

Coronavirus Antibodies in Sera From Patients With Multiple Sclerosis and Matched Controls

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• Sera from patients with multiple sclerosis and carefully matched controls were tested for antibodies to three strains of coronavirus. There was no significant difference in the levels of antibody in the patients vs the controls. We conclude that unless the strains of coronaviruses recently reported to have been isolated from patients with multiple sclerosis express important serological differences from those used in these studies, coronaviruses are not associated with the cause of multiple sclerosis.

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Burks et al¹ recently reported the isolation of coronaviruses from two patients with multiple sclerosis (MS). One isolate was recovered from mice in which CNS disease developed two to ten months after intracranial inoculation of human brain necropsy material. The other isolate was obtained following subculture of brain tissue from a patient with MS onto

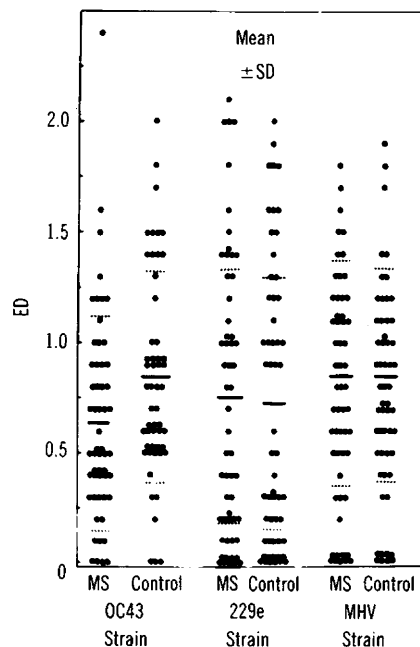
tissue culture monolayers. Coronaviruses are ubiquitous in man and animals, causing upper respiratory diseases and colds in man and encephalitis and hepatitis in mice. Antigenic analyses of coronaviruses have indicated that common group antigens exist. We report the prevalence of antibodies to three strains of coronaviruses in patients with MS and carefully matched controls.

PATIENTS AND METHODS

The patient populations have been previously described.² These patients and controls live in the Milwaukee area. The controls were chosen to minimize the possible socioeconomic and ethnic influence on the cause of MS. The factors considered in choosing the controls included (1) age match, ± 12 years; (2) sex match; (3) non-consanguinity; (4) birth and residence in the same general area for the first 15 years of life (within a 50-mile radius); and (5) acquaintance with each other for the past ten years.

Antibody levels to three strains of coronaviruses were determined by the enzyme-linked immunosorbent assay.³ The titer of

antibodies in the serum was expressed as a positive extinction deviation (ED).⁴ An ED result of 2 indicates a high level of antibodies to coronavirus; the lower the ED results, the lower the antibody level. Three antigenically different strains of coronaviruses were used. The strain OC43 is the only human isolate that hemagglutinates. The mouse hepatitis virus strain A59 (MHVA59) causes hepatitis and encephalitis in mice. The 229e strain was isolated from man. The antigenic preparations consisted of the following: (1) strain OC43 was a 10% suspension of mouse brain tissue; (2) strain MHVA59 was a cell-released virus



Occurrence of antibodies to coronavirus in serum from patients with multiple sclerosis (MS) and matched controls, as shown by enzyme-linked immunosorbent assay. Distribution of antibody titer among patients with MS and matched controls was similar for each strain. Differences were not statistically significant as shown by Student's two-tailed *t* test ($P < .05$). OC43, 229e, and MHV indicate strains of coronaviruses.

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Frequency of Occurrence of Antibodies to Coronavirus in Sera From Patients With Multiple Sclerosis and Matched Controls							
ED*	Level of Antibody	Antibodies to Strain OC43		Antibodies to Strain 229e		Antibodies to Strain MHVA59	
		Patients, %	Matched Controls, %	Patients, %	Matched Controls, %	Patients, %	Matched Controls, %
≥ 1.0	High	21.8	25.4	40	38.2	41.8	36.4
0.4-0.9	Low	34.6	58.3	10.9	9.1	36.4	36.4
< 0.4	Negative	43.6	16.3	49.1	52.7	21.8	27.9

*ED indicates extinction deviation.

prepared from the 17th clone line of BALB/C3T3 tissue culture cells and was purified on a sucrose gradient; (3) strain 229e was a 10% cell pack prepared from WI-38 tissue culture cells and purified on a sucrose gradient. Control antigens were prepared from uninoculated material and treated in a manner similar to the viral antigens.

