

8. This cell line (A-549) was described by D. J. Giard, S. A. Aaronson, G. J. Todard, P. Arnstein, J. H. Kersey, H. Dorsik, and W. P. Parks [*J. Natl. Cancer Inst.* 51, 1417 (1973)]; M. Lieber, B. Smith, A. Szakal, W. Nelson-Rees, and G. A. Todardo [*Int. J. Cancer* 17, 62 (1976)]; and K. L. Jones, N. S. Anderson III, and J. Addison [*Cancer Res.* 38, 1688 (1978)].
9. This cell line (MCF-7, estrogen-sensitive) was described by H. D. Soule, J. Vazquez, A. Long, S. Albert, and M. Brennan [*J. Natl. Cancer Inst.* 51, 1409 (1973)] and by K. B. Horwitz, M. E. Kostlow, and W. I. McGuire [*Steroids* 26, 785 (1975)].
10. Human uterine carcinosarcoma cells and endometrial adenocarcinoma cells were obtained from pathologically confirmed gynecologic tumors and developed as new cell lines. The endometrial adenocarcinoma cell line is estrogen-sensitive. Both were described by M. S. Kao and S. C. D. Lee (27th Annual Meeting of the Society for Gynecologic Investigation, Denver, 20 to 23 March 1980), abstr. 7.
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18. Parts of this report were presented at the 27th Annual Meeting of the Society for Gynecologic Investigation, Denver, 20 to 23 March 1980 (abstracts 7 and 150), and at the 73rd Annual Meeting of the Air Pollution Control Association, Montreal, 24 June 1980 (poster session 27). We thank W. Nelson-Rees for his gift of A-549 cells; the MCF-7 cells were obtained from E. M. Jensen. We also thank C. M. Copley, Jr., H. M. Camel, and T. Morgan for their constructive criticism of the manuscript. Correspondence should be addressed to F.S.

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Two Coronaviruses Isolated from Central Nervous System Tissue of Two Multiple Sclerosis Patients

Abstract. *Two coronaviruses were isolated from brain material obtained at autopsy from two multiple sclerosis patients. The viruses were neutralized by serum and spinal fluid from these patients. Although most of the population have antibody to these virus isolates, multiple sclerosis patients have slightly higher concentrations of serum antibody than controls. The results suggest that coronaviruses should be considered as one additional virus with a potential implication in the etiology of multiple sclerosis.*

Multiple sclerosis (MS) is a disease characterized by a variety of neurologic signs and symptoms resulting from damage to myelin in the central nervous system. The suspicion that MS has a viral etiology has intensified as a result of epidemiologic observations that MS occurs more frequently in specific geographic locations and that higher risk is related to exposure to an environmental agent before age 15 (1). Furthermore, the spinal fluids of MS patients contain antibody to viruses which is not present in the spinal fluids of control patients (2).

Virus isolations from MS tissue have been reported (3), but none has been confirmed and no single infectious agent has been consistently associated with the disease. When suckling mice or cell cultures particularly sensitive to coronaviruses were inoculated with fresh brain specimens obtained at autopsy from two MS patients, we were able to isolate two coronaviruses. Viruses of this family are known to cause upper respiratory infections in humans (4), but they had not previously been isolated from human brain material. Coronaviruses represent an attractive candidate for a role in the etiology of MS since viruses of this group cause a demyelinating-remyelinating disease in mice (5). Furthermore, using electron microscopy, Tanaka *et al.* have identified coronavirus-like particles in the brain of one MS patient (6).

