JOURNAL OF VIROLOGY, Mar. 1985, p. 1016–1019
0022-538X/85/031016-04$02.00/0

Vol. 53, No. 3

Differential Susceptibility of Cultured Neural Cells to the Human Coronavirus OC43

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Received 10 August 1984/Accepted 4 November 1984

By using cell-type-specific markers and neural cultures derived from various areas of the nervous system, it has been possible to identify various interactions between OC43 virus and mouse oligodendrocytes, neurons, astrocytes, and fibroblasts. Neurons derived from dorsal root ganglia produced viral antigen and infectious virus. Astrocytes and fibroblasts both produced viral antigen but not infectious virus. Oligodendrocytes produced neither infectious virus nor viral antigen. Human embryo brain cells, including astrocytes, were susceptible to OC43 infection but did not produce infectious virus.

OC43 is a human respiratory virus of the *Coronaviridae* family, which has been adapted to grow in suckling mouse brain cells and thus cause lethal infection. OC43 virus, originally isolated in organ cultures from nasal secretions, has been adapted to grow in suckling mouse brain cells; it is, however, a human virus, and its ability to grow in human brain cells is of interest. Reports of coronavirus isolations from brain cells of multiple sclerosis patients (3, 7) have stimulated our laboratory studies on the neurotropism of human coronaviruses. Infected mice display an age-related susceptibility to the virus and a striking selective vulnerability of neural cells (14). An examination of infected mouse brain cells revealed extensive infection of the cerebral cortex and particularly of neurons, e.g., in the hippocampus. Neurons of the dorsal root ganglia were also infected, and cerebellar infection was restricted to Purkinje cells. Even in extensively infected brain cells there was little or no necrosis, and it did not appear that neuronal destruction was responsible for host cell death.

Neural cultures offer an opportunity to study the basis for selective vulnerability of certain cells to OC43 infection. The age-dependent resistance of mice to OC43, moreover, may be mediated by changes in the ability of neural cells to support virus growth (14). Work on another neurotropic coronavirus, JHM, has demonstrated that intracerebral inoculation of BALB/c mice leads to death within 7 days (17), whereas SJL mice inoculated with the same dose of virus survive (12, 17). By the use of neural cell cultures, Knobler et al. (12) have shown that neurons from susceptible BALB/c mice produce infectious JHM virus, whereas those from resistant SJL mice do not. This demonstrates that resistance is determined at the level of the neuron. Cultures of astrocytes from SJL (resistant) mice, however, are permissive for JHM infection (4). It has also been shown (5, 11) that strains

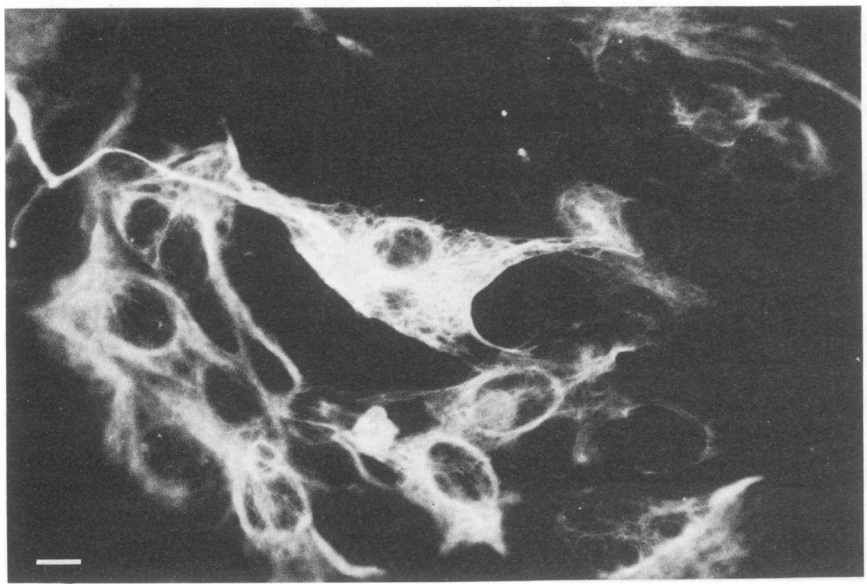

FIG. 1. MEB culture 3 days after infection with OC43 virus; immunofluorescent staining (rhodamine) with glial fibrillary acidic protein antibody. Bar = 10 μm.

