The Types of Mouse Brain Cells Susceptible to Polyoma Virus Infection

in vitro

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SUMMARY

Using the immunofluorescence technique with antisera directed against markers on mouse brain cells, polyoma virus was found to infect in vitro, type 1 astrocytes, brain fibroblasts and leptomeningeal cells but not oligodendrocytes, type 2 astrocytes or neurones.

Polyoma virus replicates in many tissues of its natural host, the mouse, including the brain when inoculated neonatally by a subcutaneous (s.c.), intraperitoneal (i.p.) or intracerebral (i.c.) route (Rowe et al., 1960; McCance & Mims, 1977). Euthymic mice appear to show no ill effects of this acute infection of the brain and virus disappears from the central nervous system by 3 weeks post-infection. In previous work (McCance, 1983) a small percentage (< 1%) of these euthymic mice (CD1 strain) exhibited rear leg paralysis and, although no infectious virus was found, polyoma DNA sequences were detected in the brain and spinal cord by DNA–DNA hybridization. However, when athymic mice (nude mice, nu/nu) were inoculated i.p. the virus replicated to high titres in the brain for at least 23 weeks post-infection (McCance et al., 1983). Hind leg paralysis developed in 8 of 16 infected nude mice and, although all eight affected mice showed early signs of primary demyelination and Wallerian (secondary) degeneration, paralysis was due in one case to a small vertebral tumour pressing on the spinal cord. Polyoma virus particles were observed by electron microscopy in oligodendrocytes (Sebesteny et al., 1980; McCance et al., 1983) and polyoma antigens were seen in other cells of the brain by immunofluorescent and immunoperoxidase staining techniques (McCance et al., 1983) including cells in the cerebral hemispheres and cerebellum. This disease was not similar to the demyelinating human disease, progressive multifocal leucoencephalopathy (PML) caused by a human papovavirus (JCV), where the virus is only found in oligodendrocytes and clinical disease results from the infection of these cells (Zu Rhein & Chou, 1965).

Apart from oligodendrocytes, the types of cells infected in vivo is not known. This study set out to investigate mouse brain cells infected in vitro using antibodies to specific markers on brain cells and double-labelling with anti-polyoma antibodies.

The corpus callosum and small pieces of attached cerebral hemispheres were dissected from 3-day-old CD1 mouse brains. Single-cell suspensions were made by a method of Raff et al. (1979) modified by J. Pearson (Dept. of Microbiology, Guy's Hospital Medical School). Briefly, the tissue was cut into small pieces using opposing scalpel blades, washed in solution I (100 ml of 0.9% NaCl, 3 ml of 1.15% KCl, 1 ml of 2.11% KH2PO4 and 21 ml of 1.3% NaHCO3) to which was added 0.3% bovine serum albumin, 0.03% MgSO4 and 0.25 g glucose and then transferred to 20 ml of fresh solution I containing 0.025% trypsin and incubated for 15 min at 37 °C. Trypsinization was stopped by addition of 20 ml of solution I containing 6-4 μg DNase (Sigma), 0.8 mg soya bean trypsin inhibitor (Sigma) and 9-6 mg MgSO4 and the tissue blocks were allowed to settle to the bottom of the container. The supernatant was discarded, the blocks resuspended in 20 ml of solution I containing 40 μg DNase, 5 mg soya bean trypsin inhibitor and 6 mg MgSO4 and pipetted up and down gently with a wide-necked Pasteur pipette. The last step was repeated until all the blocks were broken down into a single-cell suspension. The cells were collected by centrifugation at 800 g for 10 min and 2.5 × 10^5 cells were seeded onto...
polylysine-coated (20 μg/ml) coverslips in 100 μl of medium [minimal essential medium (Wellcome), supplemented with 10% heat-inactivated foetal calf serum (FCS), 0.074% KCl, 0.058% glutamine, 100 μg/ml streptomycin, 200 units/ml penicillin]. Cells adhered overnight after which time the coverslips were washed in the above medium, infected with polyoma virus (100 p.f.u./cell) for 1.5 h at 37 °C and then re-fed with 1 ml of medium as above except that 2% heat-inactivated FCS was added. The coverslips were harvested 48 h post-infection and processed according to the cell markers to be detected.