Our first virus (S.D. virus) was isolated from a 55-year-old MS patient with brainstem dysfunction. An autopsy performed within 4 hours after death revealed typical MS plaques in the cerebrum, cerebellum, brainstem, and spinal cord. Histologic sections of medulla and pons showed areas of demyelination surrounded by reactive astrocytes indicating active disease. A 10 percent homogenate of fresh, unfrozen brainstem material in saline solution was inoculated intracerebrally into weanling BALB/c mice. Seven of the ten mice died between 2 and 6 months after inoculation. Seizures, myoclonic jerks, and limb pa-

ralysis were observed. When fresh brainstem material from a mouse that died 99 days after inoculation was inoculated intracerebrally into weanling mice, they developed neurological signs in approximately 50 days. Brainstem material obtained from these mice was inoculated intracerebrally into newborn BALB/c mice, and they died in 12 days. In subsequent serial passages the time between inoculation and death decreased to 3 to 5 days. We discovered during early passage attempts that the freezing of brain material from weanling and newborn mice resulted in the loss of transmissibility. Reinoculation of suckling mice with the original brainstem homogenate, which had been prepared after autopsy and frozen, failed to produce illness.

After failing to produce viral-induced changes in 16 different cell culture systems with homogenates prepared from brain material of infected suckling mice, we observed formation of syncytial tissue (giant cells) in a spontaneously transformed 3T3 Balb/c mouse cell line (17C1-1). Electron microscopy revealed coronavirus-like particles in the cell culture system and in the livers and brains of infected suckling mice. Negative-stain electron microscopy of virus released into supernatants in the infected cell culture revealed typical coronavirus particles (Fig. 1).

The second virus (S.K. virus) was isolated from an 89-year-old woman with weakness, spasticity, cerebellar dysfunction, and extraocular movement dysfunction. The disease was slowly progressive over a 30-year period excluding an initial period of 20 years of episodes of exacerbations and remissions. An autopsy performed within 4 hours after death revealed demyelinated areas in paraventricular white matter, brainstem, cerebellum, and spinal cord. A mild glial reaction was noted around

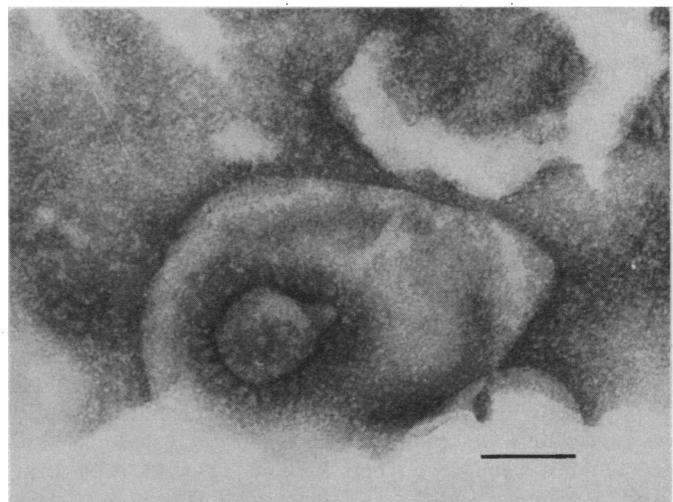


Fig. 1. Electron micrograph of virus isolate S.D. negatively stained with phosphotungstic acid. Average diameter of the virus particle is 100 nm with 20-nm corona-like peplomers. Scale bar, 100 nm.

most demyelinated areas. Homogenates of fresh autopsy material from frontal lobe, occipital lobe, parietal lobe, cerebellum, midbrain, pons, cervical spinal cord, thoracic spinal cord, lumbosacral spinal cord, and lymph nodes were inoculated into BALB/c mice and 17C1-1 cells. All mice remained healthy. However, on the 12th subculture syncytial formation was noted in the cells inoculated with material from the deep frontal lobe. Electron microscopy of the infected cells revealed coronavirus particles similar to those described for isolate S.D.

We have not succeeded in identifying coronaviruses or any other virus in homogenates of brain specimens obtained at autopsy from 11 additional MS patients and 12 patients without MS. However, our techniques only detect infectious virus particles. Coronaviruses may reside in a noninfectious or latent state in the central nervous system and, therefore, might only be intermittently detectable by our methods.

Since our virus isolates were obtained from mouse tissue, a question of primary importance is whether these viruses are of mouse or human origin. A mouse origin appears unlikely since the mice and the cell culture used to isolate the viruses showed no evidence of mouse coronavirus (mouse hepatitis virus) as judged by reactivity with mouse antibody in two tests: complement fixation on mouse serum and indirect fluorescence of mouse cells and cell cultures. Electron microscopic studies of cell cultures also showed no evidence of mouse coronavirus. In addition, endogenous coronaviruses have not been described previously in 17C1-1 cells despite their wide use in coronavirus investigations. Coronaviruses were not being studied in our laboratory, however, at the time of isolation.

