Serum Immunoglobulins in Multiple Sclerosis Patients* (34055)

C. T. UYEDA, B. GERSTL, L. F. ENG, AND J. K. SMITH

Laboratory Service, Veterans Administration Hospital, Palo Alto, California and Department of Pathology, Stanford University School of Medicine, Palo Alto, California 94304

Of the several hypotheses of the etiology of multiple sclerosis (MS), that of an autoimmune process has gained prominence (1, 2). However, attempts to demonstrate circulating antibodies in sera of MS patients against components of brain tissue have yielded negative results (3–5). A myelinolytic agent first thought to be specific for MS sera (6), has also been found in sera of patients with other neurologic diseases and even in those of normal controls (7). Seitelberger (8) recently attempted to reconcile experimental immunologic data and pathologic–anatomic findings in MS to arrive at a plausible hypothesis of its pathogenesis. He postulated a central nervous system (CNS) virus infection or other primary insult followed by entrance of CNS immunogenic proteins into the circulation with ensuing immunologic sequelae. Earlier, several authors had suggested a virus etiology (9, 10), and measles antibodies had been extensively studied in patients with MS (11, 12). Other carefully controlled virus studies (13) could not establish a statistically significant difference between MS and control sera with the exception of observations of reactions of varicella–zoster antigen.

Link (14) reported recently that the composition of immunoglobulin IgG in cerebrospinal fluid (CSF) of MS patients differed from that in their sera while in controls this globulin in CSF and sera was the same. The purpose of the present study was to provide further clarification of the immunologic properties of the sera of MS patients by determination of their immunoglobulin levels, complement-fixing activities against several viral CF antigens, capacity to neutralize staphylococcal beta hemolysin, and reaction with encephalitogenic protein (EP) by means of Ouchterlony's double diffusion (15) and passive cutaneous anaphylaxis (PCA) (16) tests.

Materials and Methods. Sera were obtained from MS patients and normal control hospital employees. Immunoplates (Hyland Laboratories) containing antibodies specific for IgA, IgG, and IgM immunoglobulins (17) were used throughout the study. Their wells were filled with 3 μl of serum delivered from a Hamilton 10-μl syringe modified to hold a disposable tip (J. K. Turner Co., Palo Alto, California). Solutions containing known quantities of the three immunoglobulins, whose concentrations corresponded to those found in the serum, were employed to furnish standard curves. The plates were placed in a moist chamber at room temperature and the IgA and IgM plates were read at 48 hr and IgG plates after 1 week. The diameters of the precipitant rings were measured under 10 times magnification to the nearest 0.1 mm and their areas were calculated. The values for the sera were taken from the standard curve.

Complement-fixing antibody titers against viruses of the adenovirus group (Type 1 through 7), herpes simplex, measles, and poliovirus Type 1 were determined by the microtiter method of Sever (18) on MS and control sera selected on the basis of IgA globulin levels, each group being composed of sera possessing relatively high and low quantities of the globulin. Control sera possessing titers of 1:32, 1:64, 1:128, and 1:512, respectively, obtained from Microbiological Associates, Bethesda, Md., were used for block titration of CF antigens. Titration of anti-

* Supported by the U. S. Veterans Administration and U. S. Public Health Service Grant NB 03821-07.

staphylococcal beta hemolysin antibodies was performed as described previously (19). Double-diffusion tests were set up in 1% agarose in 0.05*M* Tris-HCl buffer, pH 7.6, with 15 MS and 8 control sera with EP serving as antigen. The latter was obtained from purified myelin of human brain (20). The PCA test (16) was performed with four MS and two control sera against EP. Each serum was diluted 1:10 and 1:100 in saline, to avoid nonspecific reactions, and injected intradermally (0.1 ml) 3 hr prior to challenge with the intravenous antigen–dye mixture (0.5 ml, containing 0.4 mg antigen in 0.5% Evans blue in saline, per guinea pig). Falk *et al.* (21) used 0.1 mg of basic protein for similar tests with guinea pig sera. Serum (1:10 dilution) of a rabbit immunized with a total of 2 mg of EP in complete Freund's adjuvant was included as a positive control. Six MS and 20 control sera were analyzed by immunoelectrophoresis (IEP) (22) with goat anti-whole serum, anti-IgA, anti-IgG, and anti-IgM sera (Hyland Laboratories). IEP was conducted in 1% agarose in 0.05*M* veronal buffer at pH 8.6 for 40 min at 100 V and 20–24 mA.

Results and Discussion. The total protein concentrations of all sera (Table I) were within the normal range. The mean values of gamma A and gamma G globulins on the 38 control sera were slightly lower and that of gamma M slightly higher, than those reported by Fahey *et al.* (23) and by Norberg (24).