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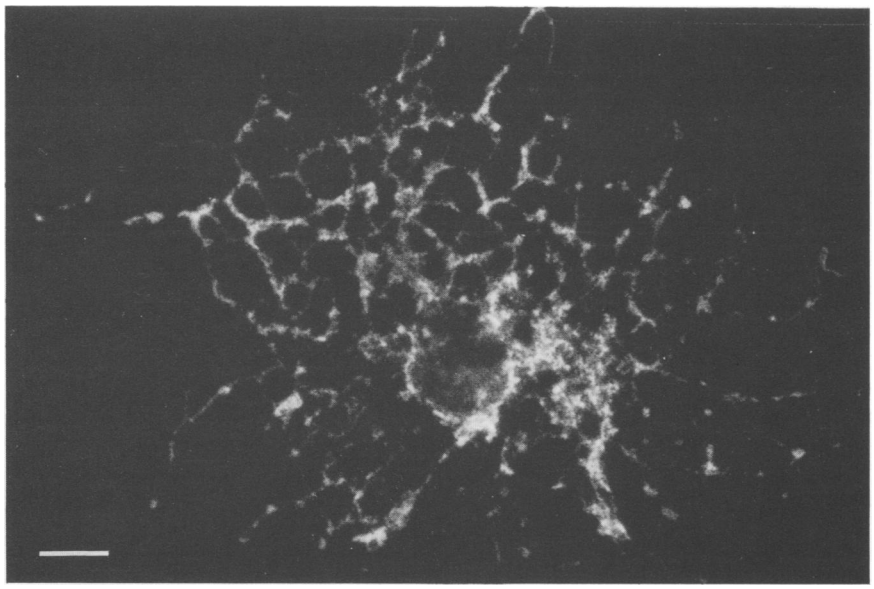

FIG. 2. Oligodendrocyte from corpus callosum; immunofluorescent staining with galactocerebroside antibody. Bar = 10 μm.

of JHM which give rise to various forms of disease infect different types of cells. Wild-type JHM infects and damages neuronal and nonneuronal cells in vitro, and animals infected with this virus usually develop a lethal encephalitis (18), with death resulting from neuronal destruction. A mutant of JHM (*ts*8) which causes primary demyelination in mice (8) infects nonneuronal cells but not neurons (5). The production of acute encephalitis or demyelination by mutants of JHM virus appears to depend on neural cell tropism in vivo (11), and tropism for neural cells in vitro predicts the disease pattern in animals.

In the studies reported here, the combination of specific neural cell markers and virus-specific antiserum was used to determine which cell types are susceptible to infection with OC43.

OC43 (a gift from D. A. J. Tyrell) stock virus was a 10% suspension of infected suckling mouse brain and had a titer of 10^9 SMic 50% lethal dose (LD_{50}) ml (equivalent to 10^8 tissue culture infective doses per ml). The LD_{50} assay was performed in 1-day-old CD1 mice by intracerebral inoculation. Neural cultures were labeled with specific antisera to identify the cell types present: astrocytes (Fig. 1) and oligodendrocytes (Fig. 2) were labeled with rabbit anti-glial fibrillary acidic protein (1, 6, 16) and rabbit anti-galactocerebroside (13, 16) (both from N. Gregson), respectively. Fibroblasts were labeled with anti-fibronectin (Cappel Laboratories, Cochranville, Pa.) and neurons (Fig. 3) by the anti-neurofilament monoclonal antibody RT97 (20) from J. Wood.

Primary mouse embryo brain (MEB) cultures were obtained after trypsinization of 18-day-old embryos of 1-day-old mice and grown on glass cover slips coated with *poly*-L-lysine. Corpus callosum from 3- to 4-day-old mice was dissected out, and a cell suspension was prepared by modi-

