. In addition, removal of antiserum could lead to the re-expression of virus proteins and the release of infectious particles. Attempts to modify other measles virus persistently infected cell lines of non-neural origin in a similar manner were unsuccessful.

**METHODS**

**Cells and virus.** The persistently infected cell lines used in this work were C6 rat glioma cells (ATCC CCL 107) infected with the SSPE virus Lec (C6/SSPE) derived by Halbach & Koschel (1979). Cells were cultivated in Dulbecco's MEM (DMEM) supplemented with 10% foetal calf serum (FCS) after splitting (1:3 to 1:4). The cells were passaged about each third or fourth day. Lu 106 cells persistently infected with measles virus Edmonston (CLu 106) derived by Norrby (1967) and Vero cells persistently infected with SSPE virus Lec (Lec-Pi) described by ter Meulen *et al.* (1981) were also used. These cell cultures were maintained in MEM supplemented with 10% FCS and regularly checked for membrane and intracellular viral antigens.

Antiserum treatment was performed using heat-inactivated serum obtained from human SSPE patients and diluted in DMEM containing 10% heat-inactivated FCS as previously described (Barrett & Koschel, 1983). Haemagglutination inhibition titres (HI) of the final dilution are stated in the text.

**Preparation of monoclonal antibodies.** Monoclonal antibodies directed against virus haemagglutinin were prepared as previously described (ter Meulen *et al.*, 1981). Cells secreting these antibodies were grown in 800 ml plastic tissue culture flasks under RPMI 1640 medium. Medium was harvested from these cells and immunoglobulins were concentrated by polyethylene glycol (PEG) precipitation at 4 °C. The HI titre was determined and the hybridoma fluids were diluted in DMEM plus 10% FCS. They were then filter-sterilized and used for antiserum treatment of the cell cultures. Antibodies from hybridomas which showed no haemagglutination inhibiting activity, but which reacted with measles virus haemagglutinin in an immune precipitation assay in combination with PAGE were used at equivalent protein concentrations.

**Fluorescent antibody staining for membrane and cytoplasmic viral antigen.** Cells to be tested for the presence of membrane-inserted antigen were gently harvested with 0.05% trypsin-EDTA and washed with cold phosphate-buffered saline (PBS). Staining was performed using hyperimmune rabbit anti-measles serum followed by fluorescein isothiocyanate (FITC)-conjugated anti-rabbit IgG. The percentage of cells displaying membrane fluorescence was then determined by u.v. microscopy. Cells expressing intracellular antigen were identified by immunofluorescent staining of acetone-fixed preparations. Staining with monoclonal antibodies was performed using acetone-fixed preparations, mouse ascites fluids and FITC-conjugated anti-mouse IgG. Membrane antigens were routinely fixed with 4% paraformaldehyde.

**Attempts to rescue virus antigen expression in C6/SSPE cell clones.** Cell fusion experiments utilized 5 × 10⁶ cells from each cell clone in suspension. These were fused to equal numbers of Vero cells using PEG-1500, and cultures were examined under the light microscope to ensure that fusion had occurred. Co-cultivation experiments were carried out by mixing 5 × 10⁶ cells from clones with an equal number of Vero cells. Temperature shift experiments were performed by incubation of cells at 33 °C. All cultures were examined weekly for the presence of antigen by immunofluorescence over a period of 2 months following the experimental procedure.
**Preparation of Fab fragments.** The immunoglobulin fraction of human SSPE serum was bound to a Protein A-Sepharose column in 0·1 M-phosphate buffer pH 7. Immunoglobulin G was then eluted in 0·1 M-glycine--HCl buffer pH 3, and immediately dialysed against phosphate buffer pH 7 (Langone, 1982). IgG was cleaved under the conditions described by Stanworth & Turner (1978) for mercuripapain with CM-cellulose-bound papain (Sigma) in phosphate buffer pH 7 containing 10 mM-cysteine and 2 mM-EDTA, for 18 h at 37°C. The enzyme was removed by centrifugation. An aliquot was analysed by SDS-PAGE to monitor the digestion. The supernatant of centrifugation was once more passed through a Protein A-Sepharose column to remove Fc components and uncleaved immunoglobulin. Fractions containing Fab fragments were identified by OD_{290} and by SDS-PAGE and pooled before H1 and neutralization titres (NT) were determined.

