DIFFERENTIAL EFFECTS OF MHV-4 INFECTION OF ASTROCYTES AND OLIGODENDROCYTES IN VITRO

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INTRODUCTION

Mouse hepatitis virus, type-4 (strain JHM) has been studied extensively as a model for neurologic disease. Intracerebral infection may lead to fatal encephalomyelitis or may be limited to demyelination (1). Prior to demyelination by myelin stripping macrophages (2), connections between myelin-forming oligodendrocytes and myelin sheaths, not ordinarily visible in the adult nervous system, become apparent (3). Of particular interest, in regard to this observation, demyelination in this model system is followed by remyelination mediated by oligodendrocytes (4,5). However, virus persistence and limited areas of recurrent demyelination may occur (5,6).

To investigate the specific oligodendrocyte response to infection with MHV-4, primary mouse brain cultures of astrocytes and oligodendrocytes were established, characterized and studied following infection with this virus.

MATERIALS AND METHODS

Primary cultures of mouse astrocytes and oligodendrocytes were prepared using an adaptation of the cell-dissociative technique for obtaining rat astrocytes and oligodendrocytes (7). The major modifications involved using 16-17 day embryonic brain which was allowed to reaggregate over a 3-4 week period, and then plating the aggregates (8). Oligodendrocytes in clusters, and some astrocytes grew out from these aggregates.

Astrocytes were identified by labeled antibody to glial fibrillary acidic protein (a gift from L. Eng). Oligodendrocytes were identified by labeling antibodies to either galactocerebroside (a gift from R. Hughes), myelin-associated glycoprotein (a gift from R. Quarles) or myelin basic protein (a gift from G. Tennekoon). Commercial conjugates of goat anti-

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rabbit IgG with rhodamine, goat antimouse IgG with fluorescein, and protein A-peroxidase were used as secondary reagents. Colloidal gold preparations were prepared in the laboratory (9). The specific enzyme activity of the oligodendrocyte marker enzyme glycerol-phosphate-dehydrogenase (GPDH) was measured as described by McCarthy and de Vellis (7).

Mouse hepatitis virus was prepared as a stock as previously characterized (6). Cultures maintained in vitro for at least one month prior to use were infected. The day of infection was considered day 0, and the cultures were observed daily over the following 10 day period.

RESULTS

Astrocytes appeared as phase-gray flat cells, although they were occasionally observed to bear broad processes. These cells were cytoplasmically labeled for glial fibrillary acidic protein (Figure 1), but not the oligodendrocyte markers. Oligodendrocytes appeared as phase-dark round cells with multiple radiating processes, and grew out from the brain aggregates (Figure 2). These cells were surface labeled for galactocerebroside (Figure 3) or cytoplasmically labeled for myelin-associated glycoprotein or myelin basic protein with each oligodendrocyte marker, likely reflecting their state of maturation.

Mouse oligodendrocytes behave differently from rat oligodendrocytes in response to exposure to hydrocortisone. In the rat, exposure of cultures to hydrocortisone, at a concentration of 1 mg/ml, induces the expression of the oligodendrocyte-specific enzyme glycerol-hposphate-dehydrogenase (GPDH). GPDH activity in the mouse glial cultures did not fluctuate in response to exposure to hydrocortisone (Table 1), even at a variety of concentrations, although rat cultures did respond (data not shown).

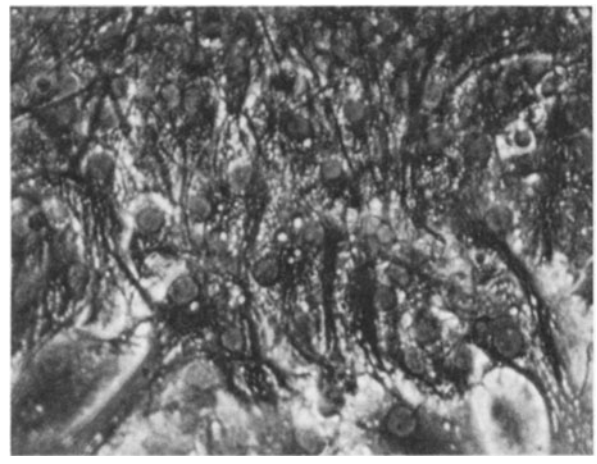

Fig. 1. Astrocytes in vitro show a fibrillar pattern of cytoplasmic staining when labelled for glial fibrillary acidic protein (above), but appear as flat phase-gray cells by phase microscopy. Magnification, 200X.

