Characterization of the Hallé SSPE Measles Virus Isolate

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SUMMARY

The Hallé subacute sclerosing panencephalitis (SSPE) measles virus isolate and its plaque-purified progeny were investigated to determine whether any unusual properties could be associated with its ability to cause persistent infection. Three types of plaque-purified progeny were isolated. One population appeared to be similar in biological and biochemical properties to laboratory-adapted measles virus and had the ability to induce syncytia (syn⁺). A second population (syn⁻) plaqued more efficiently at 39 °C than at 33 °C, did not cause normal cell fusion at either temperature, and produced particles that interfered with the replication of other measles virus isolates in vivo and in vitro. This syn⁻ virus was further plaque-purified to eliminate the interfering particles, producing the syn⁻ P2 virus. This virus also plaqued more efficiently at 39 °C than at 33 °C, but caused cell fusion only at 39 °C. Both syn⁻ viruses and the parental virus were significantly less virulent in vivo than the syn⁺ virus and caused a more prolonged infection. Biochemical analysis showed that the syn⁻ P2 population produced particles that banded at two different densities in potassium tartrate gradients; both densities were less than those of the standard laboratory measles virus and the syn⁺ virus. Although the syn⁻ P2 virus did not cause cell fusion at 33 °C, [³⁵S]methionine labelling demonstrated that the haemolysin/cell fusion protein was present in syn⁻ P2 virions. The production of interfering particles, the inability to cause cell fusion at 33 °C, and the cold-sensitive nature of the syn⁻ population appear to play a role in the ability of the Hallé SSPE virus to establish persistent infection.

INTRODUCTION

Evidence for the involvement of measles virus as the aetiological agent in subacute sclerosing panencephalitis (SSPE) has been demonstrated by immunological techniques and isolation of virus from SSPE patients (for review, see Morgan & Rapp, 1977). However, it is presently unknown why measles virus has a predilection for establishing and maintaining persistent infection. To date, there is no common characteristic for SSPE isolates. Some isolates exhibit properties similar to laboratory-adapted strains of measles virus, whereas others appear to be defective in one or more functions (Morgan & Rapp, 1977).

Previous biochemical investigations of SSPE isolates demonstrated differences in the virus-coded M protein (Hall et al., 1978; Wild & Dugre, 1978) and virus mRNA (Hall & ter Meulen, 1976). Subsequent work by Hall et al. (1978) demonstrated minor differences in the size of the smallest mRNA of SSPE-infected cells compared with measles virus-infected cells. More recently, comparisons of the proteins of SSPE and measles virus isolates

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revealed differences in electrophoretic migration of the M protein of both groups as well as in the nucleocapsid and nucleocapsid-associated proteins (Hall et al., 1979; Rima et al., 1979). These observed heterogeneities do not appear to correspond to differences in either biological or biochemical properties of the isolates.

Production of defective-interfering particles may also play an important role in establishing and maintaining persistent infections in vitro and in vivo (Huang & Baltimore, 1970). Particles containing short nucleocapsids and subgenomic RNA have been detected in cells persistently infected with an SSPE virus (Kratzsch et al., 1977) and after undiluted passage of measles virus in Vero cells (Hall et al., 1974). It appears that generation or selection of defective variants of measles virus could play an important role in the aetiology of SSPE.

The Hallé SSPE measles virus isolate was originally isolated by co-cultivation of lymph node biopsy cells from an SSPE patient (Horta-Barbosa et al., 1971a) with HeLa cells. At very early passage levels, the Hallé virus caused rapid formation of syncytia and had high infectivity and haemagglutination (HA) titres (Horta-Barbosa et al., 1971b; Hamilton et al., 1973). After passage in cell culture, however, the virus was shown to readily establish persistent infection in different cell lines (Minagawa et al., 1976; Ecob-Johnston et al., 1977; Wild & Dugre, 1978). In the present study, we have further investigated the biological and biochemical properties of the Hallé virus and have isolated and characterized three subpopulations from the Hallé virus stock. Two of these subpopulations exhibited variant properties.

