Role of Virus Variants and Cells in Maintenance of Persistent Infection by Measles Virus

LINDA E. FISHER† AND FRED RAPP*

Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

Received for publication 28 September 1978

Hamster embryo fibroblasts persistently infected with a derivative of the Schwarz vaccine strain of measles virus spontaneously released virus particles with an average buoyant density considerably lower than that of the parental virus. The released virus contained all of the measles virus structural proteins and interfered with replication of standard virus. All of the virus structural proteins were associated with a membrane-free cytoplasmic extract from the persistently infected cells. Membrane-free cytoplasmic extracts prepared from Vero cells lytically infected with Schwarz strain measles contained little or no virus envelope structural protein. Maintenance of persistent infection may involve both the presence of virus variants and a defect in the ability of the infected cell to replicate the virus efficiently.

Variant viruses, defective virus particles (5), temperature-sensitive (ts) virus mutants (10), and host cell factors (14) have been implicated in the establishment and maintenance of persistent infection in vitro. Defective interfering particles have been isolated from a number of persistently infected cells (5). Hall et al. (2) and Rima et al. (12) were able to establish persistent infections in Vero cells infected with measles virus stocks containing defective interfering particles. Rima et al. (12) found that the virus released at passage 43 from persistently infected cells was similar to the virus originally used to infect the cells. Other evidence indicates that ts virus variants may be selected during the course of persistent infection (10). Knight et al. (6) established a culture of hamster embryo fibroblasts (HEF) persistently infected with Schwarz strain measles virus (S cells). These cells spontaneously began to release small amounts of ts virus at 37°C after 18 cell passages. At passages later than 45, however, the ts trait was lost. Gould and Linton (1) described HEp2 cells persistently infected with measles which released virus capable of replicating in Vero cells at temperatures between 32 and 39°C but not at 39.5°C and above.

Wild and Dugre (14) established a line of BGM (African green monkey) cells infected with the Hallé measles isolate from a patient with subacute sclerosing panencephalitis. In their system, neither defective interfering nor ts variants appear to be present. They therefore concluded that a host cell factor plays the major role in restriction of virus replication.

As a consequence of lytic infection, virus is produced possessing six structural polypeptides with relative molecular weights between 37,000 and 80,000 (9). The 80,000-dalton polypeptide, designated G, is an envelope-associated glycoprotein. The major protein species in the purified virion is the nucleocapsid protein (NP). With different strains of measles virus the relative molecular weight of the NP varies from 60,000 to 62,000. Associated with the NP is VP2 (70,000 daltons) (9). Under reducing conditions, the envelope protein VP5 (hemolysin, fusion factor) migrates as a nonglycosylated protein (3). The smallest polypeptide (M), by analogy with other paramyxoviruses, is a nonglycosylated membrane component. The function of VP4 is unknown. There is considerable variation from strain to strain and among different preparations of the same strain in the amount of VP4 detected (9). In the cytoplasmic fraction (cell lysate with nuclei removed by pelleting) of cells lytically infected with measles virus, Wechsler and Fields (13) detected the six viral structural proteins and, in addition, a structural protein (L) with a molecular weight of 200,000 and two nonstructural, virus-induced proteins.

Until now no attempt has been made to compare the specific virus structural proteins synthesized by persistently and lytically infected cells. In this communication we characterize the virus spontaneously released from persistently infected HEF cells and investigate the expres-
sion of virus structural proteins within persistently and lytically infected cells.

MATERIALS AND METHODS

Cells. Vero cells (Flow Laboratories) were grown in glass roller bottles in medium 199 supplemented with 5% fetal calf serum, 10% tryptose phosphate broth, and 2 mM l-arginine. S cells (measles-infected HEF cells) were developed by Knight et al. (6). The cells were maintained in glass prescription bottles in Eagle's minimum essential medium as described previously (6). All experiments described here were carried out between passages 89 and 95 of the S cells. HEF cells were used as uninfected controls and were prepared from 13-day-old Syrian hamster embryos. The cells were passaged at confluence and used as secondary cultures.

Virus. Schwarz vaccine strain of measles was grown in Vero cells. The virus was plaque-purified and passaged at a low multiplicity of infection. Subacute sclerosing panencephalitis virus Hallé strain labeled with [35S]methionine was used as marker virus. Virus titers were determined by plaque titration in Vero cells as described by Rapp (11).