The most common cell type was the astrocyte, which was fixed with methanol at −20 °C for 30 min, labelled with rabbit anti-glial fibrillary acidic protein antibodies (GFAP, 1/25 dilution, a gift from Dr N. A. Gregson) for 15 min at 37 °C, washed twice in phosphate-buffered saline (PBS) and then labelled with rhodamine-conjugated goat anti-rabbit antibodies (GAR/TRITC, 1/20 dilution; Nordic Immunological Laboratories, Maidenhead, U.K.) and fluorescein-conjugated mouse anti-polyoma serum (PV/FITC, 1/4 dilution). The GFAP were produced by the method of Eng et al. (1971) and the antiserum raised in rabbits with complete Freund’s adjuvant. The anti-polyoma serum was a hyperimmune serum prepared as previously described (McCance & Mires, 1977) and detects the capsid antigens of polyoma virus. The coverslips were washed twice in PBS, fixed onto a slide and mounted in 90% glycerol–10% PBS solution. Two types of astrocyte were observed (Raff et al., 1983); type 1 astrocytes have a fibroblast-like morphology (Fig. 1a) and were infected with polyoma (Fig. 1b), whereas type 2 astrocytes, which have a smaller cell body, many processes (Fig. 1c) and resemble oligodendrocytes were not seen to be infected with polyoma. Although some astrocytes were susceptible to polyoma infection, they were not infected as frequently as GFAP-negative (GFAP−) cells in the cultures.

Oligodendrocytes are GFAP− and are labelled with rabbit antisera directed against surface galactocerebrosides (GC; Raff et al., 1983). This serum was raised against chromatographically purified human galactocerebroside containing both the hydroxy- and non-hydroxy-derivatives by intradermal injection with complete Freund’s adjuvant (Oxberry & Gregson, 1974). The serum was a gift from Dr N. A. Gregson and cells reactive to this serum represented <10% of the cells in culture. Cells were washed once in PBS with 10% FCS and labelled with anti-GC (1/20 dilution) for 30 min at room temperature, then fixed at −20 °C for 30 min with methanol and finally labelled with GAR/TRITC and PV/FITC for 15 min at 37 °C and washed in PBS.

Fig. 1. Mouse brain cells labelled with rabbit anti-glial fibrillary acidic protein antibodies (GFAP), then rhodamine-conjugated goat anti-rabbit antibodies (GAR/TRITC) and finally with fluorescein-conjugated mouse anti-polyoma virus antibodies (PV/FITC). (a) Astrocyte (type 1) when viewed with rhodamine filters; (b) same cell viewed with fluorescein filters exhibiting polyoma virus capsid antigens in the nucleus; (c) an astrocyte (type 2) viewed with rhodamine filters, showing a number of processes. Bar marker represents 11 μm in (a) to (c).
Fig. 2. Mouse brain cells labelled with rabbit anti-galactocerebroside antibodies (GC) and GAR/TRITC and finally PV/FITC. (a) Typical oligodendrocyte in culture, viewed with rhodamine filters; (b) the same cell viewed with fluorescein filters, showing lack of labelling for viral capsid antigen (arrow), but indicating infected cells surrounding this cell. Bar marker represents 11 μm for (a) and (b).

The coverslips were then mounted as above. Oligodendrocytes were identified in the cultures as shown in Fig. 2(a) and although over 1000 cells in various cultures were observed, no infected cells were seen (Fig. 2b). T antigens (early non-structural proteins) of polyoma virus were also absent when cells were labelled with a hamster anti-T serum followed by fluorescein-conjugated swine anti-hamster serum (SwHa/FITC, 1/10 dilution) instead of PV/FITC. It would therefore appear that oligodendrocytes are totally refractory to polyoma virus infection in vitro.

Another cell type recently described by Raft et al. (1983) and labelled with a mouse monoclonal antibody A2B5 [which labels the tetrasialocerebroside (GQ) and was a gift from Dr M. Adinolfi] was found to be a progenitor cell that developed, at least in vitro, into type 2 astrocytes (process-bearing) or oligodendrocytes. This antiserum also labels neurones. Corpus callosum cultures were infected with polyoma virus and 48 h later were labelled with A2B5 antibodies (1/100 dilution), fixed and stained with GAM/TRITC and PV/FITC. A2B5-positive (A2B5+) cells did not support polyoma virus replication.

Neurones were obtained by trypsinizing (0.01 % trypsin) the cerebellum of 1-day-old mice and seeding at 5 × 10⁴ cells/13 mm coverslip onto an astrocyte feeder layer. (The astrocyte feeders were set up 7 days previously by trypsinizing whole brains from 1-day-old neonates.) Twenty-four h later, these cultures were infected with polyoma virus as above and 48 h later fixed in methanol at −20 °C, labelled with a mouse anti-neurone filament antiserum (a gift from J. Wood, Wellcome Laboratories, and used at 1/50 dilution) and then with goat anti-mouse rhodamine-conjugated antibodies (GAM/TRITC, 1/10 dilution) plus PV/FITC. Neurones did not appear to be infected with polyoma virus although it was sometimes difficult to differentiate the neurones which lay on top of the feeder layer from the astrocyte and fibroblast cells beneath, since both these latter cell types are susceptible to polyoma virus.