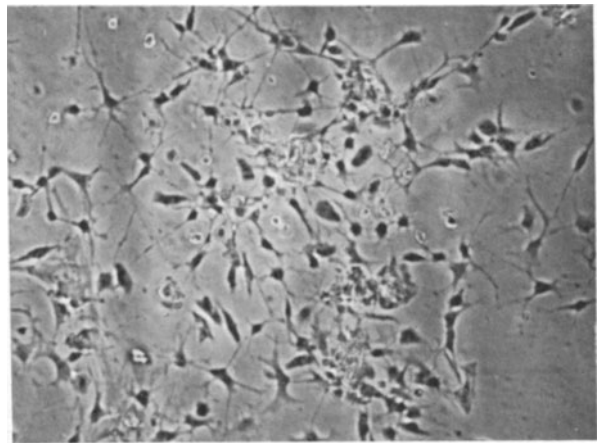

Fig. 2. Oligodendrocytes appear as round phase-dark cells with multiple radiating processes by phase microscopy, shown here growing out of brain aggregates. Magnification, 100X.

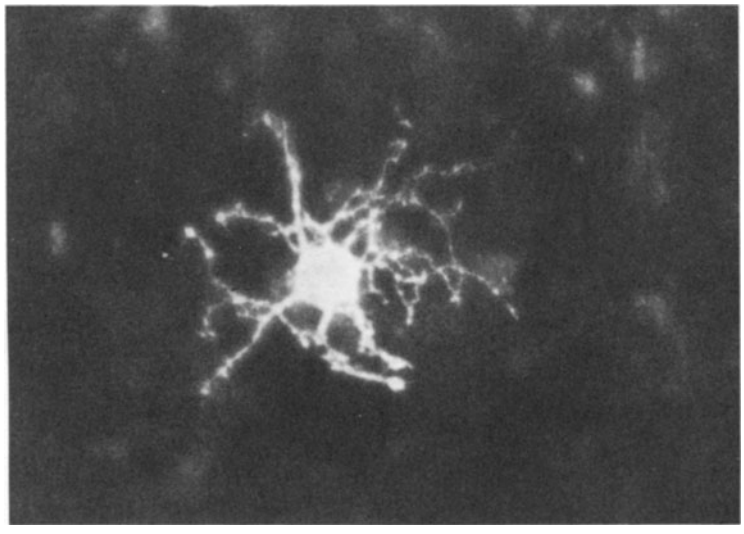

Fig. 3. Oligodendrocyte surface labeled with antibody to galactocerebroside. Magnification, 600X.

Table 1. Glycerol-Phosphate-Dehydrogenase (GPDH) Activity in Mouse Glial Cell Cultures

Stimulus	Total Protein (mg/ml)	GPDH
No Stimulus	0.80	23.48
Hydrocortisone (1 mg/ml)	1.83	22.97
MHV-4	0.64	44.94

Following infection with MHV-4, astrocytes in the cultures formed multi-nucleated giant cells (Figure 4), within 24 hours of infection. These infected syncytia could be identified as astrocytic in origin by their labeling for both MHV antigens and GFAP, but not oligodendrocyte markers. GFAP labeling was eventually lost prior to the syncytia undergoing cytolysis. In contrast, oligodendrocytes developed more and broader processes than usual 5 days after infection (Figure 5), but did not form multi-nucleated giant cells. Oligodendrocytes were not observed to undergo cell-fusion during the 10 day observation period, although there was some dropout of this cell type. Since the cultures in these experiments were neither purely of oligodendrocyte or astrocyte origin, specific quantitation of viral replication by oligodendrocytes relative to astrocytes could not be determined.