Virus purification. The medium was removed from persistently or lytically infected cells and clarified by pelleting cells at 1,000 × g. The virus was pelleted from the supernatant fluid by centrifugation at 30,000 rpm for 1.5 h (Beckman 60 Ti rotor). The virus was resuspended in TE buffer (0.005 M Tris, pH 7.4, 0.001 M EDTA) and layered on a 15 to 40% (wt/wt) potassium tartrate (in TE buffer) gradient. Virus was sedimented at 39,000 rpm with a Beckman SW41 rotor for 1.5 h (for virus purification) or for 16 to 18 h (for density determination). Fractions (12 drops) were collected by puncture of the centrifuge tube.

Preparation of membrane-free cytoplasmic supernatant. Two 150-mm plastic plates each of S cells, Schwarz strain-infected Vero cells, and secondary HEF cells were prepared. S cells and HEF cells were labeled at 24 to 48 h after cell passage, and infected Vero cells (multiplicity of infection, 0.1) were labeled at 20 to 38 h after infection with 250 μCi of [35S]methionine in 10 ml of normal maintenance medium. The medium was removed, and the cells were scraped into Tris-buffered saline and washed once by pelleting. Membrane-free cytoplasm was obtained as described by Knipe et al. (7).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All samples were run on 8% resolving slab gels with 3% stacking gels by the method of Laemmli and Favre (8). Gels were subjected to electrophoresis at 12 mA until the samples had completely entered the stacking gel, at which time the current was increased to 24 mA. Total running time was 5.5 to 6 h.

RESULTS

Spontaneous release of virus from S cells. To study the role of the virus in the maintenance of persistent infection, we first determined the amount of virus released from the cells. Medium was removed from 150-mm culture dishes that had been seeded with approximately 5 × 10⁶ S cells and assayed for cell-free virus as described above. The results of a representative assay are shown in Table 1. Virus was released continuously by the S cells; most cell-free virus was produced early after cell passage. Nearly equal amounts of virus were released within the first 4 h after passage and between 28 and 52 h. Virus released from the cells between 4 and 28 h (early) or between 28 and 52 h (late) after cell passage was partially purified and subjected to electrophoresis as described above. In Fig. 1, the early-released (lane a) and late-released (lane b) S-cell viruses are compared with purified measles virus (lane c). Virus particles released from the S cells possessed all of the normal measles virus properties.

<table>
<thead>
<tr>
<th>Time after passage (h)</th>
<th>Virus released at 37°C (PFU)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>3.18 × 10⁶</td>
</tr>
<tr>
<td>0 to 28</td>
<td>1.89 × 10⁶</td>
</tr>
<tr>
<td>28 to 52</td>
<td>3.52 × 10⁶</td>
</tr>
</tbody>
</table>

*Total virus released from approximately 5 × 10⁶ S cells at passage 95.

Fig. 1. Virus released from S cells labeled with [35S]methionine between 4 and 28 or 28 and 52 h after cell passage was partially purified and subjected to electrophoresis as described in the text. (a) S virus released 4 to 28 h after cell passage; (b) S virus released 28 to 52 h after cell passage; (c) Hallé parental virus.
virus structural proteins, although much less of some of the proteins was detectable in this virus than was present in the laboratory virus strain.

Buoyant densities of particles released from S cells. Virus released early and late after cell passage from [35S]methionine-labeled S cells was sedimented through 15 to 40% (wt/wt) potassium tartrate as described above. The results are shown in Fig. 2. Virus harvested early after cell passage (4 to 28 h) contained two components. The major component bands had an average buoyant density of 1.21 g/cm³; the minor component had an average buoyant density of 1.17 g/cm³. Virus harvested late (28 to 52 h) had an average buoyant density of 1.20 g/cm³. Both of these virus isolates had densities considerably lower than those of laboratory strains of measles virus (average buoyant density, 1.24 g/cm³).

