Measles Antibodies in the Cerebrospinal Fluid of Patients with Multiple Sclerosis (35704)

PAUL BROWN, FRANCOISE CATHALA,[1] D. CARLETON GAJDUSEK AND CLARENCE J. GIBBS, JR.
(Introduced by J. Anthony Morris)

National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014; Hôpital de la Salpêtrière, Paris, France.

This paper reports cerebrospinal fluid measles antibody levels, as measured by five different assay techniques, on 61 patients with multiple sclerosis (MS) and 59 patients with a wide variety of other neurological diseases. CSF from an additional 58 MS and 53 control patients were examined by three or four assay methods. CSF antibody titers were compared with serum titers in 50 MS and control patients, and in 100 patients enough spinal fluid was available to permit testing for antibodies to other myxoviruses.

A number of earlier studies have reported on measles antibodies in the sera of MS patients (1–16), and most authors have found significantly increased antibody titers in MS patients as compared to patients with other neurological diseases or to healthy adults. Spinal fluid antibodies have also been examined by four of these authors, and although their results have shown a trend towards a higher incidence of measles antibodies in MS patients than controls, individual series have been too small to achieve statistical significance (1–4, 7). Furthermore, no more than three and usually only one or two types of measles antibody have been measured in each study, and, except in the original study (1), antibody titers in positive CSF specimens have not been reported.

Materials and Methods. Patients. Cerebrospinal fluids were obtained from patients admitted to the neurology service of the Assistance Publique de Paris hospitals between 1966 and 1970. Both MS and control specimens were collected concurrently throughout this period. Most patients were seen at least once by one of the authors (F.C.), a neurologist, who also reviewed records from all patients.

Multiple sclerosis. Each of these 119 carefully selected patients showed a characteristic clinical picture of multifocal CNS damage with one or more relapses, and spinal fluids were collected in all cases during a relapse. The group comprised 27 males and 92 females, with a mean age of 40 years (range, 19-65 years), and a mean duration of illness of 7.5 years (range, 1–40 years).

Controls. In this group of 112 patients, there were 61 males and 51 females, with a mean age of 44 years (range, 8–82 years). Approximately one-half of the patients suffered from a tumor, infection, neuropathy, cerebrovascular accident, or trauma, with the remainder having a miscellaneous assortment of either primary or secondary diseases of the nervous system.[2]

Antibody assay methods. Cerebrospinal fluids (and sera) were stored at —20° until tested. The entire group of 231 cerebrospinal fluids was divided into three conveniently sized lots for testing of measles antibodies, each lot including specimens from both MS and control patients.

Standard methods were employed for each

[1] Guest scientist at the National Institutes of Health, Chargée de Recherches, Institut National de la Santé et de la Recherches Médicale, Paris.

[2] Medullary compression (5), idiopathic epilepsy (4), myelopathy (4), discopathy (4), encephalopathy (3), psychosis (3), metabolic disease (3), amyotrophic lateral sclerosis (3), dementia (2), cerebellar atrophy (2), ophthalmic migraine (2), and one patient with each of the following diagnoses: Carrentiel syndrome, Mills' ascending hemiplegia, Schilder's disease, polyarteritis nodosa, Arnold-Chiari syndrome, sarcoidosis, labyrinthitis, Freidreich's ataxia, syringomyelia, ophthalmic zoster, Behcet's syndrome, fever of unknown origin, and diagnosis unknown.

type of antibody test, and are fully described in the references accompanying the following brief descriptions. Measles neutralizing (N) antibody was tested in Biologics Standards Cercopithecus (BSC-1) monkey-kidney tissue culture using Edmonston strain measles virus (17). Complement-fixing (CF) and hemagglutination-inhibiting (HI) antibodies were measured by the macrotechnique using commercially prepared antigens[3] (HI antigen was Tween–ether treated) (18). Fluorescent antibody (FA) was determined by the antiglobulin method, using measles-infected human embryonic kidney tissue cultured on cover slips and a rabbit antihuman gamma globulin conjugate (19). Mixed-hemadsorbing (HAd) antibody was tested in measles-infected BSC-1 tissue culture tubes, which, after incubation with the CSF specimens, were washed and layered with human O RH(+) red blood cells freshly coated with human anti-D serum and rabbit antihuman IgG globulin (20).[4] Serial dilutions of each specimen were tested, using the following initial dilutions: undiluted CSF for N and CF tests, 1:5 for HI and FA tests, and 1:10 for the HAd test.

