Abortive Infection of Mumps Virus in Murine Cell Lines

By AKIO YAMADA, MASATO TSURUDOME, MICHIKO HISHIYAMA AND YASUHIKO ITO*
Department of Measles Virus, National Institute of Health, 4-7-1 Gakuen, Musashi-Murayama, Tokyo 190-12, Japan

(Accepted 3 February 1984)

SUMMARY
Infection of L929 or BW5147 cells with mumps virus was shown to result in an abortive type of infection in that little or no progeny virus was produced. Both cell lines could adsorb mumps virus, indicating that restricted viral growth in these cells was not attributable to the absence of mumps virus receptors. Using [35S]methionine-labelled virus, it was demonstrated that restriction of virus growth in BW5147 cells was due in part to inefficient virus penetration into the cells. Virus-specific polypeptides were synthesized in mumps virus-infected L929 cells but were not detected in infected BW5147 cells. After addition of actinomycin D or anti-interferon serum to the cultures, mumps virus was able to replicate in L929 cells, whereas no virus growth was apparent in BW5147 cells.

INTRODUCTION
In a previous report (Tsurudome et al., 1983), it was shown that murine cells could be divided into three groups according to their susceptibility to mumps virus infection. Several cell lines such as EL4, L1210 and NS-18 were shown to be highly susceptible, whereas most cells were incapable of supporting virus replication. In order to know the mechanisms responsible for the restricted virus growth, we have now investigated viral attachment, penetration and synthesis of virus-specific polypeptides in two representative cell lines, L929 and BW5147 cells. A possible involvement of interferon in the development of abortive infections was also examined.

METHODS

Cell lines. Two murine cell lines derived from T-cell lymphomas, EL4 and BW5147 cells, were maintained in Eagle's minimum essential medium (EMEM) supplemented with 10% tryptose phosphate broth (TPB) and 8% foetal bovine serum. L929 and Vero cells were maintained in EMEM containing 10% TPB and 5% calf serum (CS). L929 cells were obtained from the Kitazato Institute (Japan).

Virus. A Vero cell-adapted (EY) Enders strain of mumps virus was prepared as described previously (Tsurudome et al., 1983). The infectivity of stock virus was $10^{7.5}$ TCID$_{50}$/ml.

Single-step growth of mumps virus. Cells (2 x 10$^6$) were infected with EY at an input multiplicity of approximately 1 p.f.u./cell. After adsorption at 37 °C for 1 h, infected cells were washed three times with EMEM and incubated with maintenance medium (EMEM containing 2% CS). L929 cells were obtained from the Kitazato Institute (Japan).

Virus titre. Virus titres were determined using Vero cell monolayers by plaque formation assay or haemadsorption with guinea-pig erythrocytes and are expressed as p.f.u./ml or TCID$_{50}$/ml.

Detection of receptors for mumps virus by cytoadsorption. Single-cell suspensions of EL4, BW5147 and L929 cells in EMEM at concentrations of about 1 x 10$^6$ cells per ml were added to the monolayers of Vero cells (approx. 2 x 10$^6$ cells) which had been infected with EY virus (m.o.i. 10$^{-3}$) 48 h before. After incubation at 37 °C for 1 h, Vero cells were gently washed with EMEM, and were stained with May–Grünewald–Giemsa solution.

Detection of receptors for mumps virus by radioactively labelled reconstituted virus vesicles. Egg-adapted virus was purified and solubilized by incubation with n-octyl-b-glucoside (OG). Purified virus suspension was made 2% with OG and allowed to stand at room temperature for 60 min. After centrifugation at 35000 rev/min for 45 min in a Spinco SW41 rotor, the solubilized viral envelope was dialysed together with 150 μCi [3H]cholesterol against phosphate-buffered saline, pH 7.2 (PBS). After complete removal of the detergent, reassembled viral envelope was isolated by centrifugation at 42000 rev/min for 1 h in a Spinco SW51 rotor. The resulting pellet dissolved in 1 ml

---

Key words: mumps virus/abortive infection/L929/BW5147
PBS showed radioactivity of $2.5 \times 10^5$ ct/min/5 μl. Cells (5 x 10^5) were incubated with 3 x 10^5 ct/min of reconstituted vesicles at room temperature for 2, 10 and 30 min, then washed four times with PBS. Cell pellets were emulsified in 0.2 ml of Soluen (Packard Instrument Co.) and radioactivities were determined in a liquid scintillation counter (Beckman). As controls, cells were treated with 10 μM of N-acetylneuraminidase (sialidase) (Sigma) at 37 °C for 2 h and processed as described above.