**Radiolabelling and immunoprecipitation of membrane antigens.** Cell monolayers were washed in PBS without Ca^{2+} and Mg^{2+} and dispersed with Tris-EDTA buffer (0·01 M-Tris-HCl, 0·14 M-NaCl, 0·05 mM-EDTA, pH 7·2). Cells were washed three times with PBS and finally resuspended in 1 ml PBS containing 10^{-5} M-potassium iodide. One unit lactoperoxidase (Sigma) was added, followed by 1 mCi 125I (Amersham Buchler). Ten μl of a 0·03% solution of H_2O_2 was then added, the mixture was agitated for 2 min, after which time fresh H_2O_2 was provided. This cycle was repeated once more, and cells were then washed twice in PBS. Cell proteins were then solubilized in a buffer containing 1% NP40 and 0.2% sodium deoxycholate and non-solubilized debris was removed by centrifugation at 15000 r.p.m. for 10 min. The supernatant was used for immunoprecipitation procedures as described by Hall et al. (1979).

**Gamma irradiation of C6/SSPE cells and measurement of cell division.** C6/SSPE cells were trypsinized, suspended in DMEM and divided into two parts in polypropylene test tubes (Falcon). One sample was then irradiated with 5000 rad 60Co γ radiation. Aliquots of irradiated and non-irradiated control C6/SSPE cells were seeded in duplicate into 3 cm diam. tissue culture Petri dishes (Nunc) for [3H]thymidine incorporation measurements, into 3 cm diam. tissue culture Petri dishes containing glass coverslips for immunofluorescence and into 30 ml vol. plastic tissue culture flasks (Nunc) for cell growth measurements.

After adhesion of the cells, the medium was removed and substituted by 3 ml fresh DMEM with 10% heat-inactivated FCS or DMEM with inactivated FCS and 50 HI units/ml measles antiserum. The percentage of cells displaying measles virus antigen in irradiated and control cells with and without measles antiserum was regularly monitored by immunofluorescence techniques as described above. Cell division was measured by counting cell numbers from the tissue culture flasks over a time period of 14 days and DNA synthesis was measured as 18 h [3H]thymidine incorporation (addition of [3H]thymidine at 2 Ci/mmol, 1 mCi per Petri dish 5 h after plating). These Petri dishes were then washed twice with PBS, cells were precipitated by 2 ml TCA (5%), collected on Whatman GF/C filters (25 mm diam.) and measured after drying in a Packard liquid scintillation counter (type 460 C) using toluol–POPOP/PPO mixture as scintillation fluid.

**RESULTS**

**Effect of SSPE serum on virus antigen expression in C6 cells**

C6/SSPE cells were grown in medium containing 50 HI units of SSPE patients' serum. After six passages in this medium, no measles virus membrane antigen could be detected in these cells by immunofluorescence techniques. Cell surface proteins were labelled at this time by the lactoperoxidase technique in order to confirm this observation. Antiserum was then removed from a duplicate culture which was passaged further. After four more passages there was a full recovery in membrane immunofluorescence and lactoperoxidase labelling was repeated. Proteins labelled in this manner were immunoprecipitated using rabbit hyperimmune anti-measles virus serum and separated on a 10% SDS–polyacrylamide gel (Fig. 1). This technique labelled both the haemagglutinin (H) and fusion (F) protein present in the parent C6/SSPE cell membranes, but these molecules were absent following antiserum treatment. After antiserum removal, both proteins could once more be detected.

Treatment of C6/SSPE cells with antiserum also decreases the expression of intracellular virus antigen (Barrett & Koschel, 1983). The influence of antibody concentration on this process was determined by maintenance of cultures in MEM containing appropriately diluted human SSPE serum. The percentage of fixed cells displaying fluorescence was then estimated. It was found that this effect was clearly dependent on the concentration of virus-directed antibody used (Fig. 2). The culture was cleared of intracellular virus antigen after six passages in medium containing 200 HI units/ml of antiserum, but required 15 passages in medium containing 3 HI units/ml for the same effect to be achieved. The recovery of virus-specific antigen expression was also examined. After 10 passages in antiserum (50 HI units/ml), C6/SSPE cells were negative in
Fig. 1. SDS-PAGE (10% acrylamide) of 125I-labelled viral membrane antigens isolated by immune precipitation, using rabbit hyperimmune anti-measles virus serum. The surface antigens were labelled in living cells by the lactoperoxidase method. Lane 1, C6/SSPE cells; lane 2, C6/SSPE cells after 10 passages treated with 50 HI units/ml antiviral serum; lane 3, C6/SSPE cells treated for 10 passages with antiviral serum which was then removed. Cells were grown 10 passages without antiviral serum. The three samples were labelled on the same day using identical conditions.

immunofluorescence tests for the expression of virus antigen. These cells were trypsinized and resuspended in medium lacking antiviral antibodies. The medium was assayed for the presence of released virus each day and the percentage of cells displaying intracellular virus-specific fluorescence was also measured (Table 1). Virus production was not detected until the 4th day after removal of antiserum, virus antigen was also observed at this time and both functions increased slowly over the following days. All of the cells were immunofluorescent 9 days after removal of the antiserum.