Testing of antibodies to myxoviruses other than measles was performed on 1:10 dilutions of CSF only. Neutralizing antibody was tested in primary rhesus monkey kidney tissue culture against influenza viruses (A/PR/8/34, A1/AA/1/57, A2/TW/1/57, B/Lee/-/40, and B/GL/1739/54), parainfluenza viruses (types 1–4), and respiratory syncitial virus (Long strain) (21). HI antibody was tested by the macrotechnique for rubella and mumps viruses (22).[5]

CSF and serum specimens were inactivated at 56° for 30 min for the CF test, but neither heat inactivation nor absorptions were done on CSF aliquots used for other tests (earlier experiments had shown no difference between tests of treated and untreated specimens). Sera tested for measles HI antibody were absorbed in standard fashion with kaolin and rhesus monkey red blood cells before testing.

CSF proteins. Total protein content was measured chemically with the Folin–phenol reagent according to the method of Lowry (23), adapted for use with an autoanalyzer (24). After determination of the total protein, gamma globulin was precipitated by zinc sulfate at pH 7.3 in the cold, and the amount of precipitate again determined by the Lowry method. Results of several thousand examinations, performed in the laboratories of the Hôpital de la Salpêtrière, have established the value of 40 (6) mg of total protein/100 ml of normal spinal fluid. Gamma globulin constitutes 5 (1.6)% of the total protein in normal spinal fluid, and any value above 9% is considered abnormal.

Results. In the group of 61 MS patients on whose CSF all five tests were done, 27 (44%) had measles antibody detected by one or more conventional tests (N, CF, HI, FA). All but three of these 27 patients also had HAd antibody. In another 18 patients (30%), antibody was detectable only by the HAd test. In the remaining 16 patients (26%), no antibody was detected by any of the five tests used. Thus, more than half of the MS patients had no conventional measles antibody, and one-half of these had no antibody detectable even by the unusually sensitive HAd test.

In the group of 59 control patients studied by all five tests, only 10 (17%) had conventional measles antibody, and all 10 also had HAd antibody. In another 19 patients (32%), antibody was detectable only by the HAd test, and the remaining 30 patients (51%)

[3] Microbiological Associates, Bethesda, Maryland.

[4] The specificity for measles antibody in this extraordinarily sensitive test was verified by the finding that no HAd measles antibody could be detected at an initial dilution of 1:10 (or at higher dilutions) in control sera from 15 children living on isolated Pacific islands, known on epidemiological grounds never to have been exposed to measles virus, and with no detectable N or HI antibody in undiluted sera.

[5] Tween–ether treated rubella antigen was kindly furnished by Mrs. Eva Brown, and egg-grown sonicated mumps antigen by Dr. James Simsarian, both of the Division of Biologics Standards, National Institutes of Health.

TABLE I. Percentage of Cerebrospinal Fluid Specimens Positive for Measles Antibodies in Multiple Sclerosis and Control Groups.

		Antibody tests[a]			
Multiple sclerosis	N	CF	HI	FA	HAd
Number tested	116	61	119	89	119
Number positive	15	24	13	11	94
Percent positive	13	39	11	12	79
Controls					
Number tested	109	61	112	95	112
Number positive	11	6	5	4	51
Percent positive	10	10	4	4	47

[a] Initial dilutions of CSF as follows: Undiluted for N and CF, 1:5 for HI and FA, and 1:10 for HAd. Individual significance levels for comparisons of MS and control groups: $P < 0.05$ for HI and FA tests, $P < 0.001$ for CF and HAd tests, and (by a permutation test) $P < 0.001$ for combined antibody tests.

had no detectable antibody by any test.[6]

Percentages of CSF specimens containing measles antibody detected by each of the five tests are shown in Table I. Each type of antibody was found significantly more often in the group of MS patients than in the control group, except neutralizing antibody, which occurred about as often in MS (13%) as control (10%) patients. HI and fluorescent antibodies were found almost as frequently as neutralizing antibody in MS patients, but occurred less often (4%) in control patients. CF antibody was more frequent in MS patients (39%) and appeared in 10% of controls.