**METHODS**

**Cells and media.** BSC-1 cells, originally obtained from R. Dulbecco (La Jolla, Ca., U.S.A.) and Vero cells (Flow Laboratories) were grown in Eagle's medium in closed culture or in Medium 199 in Petri dishes in a 5% CO₂ atmosphere. Both media were supplemented with 10% foetal calf serum (FCS), 10% tryptose-phosphate broth, NaHCO₃ (1 mg/ml in closed culture; 2 mg/ml in a 5% CO₂ atmosphere), penicillin (100 units/ml), streptomycin (100 μg/ml), fungizone (1 μg/ml), mycostatin (10 units/ml), and 1 mM-L-arginine. BSC-1 cells were used for interferon induction and assay. Vero cells were used for virus assay.

**Viruses.** The Hallé SSPE virus isolate was originally obtained from lymph node biopsy cells of an SSPE patient (Horta-Barbosa et al., 1971a) following cocultivation with HeLa cells. Virus stocks that had been passaged twice in Vero cells and adapted to HeLa cells (Joseph et al., 1975) were kindly provided by M. Oldstone (La Jolla, Ca., U.S.A.). Plaque progeny were selected and tested for interferon induction as described previously (McKimm & Rapp, 1977b). The Indiana strain of vesicular stomatitis virus (VSV) was used for interferon assays. Derivation of the CC measles virus strain has been described by Haspel et al. (1973) and the HA-negative variant, HAN-12, has been described by Breschkin et al. (1977).

**Induction and assay of interferon.** Detailed methods have been published previously (McKimm & Rapp, 1977a). Briefly, fluids were harvested from test BSC-1 cultures, the virus was neutralized by the addition of measles antiserum, and interferon yields were assayed in duplicate in BSC-1 cells using VSV as the indicator virus.

**Interference in vitro.** Cells were inoculated with the HAN-12 strain of measles virus at a multiplicity of infection (m.o.i.) of 1. After adsorption for 1 h at room temperature, the cells were washed with tris buffer and inoculated with the challenge virus at an m.o.i. of 1 or 0.01. After a further adsorption period of 1 h, the cells were washed, medium was added, and the cultures were incubated at the required temperature. Cells were harvested when they demonstrated maximum cytopathic effects (c.p.e.). Progeny virus was plaqued at 33 °C and 39 °C and assayed for HA activity. Cells infected with the progeny virus were also tested for haemadsorption.

**Animal studies.** To investigate interference in vivo and to study neurovirulence, newborn
hamsters were inoculated intracranially with 0.05 ml containing $3 \times 10^4$ p.f.u. of virus. Mortality was recorded from 4 days post-inoculation. For virus rescue, brains were removed and extruded through a 26-gauge needle into 2.5 ml medium. This cell suspension was used for co-cultivation with BSC-1 cells or was sonicated, centrifuged to remove cell debris, and assayed for cell-free virus.

**Virus purification and $^{35}$S]methionine labelling.** BSC-1 cells grown in 150-mm plastic Petri dishes were infected with the appropriate virus. The syn$^+$ virus was inoculated at an m.o.i. of 0.4 at 33 °C and cells were labelled from 48 to 72 h. The syn$^-$ P2 virus was inoculated at an m.o.i. of 2 to 5 at 33 °C. Cells were labelled from 2 to 4 days (early label) or 4 to 6 days (late label) post-infection. After early labelling, medium was removed and replaced with fresh cold medium, and virus was then harvested again on day 6. At 39 °C, cells were infected with syn$^-$ P2 at an m.o.i. of 0.1 and labelled from 36 to 72 h. The CC virus was labelled as described by Breschkin *et al.* (1977). Fluids were removed and virus was purified according to the procedure described by Hall & Martin (1973). Cells infected with both viruses were labelled with 1 $\mu$Ci/ml $^{35}$S]methionine (500 Ci/mmol; Amersham/Searle) in Eagle's medium containing 3 $\mu$g/ml methionine (10% normal concentration). Purified virus was dissolved in electrophoresis sample buffer. Prior to electrophoresis, samples were heated to 100 °C for 2 min.