Attempts to induce antigenic modulation in other persistently infected cell lines

Persistently measles virus-infected Lu 106 (Clu 106) and SSPE virus-infected Vero cells (Vero-Lec) were grown in medium containing 50 HI units/ml of SSPE antiserum, and the percentage of cells displaying cytoplasmic and membrane antigen was measured by immunofluorescence 1 day after each passage. Both cell types showed a loss of virus antigen from the plasma membrane with kinetics similar to those observed using C6/SSPE cells, but we were unable to produce a culture in which internal virus antigen was absent, even after 25 passages in medium containing antiserum.
Persistent measles virus infection in C6 cells

Fig. 2. Influence of the HI titre of antiviral serum on antigenic modulation in C6/SSPE cells. HI titres (units/ml) were 200 (●), 100 (○), 12 (□) or 3 (△). Percent values were calculated from counting total cells in fixed preparations in visible light and counting the fluorescing cells in u.v. excitation for FITC labelling.

Table 1. Recovery of virus antigen expression and infectious virus release after stopping antiserum treatment

<table>
<thead>
<tr>
<th>Time after removal from antiserum (days)</th>
<th>Virus titre/cell (p.f.u./cell)</th>
<th>% Immunofluorescence-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.0023</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>0.0011</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>0.26</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>0.73</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>3.1</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>0.42</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>0.19</td>
<td>100</td>
</tr>
</tbody>
</table>

Effect of other agents on virus antigen expression

The possibility that measles virus antigen expression could be altered non-specifically following the binding of any antibody to the C6/SSPE cell membrane was investigated. C6/SSPE cells were grown in medium containing heat-inactivated antiserum raised against a C6 membrane preparation. This was found to bind to 100% of persistently infected or uninfected C6 cells by membrane fluorescence. Cells were grown in medium containing this antiserum for a period of 8 weeks, and virus antigen expression was regularly examined. During the experimental period, this treatment had no effect on SSPE virus protein expression. At the end of this period, 100% of C6/SSPE cells were still stained by this antibody in immunofluorescence tests.

It was also possible that the human serum used in this work contained interferon which was actually responsible for the modulation effects. To investigate this, C6/SSPE cells were grown in medium containing 100 U/ml of human β interferon. No alteration in the expression of virus antigens was observed. We also found that the human interferon was unable to confer any resistance to vesicular stomatitis virus (VSV) on the uninfected C6 parent cell line. Finally, the
antiserum-treated C6/SSPE cells were completely susceptible to infection by heterologous viruses (canine distemper virus, lymphocytic choriomeningitis virus of mice, VSV) and the sera used in this work were unable to induce any antiviral state in uninfected C6 cells. Consequently we conclude that interferon plays no role in the process of antigenic modulation.

Modulation of individual virus polypeptides

The rates with which the individual virus-specific proteins were affected by this modulation process were investigated by immunofluorescence using monoclonal antibodies specific for the H, N and M proteins, as well as hyperimmune serum. Although there was a rapid disappearance of H protein from the plasma membrane, no difference was observed between the percentage of cells stained with M and N monoclonal antibodies and with those stained by hyperimmune serum. Using these antibodies, we were therefore unable to demonstrate a preferential loss of any intracellularly located virus proteins (Fig. 3).

Mechanism of antibody-mediated alteration in virus protein expression

Walker (1964) has described the curing of persistent infections by growth in antiserum as a criterion for all persistent infections in which free virus plays a role in the maintenance of infection. Therefore, an obvious explanation for our findings might have been an incomplete curing process following addition of antiserum. To investigate this possibility, a culture of C6/SSPE cells was grown in medium containing SSPE serum (50 HI units/ml) for 15 passages. At this stage no virus antigen could be detected in the culture. The cells were then trypsinized, and subcloned by dilution into micro-well plates. Seeded wells were covered with medium containing SSPE antiserum (50 HI units/ml). In this way, 67 clones were obtained which were grown into larger cultures in the continued presence of antiserum. At this stage the cells were tested for measles-specific fluorescence and all 67 clones were found negative. Cells were then resuspended in medium without antiserum and examined regularly for expression of measles virus antigens. Four of the 67 clones spontaneously re-expressed virus after removal from antiserum. A further 20 clones developed the capacity to express virus antigen at later periods, ranging up to 9 months following removal of antiserum. Consequently, the cells which did not
Persistent measles virus infection in C6 cells

