Mumps Virus Infection of Dissociated Rodent Spinal Ganglia in vitro. Expression and Disappearance of Viral Structural Proteins from Neurons

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SUMMARY

Cultured spinal ganglia and cord from mice and hamsters were infected with mumps virus or Sendai virus. Expression of five structural proteins, the haemagglutinin-neuraminidase, fusion, nucleocapsid (NP), phospho (P) and matrix proteins was examined with monoclonal antibodies to each protein. In Sendai virus-infected mouse neurons all five viral proteins were detected. In hamster neurons infected with mumps virus all viral proteins were also expressed, but in mouse neurons only the NP and P proteins were seen. This suggests a species-specific cellular restriction of viral protein synthesis in mumps virus-infected mouse neurons. There was no, or only a slight, reduction in the number of neurons between days 4 and 20 after infection of mouse cultures with mumps virus, but the proportion of infected neurons diminished from 68% to 15% during this time.

Several paramyxoviruses cause infection of the central nervous system (CNS) (Kilham & Overman, 1953; Johnson, 1982; Kristensson et al., 1983; Kristensson & Norrby, 1986). For instance, mumps virus is a common cause of acute mild meningitis and sometimes also of encephalitis in man (Wolinsky & Server, 1985). Cases with persistent intrathecal mumps antibody production have been described (Julkunen et al., 1985). Different mumps virus strains are capable of infecting the CNS of newborn hamsters with an outcome varying from acute fatal infections to non-lethal persistent infections (Kilham & Overman, 1953; Johnson, 1968; Wolinsky & Stroop, 1978; McCarthy et al., 1980). Some mumps virus strains, which in newborn hamster brains cause a productive and fatal infection, will instead cause in newborn mouse brains an abortive or persistent infection in which the virus goes through a defective cycle of replication where only the nucleocapsid (NP) and phospho (P) proteins can be detected by immunostaining (Kristensson et al., 1984). The effect of such persistent infections on neuronal development and function is not known.

Embryonic neurons can be maintained in vitro for several weeks at least (Sotelo et al., 1980) and be used for structural, functional and pathogenetic studies of virus-infected neurons without influences from the host animal's defence system (Dubois-Dalcq et al., 1982). The aim of the present study was to establish a long-term mumps virus infection of neurons in vitro and to study the expression of the different virus structural proteins of two mumps strains and Sendai virus during infection of mouse and hamster neurons.

The tissue culture technique described by Sotelo et al. (1980) was principally used. The spinal cord and ganglia were dissected from mouse or hamster foetuses on days 12 to 14 of gestation. The tissue was suspended in medium and dissociated by running it gently through a 0.8 mm syringe or a narrow Pasteur pipette several times. The tissue suspension was seeded into Petri dishes (diameter 34 mm, Falcon) previously coated with calf skin collagen (Flow Laboratories). The medium used during the first week was Eagle's minimal essential medium (Flow...
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Laboratories) supplemented with 10% horse serum (Gibco), 10% foetal calf serum (FCS) (Gibco), 2% chicken embryo extract (Flow Laboratories), 5 mg/ml glucose, 200 mM L-glutamine at 1 ml/100 ml and 15 μg/ml gentamicin. From the second week no FCS was added to the medium. The medium was changed three times a week. A cytostatic agent, cytosine arabinoside (3 μg/ml) was added to the medium during days 6 to 8 in vitro. The cultures were incubated in 5% CO₂ at 37°C. Thirteen days after establishment, the cultures were used for infection.

The viruses used were the Kilham and RW mumps virus strains (kindly provided by Dr Jerry Wolinsky, University of Texas Health Science Center at Houston, Houston, Tx., U.S.A.). For infection with Sendai virus, the Z strain grown in embryonated eggs was employed. The virus suspensions were adjusted to a titre of 4 × 10⁷ p.f.u./ml. The m.o.i. was at least 100 p.f.u./neuron. After adsorption of 1 ml of viral material for 10 min, 2 ml of medium was added. Virus infectivity was determined by plaque titration in Petri dishes as previously described (Wolinsky & Stroop, 1978; Orvell & Grandien, 1982; Löve et al., 1985).