In contrast to these relatively low frequencies, HAd antibody occurred in 79% of the MS patients, and 47% of the controls. Comparison of HAd antibody frequency in MS and control males, or MS and control females, yielded values nearly identical to those cited in comparison of the total MS with control groups. Except for HAd antibody, the titers were of a low order, as shown in Table II. For HAd antibody, calculation of geometric mean titers on the positive specimens yielded significantly different values of 1:34 and 1:25 for the MS and control groups, respectively ($p<0.05$).

Antibody levels in all specimens in which a titer of at least 1:10 was obtained in one or more of the conventional antibody tests are presented in Table III, together with their CSF protein levels. These comprise 10 MS and three control subjects. When these 10 MS patients with the highest antibody titers were compared to 22 MS patients without any detectable antibody, the difference in the average gamma globulin contents of 16.3 and 3.9 mg %, respectively, was highly significant ($p<0.001$).[7] A lesser degree of overall correlation was found between HAd antibody titer and CSF gamma globulin content in both the MS ($R = 0.436$, $p<0.05$).[7]

In the 50 patients (33 MS and 17 controls) for whom matching CSF and serum specimens were available, the highest CSF titers were associated with high serum titers for those antibodies measured (CF, HI, HAd). However, it should be noted that many other patients with equally high serum titers had little CSF antibody, and that results of the HAd test, which permitted statistical analysis, did not show a significant overall correlation between CSF and serum antibody levels ($R = 0.201$).[8]

Finally, the frequency of antibodies to

[6] If all of the 119 MS and 112 control patients tested for three or more types of antibody are included for analysis, the following values are obtained: 32% MS and 13% control patients were positive for conventional antibody, 50% MS and 35% control patients were positive only for HAd antibody, and 18% MS and 52% control patients were negative for all antibodies.

[7] Comparable figures for the three high titer control subjects and 51 control subjects without antibody are 18.3 and 8.7 mg %, but the small number of high-titer subjects renders statistical analysis meaningless. Furthermore, of the three, one had SSPE, and in the other two (one with polyradiculitis and one with a subdural neurinoma) the very high CSF protein levels suggest serum leakage through the affected meningeal surfaces. Also, 10 of the 51 control patients without antibody had gamma globulin levels actually higher than the mean value in the high titer group of MS patinets. A lesser degree of overall correlation was found between HAd antibody titer and ESF gamma globulin content in both the MS ($R = 0.436$, $p < 0.05$) and control groups ($R = 0.484$, $p < 0.05$).

[8] Sample correlation coefficients based on Fisher's z transformation, which provides only an approximate significance level, owing to the discrete nature of the data.

TABLE II. Distribution of Measles Antibody Titers in Positive CSF Specimens.

Titer	Patients	Number of specimens with reciprocal antibody titer of:								
		1	2	4	5	10	20	40	80	≧ 160
N	MS	12	1	0	1	1	0	0	0	0
	Control	8	0	0	1	2	0	0	0	0
CF	MS	9[a]	11	4	0	0	0	0	0	0
	Control	1[a]	3	2	0	0	0	0	0	0
HI	MS	ND[b]	ND	ND	5	4	3	1	0	0
	Control	ND	ND	ND	1	2	1	1	0	0
FA	MS	ND	ND	ND	8	3	0	0	0	0
	Control	ND	ND	ND	2	1	0	1	0	0
HAd	MS	ND	ND	ND	ND	22	27	17	14	14
	Control	ND	ND	ND	ND	20	14	10	4	3

[a] Titer ≧ 1.
[b] Not done.

myxoviruses other than measles did not differ significantly in the 65 MS and 35 control patients whose CSF was tested (1:10 dilution only). In the MS group, five patients had antibody to influenza type A2, one to influenza type B, three to parainfluenza type 4, one to RSV, and two to rubella. In the control group, three patients had antibody to influenza type A2, and one to RSV. All specimens with influenza type A2 antibody were collected after a type A2 epidemic in the Winter of 1967–1968.