**Preparation of [35S]-methionine-labelled mumps virus.** Vero cell monolayers in plastic dishes (100 mm diam., Falcon 3003) were infected with EY virus (m.o.i. 1). After adsorption at 37 °C for 1 h, 5 ml methionine-free EMEM containing 2% foetal calf serum was added and incubated at 37 °C for 1 h. Then infected cells were labelled with [35S]methionine (20 μCi/ml) at 37 °C for 48 h. The culture fluids were obtained and purified by sequential centrifugations.

**Penetration of mumps virus into murine cells.** The assay for penetration of virus into murine cells was performed essentially according to Whitaker-Dowling et al. (1983). Between 5 x 10^5 and 10 x 10^5 cells were incubated with 3 x 10^4 ct/min 35S-labelled virus at 37 °C for 60 min in the presence or absence of receptor-destroying enzyme (RDE, Takeda Chemical Industries Inc., Osaka, Japan). The cells were washed twice with 10 mM-HEPES pH 7.2 containing 150 mM-NaCl (HEPES/saline), and then incubated with 0.05% trypsin solution at room temperature for 7 min. Cells were washed twice with HEPES/saline containing 10% CS then lysed with 200 μl HEPES/saline containing 0.5% SDS. Radioactivities were determined by dissolving the lysates in 5 ml of ACSII (Amersham) followed by counting in a liquid scintillation counter.

**Radiolabelling of mumps virus-infected cells.** Cells (5 x 10^5) infected with virus (m.o.i. 10) were labelled with [35S]methionine (20 μCi/ml) for 7.5 h at 14 h after infection. Cells were washed and lysed in 200 μl RIPA buffer (150 mM-NaCl, 0.1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM-Tris-HCl pH 7.4, 100 KIU/ml Trasylol) and clarified by centrifugation.

**Immunoprecipitation and SDS-polyacrylamide gel electrophoresis (SDS-PAGE).** 35S-labelled cell extract (40 μl) was incubated with 50 μl of anti-mumps virus rabbit serum (1:10) at 4 °C for 60 min and further incubated with 50 μl Protein A-Sepharose (Pharmacia) at 4 °C for 60 min. After repeated washings the Sepharose was resuspended in 50 μl sample buffer (62 mM-Tris-HCl pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.0025% bromophenol blue) and heated at 100 °C for 4 min. After centrifugation the supernatant was electrophoresed in a 10% polyacrylamide gel under the conditions described by Laemmli (1970); gels were processed for fluorography (Bonner & Laskey, 1974).

**Assay for interferon (IFN).** IFN was assayed by a cytopathic effect inhibition microassay method in L929 cells with vesicular stomatitis virus as the challenge virus. The reciprocal of the highest dilution of the sample causing 50% protection was taken as the IFN titre. One IFN unit in our laboratory is equivalent to 2.7 reference research units of the mouse IFN reference preparation, NIH catalogue number G002-904-511.

**Assay for 2',5'-oligoadenylate (2-5A) synthetase activity.** 2-5A synthetase activity was assayed as described previously (Ball, 1979) and enzyme activity was expressed as moles ATP incorporated into oligomers of two or more adenylates after incubation at 37 °C for 2 h.

**Anti-interferon serum.** Rabbit anti-interferon serum (anti-IFN) was donated by Dr S. Saito (NIH, Japan) and characterized elsewhere (Fujita et al., 1979).

**Fusion induced by mumps virus-infected Vero cells.** Monolayers of L929 and BW5147 cells were overlaid with a single-cell suspension of Vero cells which had been infected with EY virus (m.o.i. 1) 12 h before. The ratio of Vero cells to BW5147 or L929 cells was 1:20. The cultures were incubated at 37 °C for 12 h, the cells were washed and stained with 2% Giemsa solution. Since neither L929 nor BW5147 cells infected with virus showed cell fusion (fusion from within), fusion of these cells induced by infected Vero cells was considered to be similar to fusion from without.