S-cell virus interference. A population of low-density virus particles is suggestive of the presence of defective interfering particles. If this is the case, the preparation should contain a large proportion of noninfectious particles and interfere with the replication of measles virus. Pools were made from early- and late-harvested S-cell virus. The virus was processed for purification, and gradient fractions were assayed for plaque-forming ability (infectious particles) and hemagglutination activity (both infectious and noninfectious particles). As Table 2 shows, the expected ratio of hemagglutination units (HAU) to PFU in an infectious virus preparation was approximately 10⁴. Several of the S-cell virus gradient fractions (SL6, SE7, and SE9, for example) had higher ratios of HAU to PFU, indicating that they contained a large proportion of noninfectious particles.

Defective interfering particles by definition are noninfectious viruses having normal structural proteins, buoyant densities lower than standard virus, and the ability to interfere with the replication of closely related viruses. The gradient fractions containing a high proportion of noninfectious particles were assayed for production of virus against the Schwarz vaccine strain of measles and other S-cell virus gradient fractions with normal and near-normal ratios of HAU to PFU. In each case, the observed yield of virus was reduced 2 to 4 log₁₀ compared with the expected additive yield (Table 3).

Expression of structural proteins in persistently and lytically infected cells. From the previous experiments it seems clear that defective interfering virus particles are produced by S cells. We were interested in determining

![Fig. 2. Virus released from S cells was processed as described in the legend to Fig. 1. The virus was sedimented through 15 to 40% (wt/wt) potassium tartrate at 39,000 rpm (SW41) for 18 h and fractionated as described in the text. Symbols: O, virus released between 4 and 28 h; •, virus released between 28 and 52 h; □, virus gradient density.](http://jvi.asm.org/)

**TABLE 2. Presence of noninfectious particles in virus from persistently infected cells**

<table>
<thead>
<tr>
<th>Virus fraction</th>
<th>PFU/ml</th>
<th>HAU/ml</th>
<th>HAU/PFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwarz</td>
<td>1.80 × 10⁷</td>
<td>32</td>
<td>1.78 × 10⁻⁶</td>
</tr>
<tr>
<td>SE7⁻</td>
<td>1.95 × 10⁷</td>
<td>2</td>
<td>1.03 × 10⁻⁵</td>
</tr>
<tr>
<td>SE8</td>
<td>4.20 × 10⁶</td>
<td>8</td>
<td>1.90 × 10⁻⁵</td>
</tr>
<tr>
<td>SE9</td>
<td>2.74 × 10⁶</td>
<td>4</td>
<td>1.46 × 10⁻⁵</td>
</tr>
<tr>
<td>SL6⁻</td>
<td>7.10 × 10⁵</td>
<td>2</td>
<td>2.82 × 10⁻⁴</td>
</tr>
<tr>
<td>SL7</td>
<td>1.90 × 10⁵</td>
<td>16</td>
<td>8.42 × 10⁻⁶</td>
</tr>
<tr>
<td>SL8</td>
<td>6.80 × 10⁴</td>
<td>16</td>
<td>2.35 × 10⁻⁵</td>
</tr>
</tbody>
</table>

* Virus was titrated for agglutination of African green monkey erythrocytes at 37°C for 1 h.
* Gradient fractions from S-cell virus harvested up to 28 h (early) or between 28 and 52 h (late) after cell passage.

**TABLE 3. Interference by measles virus from persistently infected cells with virus replication**

<table>
<thead>
<tr>
<th>Virus fraction</th>
<th>Expected yield (PFU/well)</th>
<th>Observed yield (PFU/well)*</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwarz-SL6</td>
<td>2.47 × 10⁶</td>
<td>1.47 × 10²</td>
<td>99.9</td>
</tr>
<tr>
<td>Schwarz-SL8</td>
<td>2.49 × 10⁶</td>
<td>1.86 × 10⁴</td>
<td>99.2</td>
</tr>
<tr>
<td>SE7-SL7</td>
<td>1.26 × 10⁶</td>
<td>3.40 × 10²</td>
<td>99.7</td>
</tr>
<tr>
<td>SE8-SL6</td>
<td>1.96 × 10⁴</td>
<td>1.65 × 10²</td>
<td>99.2</td>
</tr>
<tr>
<td>SE8-SL8</td>
<td>4.03 × 10⁴</td>
<td>3.58 × 10³</td>
<td>91.1</td>
</tr>
<tr>
<td>SE9-SL7</td>
<td>1.26 × 10⁴</td>
<td>2.71 × 10³</td>
<td>99.8</td>
</tr>
<tr>
<td>SE9-SL8</td>
<td>2.99 × 10⁴</td>
<td>2.94 × 10³</td>
<td>99.9</td>
</tr>
</tbody>
</table>