**Haemadsorption and HA assays.** For haemadsorption of infected cultures, the medium was removed and the cultures were flooded with a 0.5% suspension of African green monkey erythrocytes (Flow Laboratories). After incubation at 37 °C for 1 h, cultures were washed with pre-warmed tris or phosphate-buffered saline (PBS). For HA assays, serial twofold dilutions of virus were made in PBS buffer in plastic microtitre plates. An equal volume of erythrocytes was added and the plates were incubated for approx. 1 h. The HA titre was the reciprocal of the highest dilution showing 50% HA.

**RESULTS**

**Properties of the Hallé virus and plaque progeny**

The Hallé parental virus induced interferon very efficiently at an m.o.i. of 0.01 compared with other measles virus isolates (McKimm & Rapp, 1977b); however, there was little increase in the level of interferon induced with increasing m.o.i. Virus yield and syncytia formation decreased with an increase in m.o.i. This inhibition could not be due to growth inhibition by induced interferon since the same amount of interferon was induced at the lower m.o.i. These observations suggest the presence of particles in the parental stock that were not fully infectious, but could contribute to interferon induction and might interfere with replication of infectious virus.

Plaque progeny from the Hallé virus parental stock were then selected and tested for interferon induction according to the procedure described by McKimm & Rapp (1977b). Of the plaque progeny isolated, the first (syn$^+$) caused rapid syncytia formation and cell death within 48 h at 37 °C but induced little interferon. The second type (syn$^-$) produced no apparent c.p.e. at high multiplicities, and only rare isolated syncytia at a low m.o.i. This virus was initially detected only because interferon was released into the supernatant fluids, thus suggesting some virus replication had occurred. These variants were shown to be measles isolates and not another contaminating virus since their infectivity was neutralized by the addition of measles antiserum. Interferon induction, virus yields and c.p.e. of the two isolates and the parental stock were then compared (Table 1).

The low levels of interferon induced by the syn$^+$ virus may be related to loss of interferon-inducing ability observed in other measles viruses after plaque purification (McKimm & Rapp, 1977b). Increase in m.o.i. of syn$^-$ caused a dramatic reduction in virus yield. However,
Table 1. Interferon, virus yields and c.p.e. of plaque progeny of the Hallé SSPE isolate

<table>
<thead>
<tr>
<th>Virus</th>
<th>M.o.i.</th>
<th>Virus yield* (p.f.u./ml)</th>
<th>Interferon yield (PRD&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Syncytia‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hallé</td>
<td>0-5</td>
<td>3 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>160</td>
<td>-</td>
</tr>
<tr>
<td>parental</td>
<td>0-1</td>
<td>1 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>160</td>
<td>+</td>
</tr>
<tr>
<td>syn&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0-01</td>
<td>1 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>80-160</td>
<td>++ +</td>
</tr>
<tr>
<td>syn&lt;sup&gt;+&lt;/sup&gt;</td>
<td>10-0</td>
<td>1 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>40</td>
<td>+++ +</td>
</tr>
<tr>
<td>syn&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1-0</td>
<td>2 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>20-40</td>
<td>+++ +</td>
</tr>
<tr>
<td>syn&lt;sup&gt;+&lt;/sup&gt;</td>
<td>0-1</td>
<td>1 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>5</td>
<td>+++ +</td>
</tr>
<tr>
<td>syn&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1-0</td>
<td>1 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>160-320</td>
<td>-</td>
</tr>
<tr>
<td>syn&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0-5</td>
<td>4 x 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>syn&lt;sup&gt;-&lt;/sup&gt;</td>
<td>0-05</td>
<td>2 x 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>40</td>
<td>+</td>
</tr>
</tbody>
</table>