The mean concentration of IgA in the sera of the two MS groups (Martinez and Palo Alto) was significantly higher ($p = .05$) than that of the controls (Tables II and III). The difference in the mean value of IgA globulins between the two groups of MS cases, and the difference in the mean of IgG levels between the MS and control groups, were not significant. The means of the IgM globulin levels in the two MS groups were nearly identical, and significantly lower, than that of the controls ($p = .01$).

Although it has been reported that antiviral activity resides in the IgA globulins of serum and secretions (25–28), a relationship between the IgA levels and antiviral complement-fixing antibody titers could not be established either for the MS or the control sera (Table IV). Although not disproving a virus etiology of MS, these results are of interest in view of the suggested epidemiologic correlation of the two disease groups (29, 30). Also, there was no apparent correlation of the IgA levels with titers of anti-

TABLE I. Description of Data.

	N	Mean	Standard deviation	Geometric mean
Age				
1 MS (PA)[a]	21	46.6	5.4	
2 MS (M)	33	47.2	13.7	
3 Control	35	37.3	14.6	
Total protein				
1 MS (PA)	21	6.7	0.5	
2 MS (M)	35	6.7	0.5	
3 Control	38	6.8	0.5	
Gamma A				
1 MS (PA)	21	207.1	93.3	187.3
2 MS (M)	36	267.5	162.1	230.8
3 Control	38	177.1	87.2	161.0
Gamma G				
1 MS (PA)	21	1136.2	571.9	1010.0
2 MS (M)	36	831.9	327.7	755.1
3 Control	38	1019.0	374.9	946.4
Gamma M				
1 MS (PA)	21	100.7	38.2	95.2
2 MS (M)	36	103.6	53.4	94.5
3 Control	38	136.7	58.4	124.7

[a] VA hospitals: (PA, Palo Alto, California; M, Martinez, California).

TABLE II. Results from Analysis of Variance.

Variable	*F*
Age	16.95[b]
Total protein	1.19 NS
Gamma A	5.45[b]
Gamma G	3.83[a]
Gamma M	5.56[b]

[a] Significant difference between groups at .05 level.

[b] Significant difference between groups at .01 level.

NS, not significant.

TABLE III. Results from Planned Comparisons between Groups.

	Age	TP	gamma A	gamma G	gamma M
MS (PA) vs. MS (M)	NS	NS	NS	[a]	NS
MS (PA + M) vs. control	[b]	NS	[a]	NS	[b]

[a] Significant at .05 level.
[b] Significant at .01 level.
NS, not significant.

staphylococcus beta hemolysin antibodies.

Anti-EP antibodies could not be demonstrated in MS or control sera, either by the agar diffusion or by the PCA test. The control rabbit serum did give a positive PCA test within 30 min of the antigen–dye injection.

The increased levels of IgA in MS sera are of interest in view of the finding of an IgA-like protein in sera of rabbits immunized with EP, which possess gliotoxic activity *in vitro*, that could be neutralized by prior incubation with EP (31). It is unlikely that the elevated IgA levels in the MS sera signify presence of antibody to brain protein since these sera failed to react with EP in the double diffusion, as well as in the PCA tests. IgA globulins, however, have been reported to be poor precipitants (25) and according to Bloch (32) are incapable of reacting in the PCA test in guinea pigs. This problem could possibly be resolved by comparing the gliotoxic effect of MS sera of high, with those of low, IgA content.

IgM globulin levels in MS sera were significantly lower than in controls. In a previous study (19) we found that MS patients possessed lower titers of anti-staphylococcal beta hemolysin antibodies than did a control

TABLE IV. Summary of Anti-Viral C-F and Anti-Staphylococcal Beta Hemolysin Antibody Titrations.

	Adenovirus group (types 1–7)		Herpes simplex		Measles		Poliovirus (type 1)		Anti-staphylococcal beta hemolysin	
	Titer	No.	Titer	No.	Titer	No.	Titer	No.	Titer	No.
MS group										
Low IgA	<1:8	(5)	1:16	(4)	<1:8	(3)	<1:8	(6)	1:16	(1)
	1:16	(1)	1:32	(1)	1:8	(1)	AC[a]	(1)	1:64	(1)
	1:32	(1)	AC[a]	(2)	1:16	(1)			1:128	(1)
					1:64	(1)			1:256	(1)
					AC[a]	(1)			1:2048	(1)
High IgA	<1:8	(4)	<1:8	(1)	<1:8	(3)	<1:8	(7)	1:16	(1)
	1:8	(1)	1:16	(1)	1:8	(2)			1:64	(1)
	1:16	(2)	AC[a]	(4)	1:32	(1)			1:512	(1)
									1:1024	(1)
									1:2048	(1)
Control group										
Low IgA	<1:8	(1)	<1:8	(2)	<1:8	(2)	<1:8	(3)	1:16	(1)
	1:8	(3)	1:8	(2)	1:32	(1)	1:8	(1)	1:128	(3)
	1:16	(1)	1:16	(2)	AC[a]	(2)			1:256	(1)
									1:512	(1)
High IgA	<1:8	(3)	1:16	(3)	<1:8	(3)	<1:8	(5)	1:256	(1)
	1:8	(2)	1:32	(1)	1:8	(2)			1:1024	(1)
			AC[a]	(1)					1:2048	(1)