Table 2. Properties of monoclonal antibodies from mouse hybridomas against virus H protein and their effect on intracellular virus antigen expression

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>HI titre (1:X)</th>
<th>Neutralization of virus</th>
<th>Capping of measles virus antigens on cell surface</th>
<th>Effect on intracellular virus antigen expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC 32</td>
<td>2048</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NC 26</td>
<td>2048</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DW 15</td>
<td>256</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DW 17</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L 77</td>
<td>4096</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L 33</td>
<td>1024</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K 83</td>
<td>4096</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L 54</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K 53</td>
<td>256</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

express virus antigen nonetheless harboured the SSPE virus. Cells which did not re-express this virus could be induced to do so by other means (see below). Thus, the modulation effect was not mediated through any true curing process.

Modulation in γ-irradiated C6/SSPE cells

The possibility also existed that modulation was due to neutralization of virus released by virus-producing cells. Addition of antibodies could lead to the selection of virus antigen-free cells in the culture if they had a faster rate of division than cells containing virus antigen. To investigate this possibility, cell division was inhibited by irradiation (5000 rad). Cell division was measured by cell counting and by [3H]thymidine incorporation. There was no increase in cell numbers of irradiated cultures during the period of antibody modulation and [3H]thymidine incorporation was greatly reduced compared to unirradiated controls. The residual incorporation was assumed to be due to DNA repair processes. Both irradiated and control cultures maintained in medium containing measles antibodies (50 HI units/ml) displayed a loss of viral antigens with identical kinetics and measles virus antigen could not be detected in either culture after a period of 11 to 14 days. Therefore, modulation was not due to any selection and outgrowth of a faster-growing virus antigen-free cell population in the culture.

Modulation by monoclonal antibodies and monovalent Fab fragments

Attempts were made to determine which fraction of the measles virus antiserum was responsible for the regulation of intracellular virus antigen expression, using monoclonal antibodies directed against different virus proteins (H, N or M), or influencing different functions of the same protein (H). Only monoclonal antibodies directed against the haemagglutinin of measles virus were effective in modulation. A number of different monoclonal antibodies directed against the H protein have been obtained in our laboratory (ter Meulen et al., 1981; Carter et al., 1983). Antibodies were selected which were all known to bind to the membranes of cells persistently infected with SSPE virus Lec or to immunoprecipitate the H protein of this virus (ter Meulen et al., 1981; Carter et al., 1983). All of these antibodies were able to induce capping reactions on the C6/SSPE cell membrane and to strip membrane antigen from the surface of the cell; however, they differed in their effects on intracellular virus antigen expression (Table 2). All cell cultures grown in medium containing an antibody which could neutralize SSPE virus became negative for the expression of virus antigen after six passages in medium containing that antibody. However, the same protein concentration of monoclonal antibodies DW 173 and L 54, neither of which had neutralizing activity, had no detectable effect on internal antigen expression in these cells even after 15 passages in antibody-containing medium.

These experiments suggested that virus neutralization activity was more important than ability to cross-link antigens on the cell membrane. This possibility was further investigated using immunoglobulin Fab fragments prepared from SSPE patient serum as described in Methods. These preparations were found to retain HI and neutralization activities but failed to
Table 3. *Challenge of virus antigen-negative cell clones from 'down modulated' C6/SSPE cells and uninfected C6 cells with standard SSPE (Lec) virus (1 p.f.u./cell)*

<table>
<thead>
<tr>
<th>Clone no.</th>
<th>Days required for 100% infection after challenge detected by immunofluorescence</th>
<th>Development of viral antigens in parallel control cultures after 20 days</th>
<th>Time required for spontaneous development of viral antigens in continuously cultured cells (months)</th>
<th>Spontaneous development of viral antigens after freezing and thawing</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>21</td>
<td>0</td>
<td>− (5)*</td>
<td>NT†</td>
</tr>
<tr>
<td>6</td>
<td>17</td>
<td>0</td>
<td>+ (5)</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>0</td>
<td>− (12)</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>0</td>
<td>+ (9)</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>0</td>
<td>− (12)</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>0</td>
<td>+ (3)</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>0</td>
<td>+ (4)</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>14</td>
<td>0</td>
<td>+ (8)</td>
<td>+</td>
</tr>
</tbody>
</table>