* Multiwell 16-mm culture dishes were inoculated with a mixture of 0.16 HAU of each virus. After 48 h at 37°C, virus yield was determined by plaque titration.
whether there was also some difference in expression of virus structural proteins in persistently infected S cells compared with cells lytically infected with the same virus strain used in the initial infection of the S cells. Membrane-free cytoplasmic extracts were prepared and subjected to electrophoresis as described above to compare uninfected secondary HEF cells, S cells, and Vero cells infected with Schwarz strain measles. Structural proteins which are part of the virus envelope (G [the hemagglutinin], VP5 [the hemolysin or fusion factor], and M [the matrix protein]) were greatly reduced in amount or absent from the cytoplasm of lytically infected cells (Fig. 3b). In contrast, these virus structural polypeptides along with the NP remained in the soluble cell cytoplasm (Fig. 3d) of the persistently infected cells. Figures 3a and c are electrophoretic patterns of the purified virus marker and membrane-free cytoplasm from uninfected secondary HEF cells, respectively. Additional experiments showed that in lytic infection, all of the virion polypeptides became membrane associated, whereas in persistent infection, little or no virus envelope structural proteins were membrane associated (Fisher and Rapp, unpublished data).

**DISCUSSION**

Persistently infected hamster cells release virus particles that possess the normal complement of measles virus structural proteins. The released virus, however, contains a large number of noninfectious particles that interfere with virus replication. The particles have an average buoyant density much lower than that of standard measles virus. Whereas cytoplasmic extracts from Vero cells lytically infected with Schwarz strain of measles virus (the same strain used originally to produce the persistently infected cells) contain little or no detectable virus envelope structural proteins, cytoplasmic extracts from S cells contain all of the major structural polypeptides. All of the viral polypeptides in lytically infected cells become membrane associated, but only some of the polypeptides are membrane associated in the persistently infected cells.

There is evidence from these results that two factors may play a role in the maintenance of virus persistence. Although Haspel et al. (4) originally were able to isolate a ts virus from S cells, at later cell passages the ts trait was lost from the spontaneously released virus. Analysis of the virus released at late cell passages indicates a large proportion of low-density, interfering particles. These virus variants may be unable to replicate normally in HEF cells.

Persistence of measles virus in S cells can possibly be explained by a defect in the ability of the cells to replicate the virus. This need not require that variant virus also functions in maintenance of the persistent infection. A small amount of infectious virus is spontaneously released from S cells. In order for this to occur, virus proteins must be properly inserted into the cell membranes so that virus can mature by budding. On the average, less than one infectious particle is released per cell per 24 h. The fact that, for the most part, virus structural proteins tend to remain in a soluble phase within the infected-cell cytoplasm rather than becoming membrane associated may indicate that a defect exists that prevents the virus from being assembled as it would be in a lytic infection. The failure of envelope proteins to be properly glycosylated or otherwise modified after their synthesis is an example of an occurrence that could result in defective virus assembly.

It is not known whether defects in virus maturation exist in other persistently infected cell lines. In addition to differences in expression of virus structural proteins, induced, nonstructural proteins may also play a role in persistence. Studies on the expression of both virus structural and induced proteins in persistently in-

---

**Fig. 3.** As described in the text, membrane-free cytoplasmic extracts were prepared from secondary HEF; S cells, or Vero cells infected with Schwarz strain measles virus. All cells were labeled with [35S]methionine. The extracts were run on an 8% sodium dodecyl sulfate-polyacrylamide gel with a 3% stacking gel for 5.5 h at 24 mA. (a) Hallé strain virus marker; (b) Schwarz strain-infected Vero cells; (c) uninfected secondary HEF cells; (d) S cells.
fected cells may help to clarify the mechanism of induction and maintenance of virus persistence.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI 12203 from the National Institute of Allergy and Infectious Diseases. L.E.F. is the recipient of Public Health Service fellowship F 32 AI 05540 from the National Institutes of Health.

LITERATURE CITED