The failure of fusion to occur by MHV-4 infected oligodendrocytes was not related to lack of expression of the surface glycoprotein of the virus with fusion activity, E2. Both viral

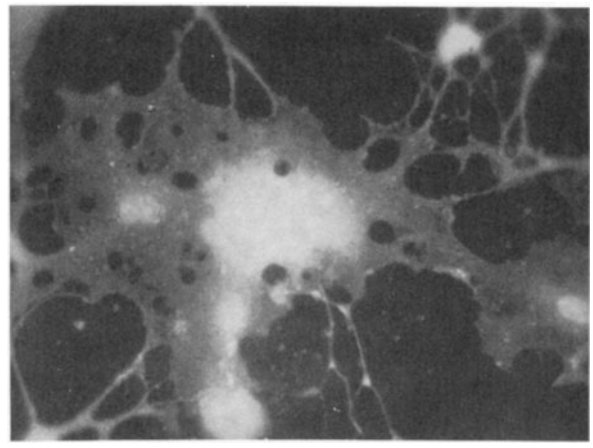

Fig. 4. Astrocytes fuse to form multi-nucleated giant cells (syncytia) 24 hours after infection, shown here with a fluorescein-label of MHV antibody. Magnification, 100X.

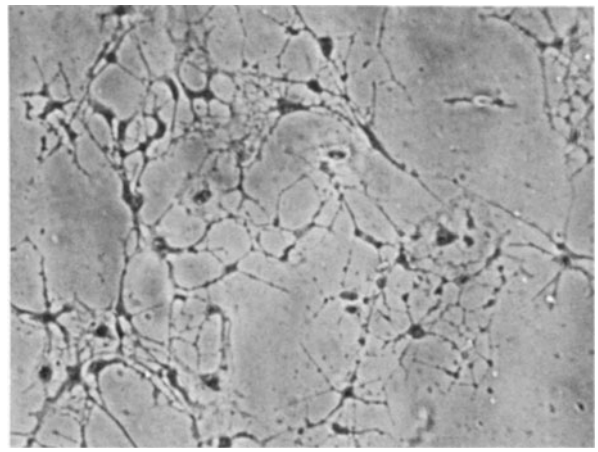

Fig. 5. Oligodendrocyte showing broader and thicker processes five days after infection with MHV-4. Magnification, 100X.

glycoproteins, E1 and E2, were detected on the surface of infected oligodendrocytes (Figure 6). Because MHV-4 infected oligodendrocytes contained more and broader processes, and thus appeared stimulated, activity of the oligodendrocyte-specific marker enzyme GPDH was measured. GPDH activity was doubled over baseline levels following MHV-4 infection (Table 1).

DISCUSSION AND CONCLUSIONS

MHV-4 infection of primary cultures of mouse astrocytes and oligodendrocytes was studied to learn about the direct responses of these cells to infection with this virus. Astrocytes fuse within 24 hours of the infection, forming multinucleated giant cells which then lyse. In contrast, oligodendrocytes were not observed to fuse and rarely lysed. The

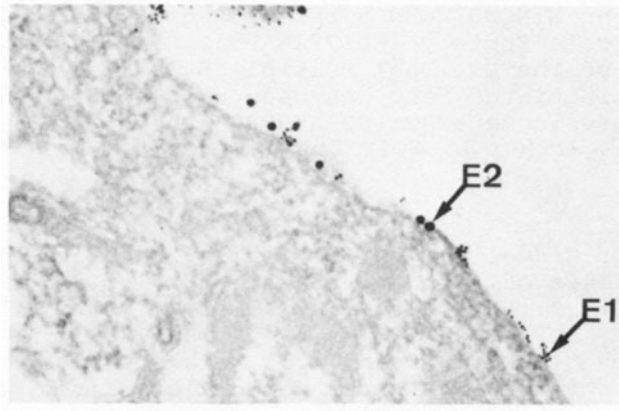

Fig. 6 Electron micrograph of an MHV-4 infected oligodendrocyte, immuno-labeled with colloidal gold to show E2 (large spheres) and E1 (small spheres). Magnification 28,000X.

lack of fusion observed in infected oligodendrocytes is not presently understood, but is not due to a lack of expression of the E2 (fusion) glycoprotein on these cells.