* Cells were harvested between 48 and 60 h post-infection; virus was plaqued at 37 °C.
† One PRD<sub>50</sub> is the amount of interferon that reduces the VSV plaque count of the control plates by 50%.
‡ --, No syncytia; +, 25% of culture showed syncytia; +++, 75% of culture demonstrated syncytia; +++++, 100% of culture demonstrated syncytia.

plaque efficiency at 39 °C was 100-fold greater than at 33 °C, producing much larger plaques at 39 °C. The syn<sup>-</sup> virus might, therefore, be cold-sensitive.

To remove interfering particles, the syn<sup>-</sup> virus was further plaque-purified, yielding the syn<sup>-</sup> P2 stock. Growth properties of the syn<sup>+</sup>, syn<sup>-</sup> and syn<sup>-</sup> P2 viruses were compared at 33 °C and 39 °C, using an m.o.i. of 0-1 (Fig. 1). Cells were harvested for virus titrations until the monolayer demonstrated 100% c.p.e. or until cells began sloughing in large numbers. Progeny were assayed at both 33 °C and 39 °C. With all three viruses c.p.e. and cell destruction appeared faster when virus was grown at 39 °C than at 33 °C, which probably accounts for the lower syn<sup>+</sup> and syn<sup>-</sup> P2 virus yields obtained at 39 °C. The syn<sup>+</sup> virus exhibited rapid syncytia formation at both temperatures. The syn<sup>-</sup> P2 virus caused spreading syncytia at 39 °C, and primarily swollen and rounded cells with only occasional syncytia at 33 °C; the syn<sup>-</sup> virus produced only isolated syncytia at 39 °C and none at 33 °C.

When virus yields were compared (Fig. 1), the highest titres were obtained with the syn<sup>-</sup> P2 virus at 33 °C. This correlates with the lack of cell destruction at this temperature and elimination of interfering particles after plaque purification of the syn<sup>-</sup> virus. When plaquing efficiency of the progeny was compared, the syn<sup>+</sup> progeny plaqued equally well at both temperatures. In contrast, progeny of both syn<sup>-</sup> viruses grown at either temperature plaqued approx. 100-fold higher at 39 °C than at 33 °C. The syn<sup>-</sup> virus was strongly positive for HA and syn<sup>-</sup>-infected cells were haemadsorption-positive. HAN-12, which grew as efficiently as the standard laboratory CC virus and was haemadsorption-negative, was, therefore, used to test for interference. Pronounced homologous interference occurred between the syn<sup>-</sup> virus and both the HAN-12 and CC strains of measles virus, especially at 39 °C (data not shown).

**Neurovirulence and interference in vivo**

The possible relationship of these plaque isolates to the aetiology of SSPE was investigated by studying neurovirulence in newborn hamsters. Animals were inoculated intracranially with the syn<sup>+</sup>, syn<sup>-</sup>, syn<sup>-</sup> P2 or Hallé parental virus; interference was also investigated by co-infection of hamsters with the syn<sup>+</sup> and syn<sup>-</sup> viruses (Fig. 2). Mortalities caused by inoculation of syn<sup>-</sup> P2 and Hallé viruses were similar to those of the syn<sup>-</sup> virus and are not shown. The syn<sup>+</sup>-inoculated animals demonstrated signs of acute encephalitis and rapid death. Mortalities in the syn<sup>-</sup>-inoculated animals were significantly greater than in all groups. The syn<sup>+</sup>-inoculated animals that survived to 20 days post-inoculation remained alive for the duration of the experiment. In the syn<sup>-</sup>-inoculated group, infection followed a more pro-
**Halé SSPE variants**

Fig. 1. Growth curves of syn⁺, syn⁻ and syn⁻ P2 viruses. BSC-1 cells were infected with an m.o.i. of 0.1. Results represent the average of two experiments. (a) Virus grown at 33 °C and plaqued at 33 °C (●) and 39 °C (□); (b) Virus grown at 39 °C and plaqued at 33 °C (●) and 39 °C (□). Growth was assayed for each virus until most of the cell monolayer was sloughing.