Although 5 of the 16 cell lines used in the identification and isolation procedures were of human origin, our virus isolates only grew in cells of murine origin. This might suggest that our isolates are of mouse origin, but we should point out that human coronaviruses are notoriously difficult to grow in human cell culture. For example, none of the 16 cell lines we used are sensitive to the human coronavirus OC43, which grows only in human tracheal organ culture or in the brains of suckling mice (7). This suggests that human coronaviruses might be adaptable to mouse tissue or cell cultures.

In an effort to confirm a human origin of the isolates, we obtained serums and spinal fluids from MS patients S.D. and S.K. at autopsy and assayed them for

Table 1. Plaque neutralization of virus isolates S.D. and S.K. by serum and cerebrospinal fluid (CSF) of patients S.D. and S.K. End-point serum titers represent the reciprocal of the highest dilution retaining greater than 50 percent plaque neutralization.

Patient	Source	Neutralization	
		Virus S.D.	Virus S.K.
S.D.	CSF	—	2
	Serum	160	80
S.K.	CSF	2	2
	Serum	320	320

viral antibody activity. Measuring antibody concentrations to our coronavirus isolates by plaque neutralization, we detected antibody to isolate S.K. in the spinal fluids of both patients. In addition, the concentration of serum antibodies to the respective isolates was high in both patients (Table 1). These data suggest that these patients had been exposed to our isolates or serologically related viruses. Further serological investigations (8) have revealed neutralizing antibody titers to our virus isolate S.K. in the serums of 85 percent of 22 normal patients tested and up to 97 percent of 65 MS patients tested.

Serum antibody titers to our coronavirus isolates in the serums of MS patients were elevated relative to those observed in control patients ($P < .01$, Wilcoxon rank sum test). Further studies (8) on 21 matched pairs of MS patients and controls revealed only borderline significance with our isolates ($.05 < P < .10$, Wilcoxon signed rank test). However, analysis for antibody to measles virus in matched pairs of MS patients and controls revealed no significant differences ($P > .10$).

We have also compared our virus isolates to known human coronaviruses (OC43, 229E) and mouse coronaviruses (JHM, A59) by plaque neutralization or immunoprecipitation studies (9). For the S.D. and S.K. isolates, we used normal serums (preimmune) and immune antisera produced in rabbits and guinea pigs as well as mice to eliminate mouse coronavirus contamination of immunized animals. The results indicate that neither isolate is related to 229E. Antiserum to human virus OC43 cross-reacts with our virus isolates and not with mouse coronaviruses A59 or JHM by plaque neutralization. However, when we used the more sensitive technique of immunoprecipitation, the same polypeptides precipitated from S.K.- or S.D.-infected cells with antiserum specific to OC43, A59, S.D., or S.K. This suggests that there is an extensive cross-reactivity

between isolates S.D. and S.K. and human strain OC43 as well as mouse strain A59.

Even if we could prove that our isolates originated in the brains of MS patients, the relation of coronaviruses and MS would not be established. For example, a latent coronavirus may have been activated in these patients but may not be responsible for MS. Latent herpesvirus is found in the trigeminal ganglion in up to 80 percent of the general population (10). Confirmation of the association of coronaviruses with MS will require repeated isolations or the direct demonstration of viral specific antigens or nucleic acid in demyelinated areas of brain material of MS patients.

The unusual sensitivity of our isolates to freezing during the initial isolation studies may explain the lack of successful isolations of coronaviruses from similar studies. Another explanation might be that cells selected for MS virus studies could not propagate coronaviruses.

To claim that coronaviruses play a role in the etiology of MS is premature. However, our results complement observations of earlier investigations that coronaviruses cause a demyelinating-remyelinating disease in animals (5) and that coronavirus-like particles were present in brain material of an MS patient (6).

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