C6 Only 5% positive after 56 days

* Numbers give the time (months) of observation.
† NT, Not tested.

cause capping of virus membrane antigens as described by Joseph & Oldstone (1974). The purity of the Fab preparations was confirmed by polyacrylamide gel electrophoresis and no uncleaved molecules could be detected. Persistently infected cells were grown in medium containing Fab fragments (25 HI units/ml) and the percentage of cells displaying intracellular virus antigen was determined at each passage. After five passages in medium containing these fragments, less than 5% of cells in the culture still displayed virus antigen and it was concluded that modulation had occurred.

*Attempts to rescue virus antigen expression in antiserum-treated C6/SSPE clones*

Physiological shock is sometimes able to induce the expression of SSPE virus (Carter et al., 1984). We therefore subjected 35 out of the 67 cell clones described above which had not spontaneously expressed virus antigens to a variety of treatments. From these 35 cell clones, 17 clones could be stimulated to produce virus antigen following resuscitation from liquid nitrogen storage, but 18 clones remained negative for virus antigen expression after this procedure, and were still negative at the end of the test period (9 months). Attempts made to stimulate virus production in the 35 long-term non-expressing clones by other physiological methods such as cell fusion, co-cultivation and temperature shift experiments as described in Methods, or growth in the absence of FCS but using newborn calf serum or lactalbumin hydrolysate, were unsuccessful. However, some clones found to be unresponsive to all of these procedures did later re-express virus antigen spontaneously, thus proving that they were indeed infected.

*Susceptibility of 'virus antigen-negative' cell clones to homologous virus infection*

Eight antigen-negative clones and uninfected C6 control cultures were challenged with 1 p.f.u./cell of standard SSPE (Lec) virus, and examined every 3 days for membrane fluorescence. The time required for 100% of cells to express virus antigen was then determined (Table 3). All clones were found to be more readily infected than control C6 cells. This could indicate that challenge with standard virus led to the stimulation or rescue of a defective measles virus which was maintained within the C6/SSPE cell clones. To investigate this possibility we attempted to rescue such a defective virus using u.v.-inactivated SSPE virus Lec as challenge virus. Measles SSPE antiserum-treated immunofluorescence-negative C6/SSPE cell clones, and Vero cells were inoculated with this irradiated virus preparation at a multiplicity of 0.01 to 5 p.f.u. equivalents/cell. The cultures were then examined for the development of c.p.e., and the medium for the presence of infectious virus. These experiments produced no evidence for reactivation of a latent virus over and above background multiplicity reactivation of the infecting u.v.-irradiated virus.
DISCUSSION

The experiments presented here describe the maintenance of a measles (SSPE/Lec) persistent infection in the CNS-derived C6 rat glioma cell line, in the absence of expression of any measles virus antigens. This state was brought about by the growth of the persistently infected cells in medium containing antibodies directed against measles virus antigens. Antibody has been previously reported to help establish (Gould & Linton, 1975), cure (Rima & Martin, 1977) or alter the characteristics of measles virus infection (Rustigian, 1966; Joseph & Oldstone, 1975). Rustigian (1966) reported that antiserum treatment of HeLa cells persistently infected with measles virus produced a culture which expressed little surface antigen but still displayed intracellular virus-specific fluorescence. After removal of antiserum from these cells, however, there was no recovery in the expression of virus proteins in the membrane, and no production of infectious virus, indicating that the virus population was now defective in some property involved in the maturation process. These cells were fully resistant to superinfection with homologous Edmonston virus. This report therefore differs from the present communication since we have found that the antiserum-induced C6/SSPE cell effect was fully reversible and that non-expressing cell clones were even more susceptible to infection with SSPE virus than the original C6 cells. This is surprising in view of the fact that most persistent infections are resistant to superinfection with homologous virus (Rima & Martin, 1977). However, one has to bear in mind that in untreated cultures of persistent infections, virus replication takes place with the expression of the major structural proteins and it is under these conditions that interference occurs.