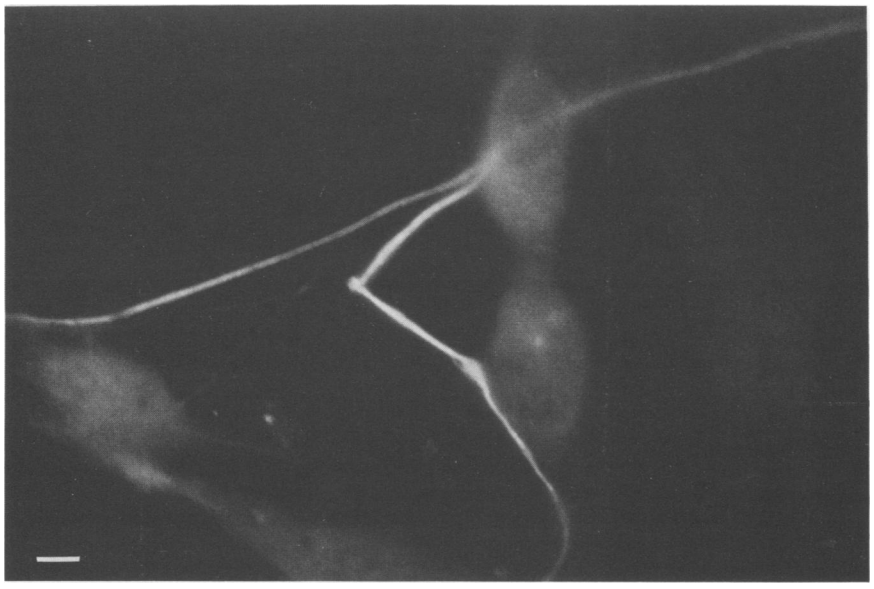

FIG. 3. Dorsal root ganglion neuron; immunofluorescent staining with RT97 neurofilament antibody. Bar = 10 μm.

TABLE 1. Types of cells found in cultures derived from various areas of the nervous system

Sample	% of various cell types in culture				
	Astrocytes	Fibroblasts	Oligodendrocytes	Neurons	Schwann cells[a]
MEB cultures	70–80	20–30	—[b]	—[b]	0
Corpus callosum	60–70	20–25	10–15	0	0
Dorsal root ganglia	0	20–25	0	10–15	60

[a] Identified by bipolar morphology.
[b] Very occasionally seen in cultures up to 4 days after seeding but rarely present later than this.

TABLE 2. Infectious virus titers in various cell types

Type of cell	Marker	% of cells displaying viral antigen	Titer of infectious virus in supernatant (SMic LD_{50}/ml 3 days after infection)	CPE
Astrocytes	Glial fibrillary acidic protein	20–30	$<10^2$	No
Oligodendrocytes	Galactocerebroside	0	$<10^2$	No
Fibroblasts	Fibronectin	15	$<10^2$	No
Neurons	Neurofilament RT97	25	$10^{4.3}$	No

fications of the method described by Raff et al. (15). MEB and corpus callosum cultures were grown in minimal essential medium containing 10% heat-inactivated fetal calf serum, 0.6% glucose, and 20 mM L-glutamine. Dorsal root ganglia of 1-day-old mice were dissected out and incubated in trypsin and collagenase for 20 min, and a cell suspension was produced by passing the tissue several times through a syringe fitted with a 21-gauge needle and then through a 23-gauge needle. As described for MEB, cells were grown on collagen-coated cover slips in medium supplemented with 25 mM KCl and nerve growth factor from A. Lumsden. All cultures were labeled with specific neural cell markers, and the proportions of the various cell types found in each culture are shown in Table 1.

Neural cell cultures were infected with OC43 within 3 to 4 days after seeding, because oligodendrocytes and neurons did not survive for long periods of time in culture. MEB cultures infected with 10^6 tissue culture infectious doses of OC43 first displayed viral antigen in the cytoplasm by 14 h, as demonstrated by the staining of fixed cell preparations by the fluorescent antibody technique with hyperimmune mouse serum. Cell-surface-associated viral antigen was first detected by fluorescent antibody staining on live cells at 20 h after infection. Both fibroblasts and astrocytes were infected, but it was clear in double-labeling experiments (Fig. 1 and 4) that many of these cells remained uninfected. The cultures were monitored for up to 6 weeks after infection; during this period the cells were checked at weekly intervals for viability by trypan blue exclusion, for viral antigen, and for cytopathic effect (CPE). Astrocytes and fibroblasts remained infected but did not display CPE and continued to exclude trypan blue. Fluids and cells from these infected cultures were harvested at various times and assayed for the presence of virus by intracerebral inoculation into 1-day-old mice. In each case, control mice were inoculated with the last wash from cultures after virus absorption. No infectious virus was detected at any time (Table 2) in cultures maintained at either 37 or 33.5°C. It would appear, therefore, that although astrocytes and fibroblasts are positive for viral antigen, they do not produce infectious virus.