**Chemicals.** [3H]Cholesterol (47.7 Ci/mmol) and L-[35S]methionine (1340 Ci/mmol) were obtained from Amersham Japan. N-Acetylneuraminidase (Vibrio cholerae) was purchased from Behringwerke. Actinomycin D was purchased from Sigma.

## RESULTS

**Growth of mumps virus in EL4, L929 and BW5147 cells**

EL4, L929 or BW5147 cells (2 x 10^5) were infected with mumps virus at an input multiplicity of approximately 1 p.f.u./cell and incubated in the maintenance medium. At appropriate times, production of infective virus was examined. The yield in L929 and BW5147 cells was less than 10^3 TCID50/ml of progeny virus throughout the infection, whereas complete growth of virus was found in EL4 cells (data not shown). No cytopathological changes were observed in L929 cells and BW5147 cells infected with the virus (data not shown).
Mumps virus abortive infection

Fig. 1. Adsorption of radioactive reconstituted vesicles. Vero, L929 and BW5147 cells were incubated with 3 × 10^5 ct/min of radioactive reconstituted vesicles at room temperature in the presence or absence of N-acetylneuraminidase. After extensive washings, cell-associated radioactivity was determined: (a) L929 cells with (△) or without (△) neuraminidase and Vero cells with (●) or without (○) neuraminidase; (b) BW5147 cells with (●) or without (○) neuraminidase.

Virus attachment to murine cells

In order to know whether incomplete growth of virus in L929 and BW5147 cells was due to a lack of attachment to these cells, we examined adsorption of these cells to infected Vero cells by a method similar to haemadsorption. Single-cell suspensions of EL4, L929 and BW5147 cells were added to Vero cell monolayers which had been infected with virus. After incubation at room temperature for 1 h, Vero cells were washed and observed for adsorption of these cells. All of these cell lines could bind to infected Vero cells but not to mock-infected cells, and treatment of infected cells with anti-mumps virus serum inhibited the cytoadsorption (data not shown). To analyse quantitatively the attachment of the virus to these cells, radioactive reconstituted vesicles were prepared. Vero cells, L929 cells and BW5147 cells were incubated with 3 × 10^5 ct/min of radioactive reconstituted vesicles at room temperature for 2, 10 and 30 min in the presence or absence of sialidase. After extensive washings, cell-associated radioactivity was determined. As shown in Fig. 1, cell-associated radioactivity in each of these cell lines without sialidase was increased with incubation time, while increase of cell-associated radioactivity of the cells with sialidase was not observed. These observations suggested that viral attachment could occur to all of the cells examined.

Fusion of L929 and BW5147 cells induced by mumps virus-infected Vero cells

The ability of L929 and BW5147 cells to fuse with infected Vero cells was examined. A single-cell suspension of Vero cells infected 12 h before was added to monolayers of L929 or BW5147 cells. After incubation at 37 °C for 12 h, cells were stained and examined for the presence of polykaryocytes. As shown in Fig. 2, syncytial cells were found in L929 cell monolayers co-cultivated with infected Vero cells. However, no apparent syncytial cells were observed in BW5147 cells co-cultivated with infected Vero cells. These observations indicated that BW5147 cells were comparatively resistant to the fusion activity of mumps virus.
Penetration of mumps virus

The evidence that BW5147 cells are more resistant to the fusion activity of the virus led us to investigate whether it efficiently penetrated BW5147 cells. For this purpose, we examined the
Mumps virus abortive infection

Table 1. Viral penetration into EL4 and BW5147 cells*

<table>
<thead>
<tr>
<th>Cells</th>
<th>+RDE (ct/min)</th>
<th>−RDE (ct/min)</th>
<th>Penetration rate†</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL4</td>
<td>151.5</td>
<td>987.3</td>
<td>6.5</td>
</tr>
<tr>
<td>BW5147</td>
<td>532.5</td>
<td>445.9</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* Cells (6 x 10⁵) were incubated with 3 x 10⁴ ct/min [35S]methionine-labelled virus at 37 °C for 1 h in the presence or absence of RDE. After treatment of these cells with trypsin, cell-associated radioactivity was determined.