Despite our inability to detect virus proteins in cultures modulated with the techniques described here it is clear that these cells were not simply cured and that re-expression following removal of antiserum was not due to spread of virus from a very small number of virus antigen-positive cells which had not been detected. The majority of cultures that were derived from cells cloned in the presence of antiserum either re-expressed virus spontaneously after the removal of antiserum or virus production could be stimulated by freezing and thawing. Furthermore, modulation was not due to selection and outgrowth of a small population of cells in the C6/SSPE culture which were already deficient in the expression of measles virus antigens. Antibody treatment of irradiated cells which did not divide resulted in a modulation with kinetics similar to that of dividing cells. This indicates clearly that modulation was a direct effect of an antibody reaction with every cell in the culture and was not the result of any selection or curing process. Moreover, intracellular antigenic modulation is clearly specific for the antigens against which the antibodies are raised and is dependent on the antibody concentration available for binding. There is no modulation of virus antigens if C6/SSPE cells are grown in serum containing antibodies against the C6 cell membrane antigens. These antibodies bind to the cell membrane but have no effect on virus antigen expression. This finding is in accordance with observations that antibodies binding to major histocompatibility antigens do not alter the expression of measles virus polypeptides in infected HeLa cells (Oldstone et al., 1983).

Fujinami and co-workers showed in a recent study that monoclonal antibodies to the haemagglutinin of measles virus decreased the expression of the phosphoprotein, fusion and membrane proteins of measles virus in the course of viral replication after binding to the haemagglutinin expressed on the surface of lysotypically infected HeLa cells, whereas monoclonal antibodies to the F protein were less effective (Fujinami et al., 1984). In the case of C6/SSPE cells, however, monoclonal antibodies to the H protein of measles virus were effective in blocking all intracellular viral protein synthesis provided these antibodies were reacting with an epitope on the H protein which elicits neutralizing antibodies. Antibodies bound to measles virus proteins inserted in the membrane at epitopes distinct from those involved in neutralization (Carter et al., 1982) are unable to initiate this reaction. Moreover, the phenomenon of capping and patching of viral antigens as described by Joseph & Oldstone (1974) is not an essential requirement of the modulation mechanism. Fab preparations which retain HI and neutralization activities but do not cause capping of viral membrane antigens were also able to interfere with the expression of intracellular antigens.

It is not clear by what mechanism antiviral antibody mediates such an effect of intracellular
viral replicative events. Certainly a transmembrane signal is provided which alters expression of different viral gene products inside the cell. It is conceivable that some structural rearrangements can occur following binding of the antibody to the specific portion of the measles virus H protein in cell membranes, leading to a transmembrane effect on virus RNA transcription. Possee et al. (1982) reported that antibody binding to influenza virus haemagglutinin (HA) protein exerted its neutralizing effect by inactivating the virion transcriptase activity. They proposed that this was brought about by an allosteric rearrangement of the HA molecule which could be transmitted across the virus envelope to the interior of the particle. We are currently examining such mechanisms with detailed studies on the regulation of intracellular virus RNA synthesis by different antibodies binding to viral antigens in the cell membrane.

The observation that measles antibodies induce modulation in persistently infected C6 cells is of particular interest since cells of non-neural origin did not reveal this phenomenon. Antibody binding to measles virus persistently infected Lu 106 cells and measles (SSPE-Lec) virus-infected Vero cells resulted in a stripping of the H and F proteins. However, prolonged maintenance of these cells in antibody-containing medium did not result in a total loss of intracellular viral antigens. This is in agreement with the reports on antibody treatment of persistently infected HeLa cells described by others (Rustigian, 1966; Joseph & Oldstone, 1975). However, it is clear that measles virus can be maintained in a cell line of CNS origin without detectable expression of virus proteins. This state can be stable in the presence of antibody but can also be spontaneously reversed. It is not known whether a process such as that described here is involved in pathogenesis in an intact organism, but such a process could permit maintenance of virus persistence within an infected host over a long period without elimination of infected cells by cytotoxic immune responses. Spontaneous re-expression of virus at a later stage could then permit virus spread or re-stimulation within the cell population as a whole, leading to the production of a CNS disease of delayed onset. Indeed, measles virus RNA has been detected in brain tissue from normal adults (Haase et al., 1981), and it has been suggested that persistent measles virus may be part of the normal human CNS virological fauna. Further experiments are necessary to characterize the state of virus expression in the persistent infection described here, and also in human brain in order to clarify this point.

We would like to thank Professor E. Wecker for many helpful discussions, Annette Rummel and Helga Sennefelder for excellent technical assistance and Helga Kriesinger for typing the manuscript. We are grateful to Professor Dr E. Günther, Max-Planck-Institut für Immunobiologie, Freiburg, F.R.G., for his generous gift of anti-C6 serum. This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 105 (Project C 3, K. Koschel) and 165 (V. ter Meulen).

REFERENCES


(Received 2 January 1985)