Discussion. Adams, who first reported that the CSF of MS patients contained increased amounts of measles antibody compared to controls (1), subsequently found no difference between MS and control CSF (7), when the MS patients were not selected because of unusually high serum antibody titers, as was true of his first series. The three other series also failed to show statistically significant differences between MS and control groups (2–4).

In our series of CSF specimens, measles

TABLE III. Patients with Highest[a] CSF Measles Antibody Titers.

Diagnosis	CSF protein		Reciprocal antibody titer				
	Total protein (mg %)	γ globulin (mg %)	N	CF	HI	FA	HAd
Multiple sclerosis	38	3	10	4	10	<5	80
Multiple sclerosis	43	10	1	2	10	<5	40
Multiple sclerosis	71	9	<1	4	<5	10	40
Multiple sclerosis	78	21	<1	4	10	<5	160
Multiple sclerosis	74	24	1	2	10	5	640
Multiple sclerosis	51	23	<1	<4	<5	10	20
Multiple sclerosis	65	23	5	<4	20	5	80
Multiple sclerosis	96	27	1	<4	20	5	320
Multiple sclerosis	92	16	2	<4	40	5	320
Multiple sclerosis	36	7	<1	<4	20	5	20
Polyradiculoneuritis	130	18	10	<4	40	5	640
Cervical subdural neurinoma	119	17	5	<4	20	10	320
Subacute sclerosing panencephalitis	128	35	10	2	10	40	40

[a] Titer ≧ 1:10 for any conventional (N, CF, HI, FA) antibody.

antibodies were present significantly more often in MS than control patients. Presence of conventional measles antibodies in high titer correlated well with the presence of high-titer HAd antibody, and whether or not we accept the HAd test as indicating only specific measles-induced antibody (no nonspecific reactant has yet been demonstrated), there still remains the highly significant difference between HAd activity in MS and control groups. In a few patients, antibody titers reached levels seen in SSPE (25); however, almost one-third of the MS patients had antibody detectable only by the HAd test, and one-fourth had no antibody detectable by any test.

The importance of the difference in measles antibody levels between our MS and control patients is also supported by CSF protein data. High levels of measles antibodies in MS patients were significantly associated with high levels of CSF gamma globulin, whereas in neurological diseases other than MS, measles antibodies were often absent despite extraordinarily high levels of gamma globulin. Moreover, an association between gamma globulin levels and antibodies to myxoviruses other than measles was not observed.

If our positive results do mean that measles virus itself is involved in the pathogenesis of MS, we may well speculate as to the reason for the inconstant occurrence of antibody.

The data suggest that in at least half of MS patients there is no evidence for the type of measles virus involvement in a slowly progressive, presumably defective, and partly antibody-blocked infection such as is occurring in SSPE. However, one may hypothesize that in MS a similar process is underway, but with the virus genetic information further repressed, with replication occurring at an asynchronous, imperfect, and incomplete level, with no, or incomplete, virion assembly, or perhaps even with only a very defective virus genome present.

It might then be possible that only occasional patients have sufficient measles antigen released to produce specific measles sensitization, and that the type and relative quantities of antigen released could well vary from patient to patient. However, it is also possible that different latent agents might cause the same MS syndrome, and that the positive results occurred only in those MS cases wherein measles virus slow infection was involved.

Summary. Measles antibodies were measured in the cerebrospinal fluids of 119 patients with multiple sclerosis and 112 patients with other neurological diseases. The frequency of measles antibody was significantly greater in the MS group than in the control group, and, in the sensitive mixed-hemadsorbing antibody test, the geometric mean antibody titer was also significantly higher in the MS than control group.

A few MS patients had antibody levels in the range of patients with SSPE. On the other hand, one-fourth of the MS patients had no measles antibody by any of five different assays employed, and nearly one-third more had none but hemadsorbing antibody. If measles virus is involved in the pathogenesis of multiple sclerosis, it must be present in an unusually latent, masked, or defective condition.