Fibroblasts and leptomeningeal cells are GFAP- and GC- but can be labelled with anti-fibronectin antibodies (Cappel Laboratories, Cochranville, Pa. U.S.A., anti-LETS antibodies; Raft et al., 1979). In cultures from the corpus callosum, fibronectin-positive cells (FN+) are <5% but in whole brain cultures from 1-day-old mice as used for neurone feeder layers, they make up nearly 50% of the cell types. When these cultures are infected with polyoma virus, harvested 48 h later, labelled with rabbit anti-fibronectin antibodies (1/10 dilution), fixed in methanol at −20 °C and stained with GAR/TRITC and PV/FITC, FN+ cells exhibited double staining as shown in Fig. 3. Fibroblasts are probably the positively stained, polyoma virus-infected, cells seen in the granular layers of the cerebellum described previously (McCance et al., 1983). Finally, there were cells infected with polyoma virus which were GFAP- and FN −, but which had a morphology similar to both type 1 astrocytes and FN+ cells. These were large
Table 1. *Mouse brain cells susceptible to polyoma virus infection*

<table>
<thead>
<tr>
<th>Cell</th>
<th>Antiserum used as marker</th>
<th>Polyoma virus replication*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligodendrocyte</td>
<td>Rabbit anti-galactocerebroside serum</td>
<td>−</td>
</tr>
<tr>
<td>Astrocytes (type 1)</td>
<td>Rabbit anti-glial fibrillary</td>
<td>+</td>
</tr>
<tr>
<td>Astrocytes (type 2)</td>
<td>acidic protein serum</td>
<td>−</td>
</tr>
<tr>
<td>Neurones</td>
<td>Mouse anti-neurone filament serum</td>
<td>−</td>
</tr>
<tr>
<td>Leptomeningeal cells and fibroblasts</td>
<td>Rabbit anti-fibronectin serum</td>
<td>+</td>
</tr>
<tr>
<td>Astrocyte (type 2) and oligodendrocyte progenitor cells</td>
<td>Mouse monoclonal anti-tetrasialocerebroside serum</td>
<td>−</td>
</tr>
</tbody>
</table>

* Cell susceptible (+) or resistant (−) to polyoma infection.

flat cells with oval nuclei and this morphology suggested that they were not A2B5+ cells which were small-bodied cells with many processes similar to type 2 astrocytes (Fig. 1c). The cell type involved is not at present known.

Table 1 summarizes the cells tested for their ability to support polyoma virus replication. It was surprising to find that oligodendrocytes are not susceptible to polyoma infection in vitro (even abortive infection was not detected, as no T antigens were observed), since virions have been found in the nucleus of oligodendrocytes in vivo (Sebesteny et al., 1980; McCance et al., 1983). A possible but unlikely explanation is that the latter studies were carried out on nude mice while the in vitro work described here was with cells from the brains of an outbred Swiss strain of CD1. The human papovavirus (JCV) is also found in oligodendrocytes in the brains of individuals with PML and it is probably the lysis of these cells during JCV replication that leads to clinical disease. No human oligodendrocytes have yet been cultured to test their susceptibility in vitro. Possibly the conditions in vitro do not reflect all in vivo properties or, since the oligodendrocytes in vivo were identified by electron microscopy and not with antiserum against specific cell markers, that they were in fact inaccurately described. Another explanation might be that the susceptibility to infection with papovaviruses in the brain and in brain cell cultures
varies, since JCV produces an abortive infection in astrocytes in PML brains (Dörries et al., 1979) but a fully infectious cycle in vitro (Fig. 4, Wroblewska et al., 1982). Perhaps the JCV abortive infection in vivo occurs in type 2 astrocytes which in the rat at least are found most commonly in the white matter (Raff et al., 1983) where virus-induced demyelination is seen in man. In this communication it has been shown that polyoma virus infects the type 1 astrocytes (Fig. 1a, b) while type 2 are resistant. Caution has to be advocated when comparing viruses of the papovavirus group, since their mode of replication may be different as is seen with their method of transforming cells (Topp et al., 1981).

The other susceptible cell types were fibroblasts and leptomeningeal cells identified with antibodies to fibronectin. Immunofluorescent staining with PV/FITC of leptomeningeal cells in vivo also shows that they are susceptible to polyoma virus infection when 1-day-old neonates are infected intracerebrally and sections cut of the whole head 7 to 10 days post-infection (D. J. McCance, unpublished data). Wroblewska et al. (1982) have reported that fibronectin-positive cells from human brains were susceptible to JCV infection in vitro.

Finally, neurones are not infected in vitro although Wallerian degeneration seen in vivo in nude mice exhibiting hind leg paralysis after infection with polyoma virus might suggest some direct effect on neurones (McCance et al., 1983; Harper et al., 1983) although studies in vivo have not shown any virus or viral antigens in these cells.

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REFERENCES


Short communication


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