A longed course; animals died up to 10 to 12 weeks post-inoculation. These animals showed signs of neurological involvement, but it was less severe than in syn⁺-inoculated animals. This pattern of infection may be more related to the slow SSPE disease process.

Animals inoculated with a combination of both viruses showed a decrease in mortality rate and total mortality (60%) compared with animals inoculated with the syn⁺ virus alone (85%) even though they actually received twice the dose of virus. Interferon induction did not play a significant role in protection since interferon was not detected in the blood or organs of infected animals. The syn⁻ virus can, therefore, interfere with the replication of other measles viruses not only *in vitro* but also *in vivo*, altering the pattern of infection from an acute to a more chronic infection.

**Virus isolation from infected animals**

Virus was isolated from infected animals either directly by plaquing cell-free supernatants of sonicated brain suspensions or by co-cultivating brain tissue with BSC-1 cells. Two
Fig. 2. Mortality curves of syn+ (○), syn− (●) and syn+/syn− (△) co-inoculated newborn hamsters. Results represent the average of at least two independent experiments in which a total of 71 syn+, 59 syn− and 45 syn+/syn− animals were inoculated with 3 × 10⁴ p.f.u. of each virus.

Table 2. Isolation of virus from brains of hamsters inoculated with syn+, syn−, or co-inoculated with syn+ and syn− viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Plaque morphology (day 9);</th>
<th>Direct*</th>
<th>Co-cultivated†</th>
</tr>
</thead>
<tbody>
<tr>
<td>syn+</td>
<td>Large, clear</td>
<td>6 × 10⁴</td>
<td>ND</td>
</tr>
<tr>
<td>syn−</td>
<td>Small, irregular</td>
<td>&lt;10¹</td>
<td>&gt;10⁴</td>
</tr>
<tr>
<td>syn+/syn−§</td>
<td>Large, clear</td>
<td>&gt;10⁵</td>
<td>&lt;10¹</td>
</tr>
<tr>
<td></td>
<td>Smaller, irregular</td>
<td>&gt;10⁵</td>
<td>&gt;10⁵</td>
</tr>
</tbody>
</table>

* Cell-free supernatants of brain extracts were titred for virus and are expressed as p.f.u./ml. Brains were all suspended in 2.5 ml media.
† Brain tissue suspensions were co-cultivated with BSC-1 cells which were subsequently titred for virus. Yields are expressed as p.f.u./ml.
§ ND, Not done.
§ Co-inoculated animals.

animals from each group were sacrificed and examined for virus on days 9 and 14 each time the experiments were performed (Table 2). By 14 days post-inoculation, virus could still be isolated directly from brains of syn+ inoculated animals. Co-cultivation of brain suspensions was necessary to recover virus from the syn− and syn+/syn− inoculated animals. Virus isolated early in infection from the co-inoculated animals formed large, clear plaques. However, virus rescued at day 14 from these animals formed smaller more irregular plaques. Plaque morphology suggests that during early infection, predominantly syn+ virus was replicating. Later in infection, the smaller, irregular plaques suggest that the syn− virus was the predominant species.

DISCUSSION

Production of defective or variant populations is thought to be important in the persistence of measles virus. Biochemical studies have demonstrated differences in SSPE genomes (Hall & ter Meulen, 1976), polypeptides (Hall et al., 1978; Wechsler & Fields, 1978), and mRNA
Hallé SSPE variants

(Hall et al., 1978). However, this heterogeneity has subsequently been shown to exist in several measles isolates as well as in SSPE isolates (Hall et al., 1979; Rima et al., 1979). The isolation of defective particles containing smaller nucleocapsids and subgenomic RNA from persistently infected cells has also been described (Hall et al., 1974; Kiley & Payne, 1974; Kratzsch et al., 1977).