† Penetration rate is expressed as the ratio of radioactivity obtained from the cells without RDE to that from the cells with RDE.

ability of radioactive infectious virus to penetrate EL4 and BW5147 cells. When EL4 cells were incubated with 35S-labelled virus in the absence of RDE, more trypsin-resistant radioactivity was retained than in the presence of RDE (Table 1). In contrast, such a difference was not seen with BW5147 cells (Table 1). This observation was confirmed when viral penetration was compared between cells incubated with 35S-labelled virus at 4 °C or 37 °C. Radioactivity retained in EL4 cells incubated at 37 °C was higher than that retained in those incubated at 4 °C, whereas such a difference was not observed in the case of BW5147 cells (data not shown). In a further experiment, when EL4, L929 and BW5147 cells pre-incubated with infective virus at 4 °C for 2 h and washed extensively were frozen and thawed, the recovery of infectious virus from BW5147 cells was more than that from the other two cell types (data not shown). These findings suggested that penetration of virus into BW5147 cells was inefficient.

Virus-specific protein synthesis

Next we analysed the synthesis of virus-specific polypeptides after infection. Infected cells were labelled with [35S]methionine for 7-5 h at 14 h post-infection. 35S-labelled cell extracts were immunoprecipitated and then analysed by SDS-PAGE. As shown in Fig. 3 (a), immunoprecipitates from EL4 cell extracts contained all of the major structural polypeptides (HN, NP, F, P, M). All polypeptides were detected in virus-infected L929 cells (Fig. 3 b), although the amount of M protein seemed to be low. There were, however, no detectable virus-specific polypeptides in immunoprecipitates from BW5147 cell extracts (Fig. 3 a). These observations suggested that the steps of virus replication defective in BW5147 and L929 cells were the early and late stages, respectively.

Role of interferon in establishing abortive infection in L929 and BW5147 cells

To examine the possibility that the failure of mumps virus to grow in L929 cells was mediated by IFN, the effects of actinomycin D on its replication were examined. Infected L929 cells were incubated in the presence of various doses of actinomycin D. After incubation at 37 °C for 48 h, virus infectivities were assayed. Actinomycin D enhanced the yield of infectious virus in L929 cells and maximal virus yield was obtained in cells treated with 0.5 µg/ml actinomycin D (data not shown). Subsequently, the kinetics of virus replication was examined in L929 cells pretreated with or without actinomycin D (0.5 µg/ml) for 2 days and infected with mumps virus (m.o.i. 1); at appropriate times post-infection, the infectivity, IFN and 2-5A synthetase activities were determined. In infected L929 cells in the presence of actinomycin D, a low but significant amount of IFN was detected in the culture fluids at early periods after the infection (Fig. 4a). Induction of 2-5A synthetase activity was also detected in L929 cells infected with virus (Fig. 4a). On the other hand, virus replication was enhanced and no IFN activity was detectable in the culture fluids of L929 cells pretreated with actinomycin D (Fig. 4b). These results suggested a possible involvement of IFN in the inability of mumps virus to grow in L929 cells. On the contrary, neither IFN nor 2-5A synthetase activity was detected in the case of infected BW5147 cells (data not shown). We therefore examined the effect of anti-IFN serum on
Fig. 3. Virus-specific protein synthesis in EL4, BW5147 and L929 cells. (a) Infected EL4 and BW5147 cells were labelled with 20 μCi/ml [35S]methionine for 7.5 h at 14 h post-infection. 35S-labelled cell extracts were immunoprecipitated and analysed by SDS–PAGE. Lanes 1 and 2, EL4 extract; lanes 3 and 4, BW5147 extract; lane 5, post-labelled mumps virus; lane 6, 14C-labelled protein markers. Cell extracts were precipitated by normal rabbit serum (NRS, 1:10) (lanes 2 and 4), or anti-mumps virus rabbit serum (1:10) (lanes 1 and 3). (b) Infected L929 cells were labelled, extracted and analysed by SDS–PAGE. Infected cells were cultured in the presence of NRS (lane 1) or anti-IFN serum (lane 2); lane 3, 14C-labelled protein markers.

the infection of L929 or BW5147 cells. As shown in Fig. 4(c), the presence of anti-IFN serum throughout the infection caused a remarkable enhancement of virus yield in L929 cells, whereas no effect was observed in BW5147 cells (data not shown). These results showed that abortive virus replication in L929 cells was a result of autointerference by the endogenously produced IFN during the course of infection and that there was no relationship between IFN production and abortive replication of mumps virus in BW5147 cells. The presence of anti-IFN serum in the culture enhanced the synthesis of virus polypeptides in virus-infected L929 cells (Fig. 3b).