For observation of cell cultures, defined areas of the Petri dishes were marked for counting of large spinal ganglion neurons which were readily identified under the phase contrast microscope. These areas were counted daily in several RW mumps and Sendai virus-infected and non-infected cultures. Three or four infected and three or four non-infected cultures were used in four separate experiments.

For immunofluorescence (IF) analysis of the expression of the different viral antigens, cell cultures were fixed 2 to 4 days post-infection (p.i.) in 4% paraformaldehyde in phosphate buffer pH 7.4 for 20 min. After rinsing in phosphate-buffered saline (PBS), the cultures were incubated with a monoclonal antibody against a specific viral protein. The antibodies used were directed against five structural proteins [haemagglutinin-neuraminidase (HN), fusion (F), NP, P and matrix (M)] of mumps and Sendai viruses (Orvell & Grandien, 1982; Orvell, 1984). The antibodies were used in a 1:50 dilution of the original ascites. This dilution of antibodies had previously given strong immunostaining of all five viral antigens in Vero cells. After incubation the cultures were washed thoroughly in PBS and fluorescein-labelled goat anti-mouse antibodies were added (Nordic Immunological Laboratories, Tilburg, The Netherlands). All incubations were performed at room temperature for 20 min. After thorough rinsing in PBS the samples were mounted in a glycerine:phosphate buffer solution (9:1) and examined under a Nikon epifluorescence microscope.

To facilitate identification of neurons in immunostained preparations, double staining with rabbit antibodies against neuron-specific enolase (Dako, Copenhagen, Denmark) (Hooghe-Peters et al., 1979) and a mouse monoclonal antibody against NP of mumps virus was employed. After incubation and washing, fluorescein-labelled swine anti-rabbit antibodies (Dako) were added. The cultures were then washed and incubated with rhodamine-labelled rabbit anti-mouse antibodies (Dako). From each of three separate sets of experiments 300 to 500 neurons from two or three Petri dishes were counted at days 4, 14 and 20 p.i. and the ratio of infected to non-infected neurons was determined.

Large spinal ganglion neurons from mice and hamsters were often arranged in clusters and grew on the surface of a confluent layer of cells (Fig. 1a). They often extended a network of long neurites. These cells were positive by IF staining for neuron-specific enolase (Fig. 1b). During the first 4 days after infection of mouse neurons with the RW mumps strain a reduction of neurons to about 70% of the initial number occurred, but after this there was only a slight gradual disappearance of cells (Fig. 2), not exceeding that of non-infected controls. Titration of virus infectivity in the mouse neuron cultures infected with the RW strain of mumps virus revealed low viral titres of 10^2 p.f.u./ml for 2 days p.i. and thereafter minimal or no titres of virus were detected.

On day 6 p.i. Sendai virus-infected mouse neurons started to degenerate and had disappeared 8 to 9 days p.i. Titration of Sendai virus in the culture fluid revealed a productive infection with a maximum titre of 10^8 p.f.u./ml at day 3 p.i.

Sendai virus antigen was detected immunohistochemically in neurons as well as in other cell types in the mouse cultures, while mumps antigen was almost confined to neurons in both hamster and mouse cultures. Double staining of mouse cultures infected with the RW mumps
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Fig. 1. (a) Mouse dorsal root ganglion neurons in cultures seen under a phase contrast microscope. (b) IF staining with anti-neuron-specific enolase of mouse neurons. (c) IF staining of NP mumps virus protein in mouse neurons from the same areas as (b). Bar markers represent 25 μm.

Fig. 2. Survival of mouse dorsal root ganglion neurons after inoculation with the RW strain of mumps virus (▲) as compared with uninfected controls (●). Decrease of cells: (RW-infected against uninfected controls from days 0 to 4 p.i., P < 0.01; RW-infected RW against uninfected controls from days 4 to 20 p.i., not significant.

strain (Fig. 1b, c) also revealed that the great majority of infected cells were enolase-positive. Four days after inoculation the majority of the mouse neurons contained viral antigen (Table 1), but after 14 days the number of infected cells was significantly reduced and at day 20 antigen persisted only in a small fraction of the neurons. The disappearance of antigen was apparently not the result of a loss of infected neurons, since there was no or only a minimal reduction in the number of neurons during this period (Fig. 2).