Our studies revealed that the Hallé SSPE measles virus isolate was composed of two stable plaque variants: (i) syn+, which was similar to laboratory-adapted measles virus, and (ii) syn-, which plaqued and formed syncytia more efficiently at 39 °C than at 33 °C and also produced interfering particles. To eliminate these interfering particles, the syn- stock was further plaque-purified, producing the syn- P2 stock. These three populations were characterized biologically and biochemically to determine their contributions to the properties of the parental Hallé virus and their possible relationship to the aetiology of SSPE.

In vitro, the syn+ virus grew rapidly in BSC-1 cells at 33 °C and 39 °C, forming syncytia and plaquing equally well at both temperatures. Biochemical analysis showed that this virus was similar to the standard CC laboratory measles virus. In vivo this virus was highly virulent, causing acute encephalitis and rapid death.

The syn- and syn- P2 viruses exhibited cold-sensitive properties, i.e. more efficient plaquing at 39 °C than at 33 °C and no syncytia formation at 33 °C. Biochemical analysis showed that the cell fusion/haemolysin protein was present in the syn- P2 virus grown at both temperatures. Furthermore, the syn- P2 virus demonstrated haemolytic activity in vitro. Thus, although the haemolysin/cell fusion protein was present, its fusion activity was not expressed in infected cells at 33 °C. The inability of the syn- viruses to cause normal cell fusion might be due to a conformational defect in the cell fusion protein, rather than to a defect in synthesis. Such a conformational defect could still allow the expression of haemolytic activity but not of cell fusion.

In addition to a defect in cell fusion, the syn- virus produced particles that interfered with the replication of other measles virus isolates in vitro and in vivo. Reductions in virus yields were shown when BSC-1 cells were co-infected with the syn- virus and either the CC or HAN-12 viruses. Greater reductions in yields were obtained when a higher m.o.i. of syn- was used. In vivo the Hallé parental, syn-, and syn- P2 viruses caused more protracted courses of infection and lower mortality rates than the syn+ virus. Mortality rates of animals co-inoculated with the syn- and syn+ viruses were reduced compared with animals inoculated with the syn+ virus alone. These results suggest that production of interfering particles is associated with the prolonged course of disease. The possibility that the defect in cell fusion also affects virulence cannot be ruled out. We currently favour the hypothesis that both factors act in conjunction to alter the nature of the infection.

Further biochemical studies of the syn- P2 virus demonstrated that virus particles of two different density classes were produced late in the infectious cycle at 33 °C, but only of one density at 39 °C. The denser H band showed 10- to 100-fold higher infectivity than the L band, yet the L band demonstrated a four- to eightfold higher HA titre (data not shown). These results indicate that the lighter fraction contained particles which were defective in infectivity, but able to demonstrate HA activity. Whether the tendency of the syn- to produce defective particles is related to a cell fusion defect and the prolonged course of infection in cell culture and newborn hamsters is still unknown. Production of defective particles by this Hallé SSPE virus further implicates genetic variants and defective measles virus particles as aetiological agents of SSPE.

Other groups have studied the properties of the Hallé SSPE virus, especially with regard to its tendency to establish persistent infections (Minagawa et al., 1976; Ecob-Johnston et al., 1977; Lucas et al., 1978; Wild & Dugre, 1978). These investigators did not report the heterogeneity of the Hallé virus we have described. Our results clearly indicate that different...
culture conditions for propagating virus (particularly m.o.i.) significantly alter the proportion of syn* and defective particles. Caution should, therefore, be exercised when examining isolates of viruses which have been adapted to cell culture.

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REFERENCES


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