Following infection with MHV-4, oligodendrocytes physically appeared stimulated developing more and broader cell processes over the course of several days. This response *in vitro* is akin to their response following MHV-4 infection described *in vivo*. In the adult nervous system, rarely observed connections between oligodendrocytes and myelin sheaths became easily observed following MHV-4 infection (3). Biochemical evidence indicating direct stimulation of oligodendrocytes following MHV-4 infection also comes from finding increased activity of the oligodendrocyte cell-specific enzyme glycerol-phosphate-dehydrogenase.

The restriction of fusion and limited lysis of oligodendrocytes but not astrocytes *in vitro*, if reflecting similar events following MHV-4 infection *in vivo*, are important new findings. They suggest that MHV-4 induced demyelination may not simply be due to a lytic infection of oligodendrocytes and subsequent passive clearing of myelin debris by macrophages. Instead, demyelination may reflect active stripping by macrophages of myelin expressing MHV antigens (10), without oligodendrocyte death, or passive stripping of myelin by activated macrophages recruited to an infected area. In either of these latter situations, some infected oligodendrocytes may survive, and possibly remain persistently infected. Surviving MHV-4 infected oligodendrocytes may contribute to remyelination, and if persistently infected may later contribute to recurrent demyelination observed in this model system (5,6). Thus, the continued investigation of specific oligodendrocyte responses following infection with MHV-4 should provide new insights into the mechanisms of demyelination, remyelination, recurrent demyelination and persistent infection characterizing this model system.

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REFERENCES

1. H. Wege, S. Siddell and V. terMeulen, The biology and pathogenesis of coronaviruses, Curr. Top. Microbiol. Immunol., 99:165 (1982).

2. P.W. Lampert, J.K. Sims and A.J. Kniazeff, Mechanism of demyelination in JHM virus encephalomyelitis, Acta neuropath., 24:76 (1973).

3. H.C. Powell and P.W. Lampert, Oligodendrocytes and their myelin-plasma membrane connections in JHM mouse hepatitis virus encephalitis, Lab. Invest., 33:440 (1975).

4. R.M. Herndon, D.L. Price and L.P. Weiner, Regeneration of oligodendroglia during recovery from demyelinating disease, Science, 195:692 (1977).

5. R.L. Knobler, P.W. Lampert and M.B.A. Oldstone, Virus persistence and recurrent demyelination produced by a temperature-sensitive mutant of MHV-4, Nature, 298:279 (1982).

6. R.L. Knobler, L.A. Tunison, P.W. Lampert and M.B.A. Oldstone, Selected mutants of mouse hepatitis virus type 4 (JHM strain) induced different CNS diseases. Pathobiology induced by wild-type and mutants ts8 and ts15 in BALB/c and SJL/J mice, Am J. Pathol., 109:157, (1982).

7. K.D. McCarthy and J. deVellis, Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissues, J. Cell Biol, 85:890 (1980).

8. R.L. Knobler, R. Cole, J. deVellis and M.B.A. Oldstone, The preparation and characterization of primary mouse astrocyte and oligodendrocyte cultures: Fetal age and preparative variations which influence outcome, in preparation (1986).

9. S.L. Goodman, G.M. Hodges, K.L. Trejdosiewicz and D.C. Livingston, Colloidal gold markers and probes for routine application in microscopy, J. Microsc. 123:201 (1981).

10. R.L. Knobler and M.B.A. Oldstone, The role of host genes and virus-cell tropism in virus induced demyelination: A unique murine experimental model for understanding human demyelinating disease, in: "Viruses and Demyelinating Diseases," C.A. Mims, M.L. Cuzner and R.E. Kelly, eds., Academic Press, London, (1983).