We thank Dr. John Hooks, Miss Sarah Anderson, Miss Marcia Applegate, and Mr. Donald Nelson for laboratory assistance; Dr. Peter Shaughnessy for statistical analaysis; and Dr. Lim Kok Ann for critical appraisal of the manuscript.

1. Adams, J. M., and Imagawa, D. T., Proc. Soc. Exp. Biol. Med. **111**, 562 (1962).

2. Sibley, W. A., and Foley, J. M., Trans. Amer. Neurol. Ass. **88**, 277 (1963).

3. Reed, D., Sever, J., Kurtzke, J., and Kurland, L., Arch. Neurol. (Chicago) **10**, 402 (1964).

4. Pette, E., and Kuwert, E., Arch. Gesamte Virusforsch. **16**, 141 (1965).

5. Ross, C. A. C., Lenman, J. A. R., and Rutter, C., Brit. Med. J. **1**, 226 (1965).

6. Clark, J. K., Dane, D. S., and Dick, G. W. A., Brain **88**, 953 (1965).

7. Adams, J. M., Neurology **17**, 707 (1967).

8. Just, M., Reider, H. P., and Ritzel, G., Klin. Wochenschr. **45**, 707 (1967).

9. Panelius, M., Acta Neurol. Scand., Suppl. 39, **45**, 57 (1969).

10. Caspary, E. A., Chambers, M. E., and Field, E. J., Neurology **19**, 1038 (1969).

11. Ross, C. A. C., Lenman, J. A. R., and Mel-

ville, I. D., Brit. Med. J. 3, 512 (1969).

12. Mai, K., Add. ad Int. Arch. Allergy Appl. Immunol. **36**, 109 (1969).

13. Henson, T. E., Brody, J. A., Sever, J. L., Dyken, M. L., and Cannon, J., J. Amer. Med. Ass. **211**, 1985 (1970).

14. Adams, J. M., Brooks, M. B., Fisher, E. D., and Tyler, C. S., Neurology **20**, 1039 (1970).

15. Brody, J. A., Sever, J. L., and Henson, T. E., J. Amer. Med. Ass. (in press).

16. Sever, J. L., Kurtzke, J. F., Alter, H., Schumacher, G. A., Gilkeson, M. R., Ellenberg, J. H., and Brody, J. A., Arch. Neurol. (Chicago) (in press).

17. Meyer, H. M., Brooks, B. E., Douglas, R. D., and Rogers, N. G., Amer. J. Dis. Child. **103**, 495 (1962).

18. Katz, S. L., and Enders, J. F., *in* "Diagnostic Procedures for Viral and Rickettsial Infections" (R. H. Lennette and N. R. Schmidt, eds.), p. 521. Amer. Pub. Health Ass., New York (1969).

19. Adels, B. R., Gajdusek, D. C., Gibbs, C. J., Albrecht, P., and Rogers, N. G., Neurology **18**, 30 (1968).

20. Fagraeus, A., Espmark, J. A., and Jonsson, J., Immunology **9**, 161 (1965).

21. Morris, J. A., Hatano, M., Robinson, R. Q., Aulisio, C. G., and Smadel, J. E., Proc. Soc. Exp. Biol. Med. **114**, 406 (1963).

22. Plotkin, S. A., and Henle, W., *in* "Diagnostic Procedures for Viral and Rickettsial Infections" (R. H. Lennette and N. R. Schmidt, eds.), p. 391 and p. 471. Amer. Pub. Health Ass., New York (1969).

23. Lowry, O., J. Biol. Chem. **193**, 265 (1962).

24. Castaigne, P., Cambier, J., and Schuller, E., Rev. Fr. Étud. Clin. Biol. **10**, 529 (1965).

25. Gibbs, C. J., Gajdusek, D. C., Hooks, J., Lowenthal, A., and Adels, B. R., Pediat. Res. **3**, 495 (1969).

Received Nov. 25, 1970. P.S.E.B.M., 1971, Vol. 137.