In corpus callosum cultures the oligodendrocytes remained uninfected; astrocytes and fibroblasts in the same culture were positive for OC43 viral antigen but did not produce infectious virus (Table 2). Dorsal root ganglia cultures contained neurons and fibroblasts which were positive for OC43 viral antigen, but those cells with typical Schwann cell morphology were negative. Neurons displayed antigens in granular form (both in the perikaryon and in the neuronal cell processes), antigen-containing neurons were still present, and no CPE was detected by day 7 after infection. Fluids from dorsal root ganglia cultures were

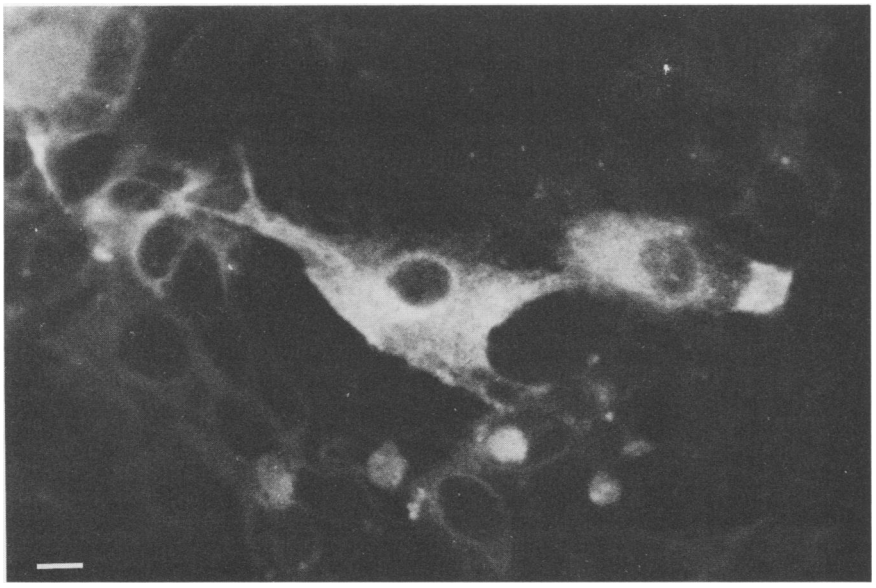

FIG. 4. Same field as shown in Fig. 1 stained (fluorescein) with OC43 antiserum showing infected cells. Bar = 10 μm.

inoculated into 1-day-old CD1 mice, and titers of virus in duplicate cultures reached $10^{4.3}$ SMic LD_{50}/ml and $10^{4.2}$ SMic LD_{50}/ml at 3 days postinfection (Table 2); however, no mice died after the inoculation of undiluted postabsorption fluids (titer of $<10^2$ SMic LD_{50}/ml). Because infected fibroblasts in MEB and corpus callosum cultures do not produce infectious virus and neurons were the only other cell type infected, this demonstrates that neurons undergo productive infection with OC43 and release infectious virus into the culture medium.

Cover slip cultures of primary human embryo brain cells were prepared from 12- to 16-week-old fetuses obtained from the tissue bank of the Royal Marsen Hospital, London, and infected with OC43. When fixed and stained on days 3 and 7 after infection, large numbers of antigen-positive cells were seen, including infected astrocytes, as detected by anti-glial fibrillary acidic protein immunofluorescent staining. Infected cells showed no CPE, and infectious virus was not detectable in the medium from these cultures. The age of the fetus and the time at which the tissue was taken after abortion make it unlikely that any neurons or oligodendrocytes were present (10); therefore, no double-labeling experiments with markers for these cells were carried out.

This study relies on the use of recently available methods for producing neural cell cultures, together with specific labeling of these cells with immunological markers. So far, these techniques have only been used to study herpes simplex virus (9), JC virus (19) infection of human neural cultures, and JHM virus infection of murine glial cultures (4). The results (Table 2) demonstrate a dramatic difference in the tropism of OC43 virus for various cells. Neurons undergo productive infection with the release of infectious virus, whereas oligodendrocytes fail to become infected. Astrocytes and fibroblasts show a defective cycle of infection with the production of viral antigen but no infectious virus. These in vitro results can be compared with those obtained for OC43 infection in vivo. High titers of virus are produced in the brains of infected suckling mice, and neuronal infection is prominent (14); preliminary observations made by electron microscopy on infected brain tissue confirm the presence of virus particles in neurons.

We failed to detect a CPE in infected neurons and astrocytes; the latter remained intact and infected for 6 weeks, and the former remained intact and infected for at least 7 days. There were also no signs of cell damage or necrosis in the brains of suckling mice dying 3 to 7 days after infection with OC43 virus (14) despite the fact that neurons were extensively infected and astrocytes were probably infected. At no time did we find demyelination in OC43-infected mice, and the in vitro results would suggest that this was because oligodendrocytes were not infected. It is, however, possible that a different strain of OC43 virus would infect oligodendrocytes and cause demyelination.

This work was supported by grant no. M/G/1-1/83 from the Multiple Sclerosis Society of Great Britain and Northern Ireland.

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