**DISCUSSION**

At least two different mechanisms appear to be involved in the establishment of abortive infections of mumps virus in murine cells. IFN appears to play an important role in establishing abortive infection in L929 cells, whereas virus penetration was found to be disturbed in the case of BW5147 cells.
Fig. 4. Role of IFN in establishing abortive infection in L929 cells. L929 cells pretreated without (a) or with 0.5 µg/ml actinomycin D (b) for 2 days were infected and, at various times post-infection, infectivity, IFN activity of culture fluid and 2-5A synthetase activity of the cells were determined. •, Virus titre; ○, IFN activity; △, 2-5A synthetase activity. (c) Infected L929 cells were cultured in the presence of 1% anti-IFN serum (○) or 1% NRS (●).
The involvement of IFN in abortive infection was reported for the infection of L929 cells with Newcastle disease virus (NDV) in which endogenous IFN interfered with the growth of the virus (Nagai et al., 1981), although Youngner & Scott (1968) reported that NDV yield did not increase even when IFN production was suppressed by actinomycin D. In our study, inhibition of IFN synthesis by actinomycin D enhanced the yield of mumps virus in L cells. Furthermore, the addition of anti-IFN-α/β serum to the infected L929 cell culture was able to overcome the abortive replication of mumps virus.

The mechanism responsible for limited growth of the virus in BW5147 cells seems to differ from that involved in infected L929 cells. No synthesis of virus-specific polypeptides was observed in infected BW5147 cells, suggesting that virus replication was inhibited at a step before translation. The observation that BW5147 cells were resistant to the fusion activity of the virus, and that BW5147 cells were limited in its uptake, strongly suggests that viral penetration was disturbed in BW5147 cells. Resistance of certain mutant cell lines to the fusion activity of Sendai virus was reported by Toyama et al. (1977). They proposed that a decrease in the amount of sialic acids was responsible for the resistance. Membrane fusion between the cell membrane and the viral envelope may be regulated by many other factors, e.g. membrane fluidity, functions of microfilaments and microtubules. The mechanism(s) by which the cell membrane of BW5147 cells resists viral envelope–cell fusion remain to be resolved.

Murine cells have been classified into three groups according to their susceptibility to mumps virus infection (Tsurudome et al., 1983). Several cell lines such as NS-18 (a neuroblastoma cell line), L1210 (null cell line), EL4 (T-cell line) and 5C3 (B-cell hybridoma) produced high titres of virus; that is, they are fully permissive cells. Two fibroblast cell lines, DK (kind gift of Dr K. Kai, Institute of Medical Science, University of Tokyo) and C243 cells, and a glioma cell line (203GL), are less susceptible, and infection of these cells results in limited replication of the virus (partially permissive cells). The majority of murine cells are unable to support mumps virus replication (non-permissive cells). Although fully permissive cells yielded high titres of virus, IFN production was scarcely observed (unpublished data). This suggests that mumps virus replication in these cells is not regulated by endogenous IFN produced during the course of infection. Addition of anti-IFN serum caused enhancement of virus replication in DK cells, a partially permissive cell line (unpublished observation). Thus, the limited growth of the virus in partially permissive cells might be due to interference by endogenously produced IFN. A preliminary experiment has shown that SP2/0 (B-cell line) and B-7-7 (T-cell hybridoma), both non-permissive cell lines, resemble BW5147 cells in the mechanism of abortive infection by mumps virus (unpublished data).

We wish to thank Dr S. Saito for her help in assaying 2–5A synthetase activity and for donating anti-interferon serum. We are also indebted to Dr A. Sugiura for his continuous advice and encouragement.

REFERENCES


(Received 7 November 1983)