A search for the individual viral proteins in infected neurons identified all five proteins in Sendai virus-infected mouse neurons and mumps virus-infected hamster neurons. However, only the NP and P proteins could be found in mouse neurons infected with either of the two mumps strains (Table 2, Fig. 1c).

Both mumps virus strains, RW and Kilham, gave a widespread infection of neurons but of only a few non-neuronal cells in the mixed population of cultured cells. Such selective infection of neurons in vitro has previously been described for measles virus (Rentier et al., 1981) and
Table 1. Enolase-positive cells infected with mumps virus

<table>
<thead>
<tr>
<th>Day p.i.</th>
<th>Positive cells (% ± s.D.)</th>
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<tbody>
<tr>
<td>4</td>
<td>68.3 ± 5.7</td>
</tr>
<tr>
<td>14</td>
<td>34.7 ± 5.1</td>
</tr>
<tr>
<td>20</td>
<td>15.7 ± 3.4</td>
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Table 2. Expression of viral proteins in infected spinal ganglia

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mouse cells</th>
<th>Hamster cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sendai virus</td>
<td>Kilham mumps</td>
</tr>
<tr>
<td>HN</td>
<td>+*</td>
<td>-</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NP</td>
<td>+</td>
<td>+</td>
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<tr>
<td>P</td>
<td>+</td>
<td>+</td>
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<tr>
<td>M</td>
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* +, Positive; -, negative; +w, weak.

rabies virus (Tsiang et al., 1983) and seems to occur also in infections in vivo by these viruses. This is in contrast to Sendai virus which infected both neurons and various non-neuronal cells in vitro but only neurons and ependymal cells harboured this infection in vivo (Kristensson et al., 1983).

Low titres of virus were found in the fluid from mouse neuronal cultures during the first 2 days after infection with the RW mumps strain. However, on the 3rd day, when most neurons contained viral antigen, virtually no virus could be detected in the culture fluid. This restricted viral replication was also reflected by a lack of demonstrable expression of three viral proteins, HN, F and M, by immunofluorescence. This is in agreement with previous findings in vivo (Kristensson et al., 1984) and suggests that the restriction of virus formation is the result of cellular mechanisms and not a function of the immunological defence mechanisms of the intact host. On the other hand, mumps virus grew to high titres in newborn hamsters (in contrast to newborn mice), and in cultured hamster neurons all mumps virus proteins were seen.

An intriguing finding in the present study was that mumps virus antigen seemed to disappear from infected neurons. Such a phenomenon has not previously attracted much attention although Bodian (1948) described that chromatolysis of spinal cord motor neurons in experimental poliovirus infections can be reversible. Also, recently Oldstone et al. (1986) described the disappearance of lymphocytic choriomeningitis (LCM) virus components from the CNS of infected mice after adoptive transfer of lymphocytes. No lymphocyte infiltration or necrosis was seen in the brain parenchyma in contrast to the liver, lung and kidney. Lymphocytes were, however, seen perivascularly in the Virchow-Robbin spaces and in leptomeninges. The data suggests that soluble factors such as lymphokines from the cytotoxic lymphocytes act on LCM virus-infected neurons and eliminate the viral material without causing cell necrosis.

In our model in vitro, however, where there is no immune response, lymphokines should not be present. Also, the phenomenon in which antibodies directed to a virus antigen can decrease the amount of both viral surface and internal proteins in virus-infected cells (Fujinami & Oldstone, 1979) does not prevail in our system. Even if interferon was induced in our cultures, its main action is not considered to eliminate viral antigens from cells already infected (Joklik, 1985). Our results may therefore indicate other mechanisms for clearance of viral material from neurons e.g. involving a direct alteration in the nerve cell body’s metabolism by which the neuron may eliminate viral antigen.

This study, along with others, suggests that viral protein expression can be restricted by
cellular factors. Such restrictions may facilitate the establishment of viral persistence in cells when surface proteins exposed to an immunological attack are not expressed. It is also suggested that neurons may by some means clear themselves of viral antigen.

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REFERENCES


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