RESULTS

The frequency of occurrence of antibodies to the three strains of coronavirus is shown in the Figure. In this population, the mean titer of antibodies to strain OC43 was slightly decreased in the patients with MS as compared with the antibody titer to strain 229e that was elevated in the patients with MS. The mean titer of the mouse hepatitis strain was similar in both the patients with MS and matched controls. None of the differences were statistically significant. This suggests that exposures of the patients with MS and matched controls to the coronaviruses were similar.

The intensity of the infections was estimated by dividing the antibody titers to coronaviruses into categories of high, low, and negative. The results

are given in the Table. The distributions of the high titers to each strain among the patients with MS and matched controls are similar. More persons had higher titers to strain 229e and the mouse hepatitis virus than to the OC43 strain. Most of the persons in the population had either a high titer or absence of titer to the 229e strain, whereas for the OC43 and the mouse hepatitis strains about one third of the population had antibody levels that fell into each group.

COMMENT

The frequency of occurrence of antibodies to these coronaviruses in the patients with MS and the matched controls differed for each strain examined, but there was no significant difference between patients and controls. These results were similar to those previously reported from other seroepidemiologic studies that have shown that 18% to 41% of the populations studied had antibodies to the 229e strain and 50% to 60% to the OC43 strain.³

Several different viruses have been isolated from the CNS tissue of

patients with MS, but none of these viruses have been shown to be associated with the cause of MS. These isolates include *Herpes hominis* type II⁶⁻⁸ and the 6/94 paramyxovirus that is very closely related to, if not the same as, Sendai virus.⁷ Seroepidemiologic studies have generally indicated that antibodies to measles virus are higher in persons with MS than in controls. The importance of this finding is unclear. In this group of patients with MS and matched controls, we found an increased level of antibodies to measles virus in these patients with MS but not to the coronavirus strains.⁸

From the data obtained, we conclude that the distribution of antibodies to the three strains of coronaviruses were similar in the patients with MS and the matched controls. Unless the coronavirus strains recently recovered from the patients with MS by Burk et al¹ are antigenically different and react separately from the human and murine strains used in this study, coronaviruses do not appear to be associated with the cause of MS.

References

- Burks J, Devald B, Jankovsky L, et al: Characterization of coronaviruses isolated using multiple sclerosis autopsy brain material. *Neurology* 1979;29:547.
- Madden DL, Wallen WC, Sever JL: Cellular immunity in multiple sclerosis—response to several viruses, in Karcher D, Lowenthal A, Strassberg AD (eds): *Humoral Immunity in Neurological Diseases*. New York, Plenum Press Inc, 1979, pp 17-36.
- Leinikki PO, Passila S: Solid phase antibody assay by means of enzyme conjugated to antiimmunoglobulin. *J Clin Pathol* 1976;29:1116-1120.
- Leinikki PO, Shekarchi I, Tzan N, et al: Evaluation of enzyme-linked immunosorbent assay (ELISA) for mumps virus antibody. *Proc Soc Exp Biol Med* 1979;160:363-367.
- McIntosh K: Coronaviruses: A comparative review. *Curr Top Microbiol Immunol* 1974;63:85-129.
- Gudnadottir M, Helgadottir A, Bjarnason O, et al: Virus isolated from the brain of a patient with multiple sclerosis. *Exp Neurol* 1964;9:85-95.
- Koprowski H, ter Meulen V: Multiple sclerosis and parainfluenza 1 virus. *J Neurol* 1975;208:175-190.
- Madden DL, Wallen WC, Houff SA, et al: Measles and canine distemper antibody: Presence in sera from patients with multiple sclerosis and matched control subjects. *Arch Neurol* 1981;38:13-15.