[a] AC, anticomplementary.

group. Although toxin neutralization is not a prominent activity of IgM globulin, it does possess the ability to combine with it. Thus, empirically at least, the lower IgM levels are in accord with the earlier results.

The similarity of IgG levels in MS and control sera corresponds to the findings on CSF by Link (14). Kolár and Zeman (33) observed abnormal IgG arcs on IEP in the sera of patients with subacute inflammatory diseases of the CNS or with MS in the absence of quantitative changes. In the IEP analysis of six MS sera, differences in their IgA, IgG, and IgM precipitant arcs when compared with controls, could not be detected.

These results lend support to the concept of participation of an immunologic phenomenon in the development of MS.

The assistance of Dr. Edward Gocka and Emiko Nakamura, Western Research Support Center, Veterans Administration Hospital, Sepulveda, Calif., for statistical analysis of the data presented here is gratefully acknowledged.

1. Pette, E. and Pette, H., *in* "Die akut entzundlichen Erkrankungen des Nervensystems," p. 597. Thieme, Leipzig (1962).

2. Wolf, A., *in* "Mechanisms of Demyelination," p. 72, McGraw-Hill, New York (1963).

3. Ritzel, G., Wuthrich, R., and Rieder, H. P., Schweiz. Med. Wochschr. **93**, 1336 (1963).

4. Caspary, E. A., J. Neurol. Neurosurg. Psychiat. **29**, 103 (1966).

5. Lisak, R. P., Heinze, R. G., Galk, G. A., and Kies, M. W., Neurology **18**, 122 (1968).

6. Bornstein, M. B. and Appel, S. H., J. Neuropathol. Exptl. Neurol. **20**, 141 (1961).

7. Hughes, D. and Field, E. J., Clin. Exptl. Immunol. **2**, 295 (1967).

8. Seitelberger, F., Patho. Eur. **2**, 233 (1967).

9. Innes, J. and Kurland, L., Am. J. Med. **12**, 574 (1952).

10. Kurtzke, J. F., Acta Neurol. Scand. **42**, Suppl. 19, 91 (1966).

11. Adams, J. and Imagawa, D., Proc. Soc. Exptl. Biol. Med. **111**, 562 (1962).

12. Just, M., Rieder, H. P., and Ritzel, G., Klin. Wochschr. **45**, 705 (1967).

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

14. Link, H., Acta Neurol. Scand. **43**, Suppl. **28**, 1 (1967).

15. Ouchterlony, O., Progr. Allergy **5**, 1 (1958).

16. Ovary, Z., Progr. Allergy **5**, 459 (1958).

17. Mancini, G., Carbonara, A. O., and Heremans, J. F., Immunochemistry **2**, 235 (1965).

18. Sever, J., J. Immunol. **88**, 320 (1962).

19. Uyeda, C. T., Gerstl. B., Smith, J. K., and Carr, W. T., Proc. Soc. Exptl. Biol. Med. **123**, 143 (1966).

20. Eng, L. F., Chao, F-C, Gerstl, B., Pratt, D., and Tavaststjerna, M. G., Biochemistry **7**, 4455 (1968).

21. Falk, G. A., Heinze, R. G., Kies, M. W., and Elvord, E. C., J. Immunol. **100**, 321 (1968).

22. Scheidegger, J. J., Intern. Arch. Allergy Appl. Immunol. **7**, 103 (1955).

23. Fahey, J. and McKelvey, E. M., J. Immunol. **94**, 84 (1965).

24. Norberg, R., Acta Med. Scand. **181**, 485 (1967).

25. Pike, R. M., Bacteriol. Rev. **31**, 157 (1967).

26. Tokumaru, T., J. Immunol. **97**, 248 (1966).

27. Bellanti, J. A., Arnstein, M. S., and Buescher, E. L., J. Immunol. **94**, 344 (1965).

28. Lehrich, J. R., Kasel, J. A., and Rossen, R. D., J. Immunol. **97**, 654 (1966).

29. Kurtzke, J. F., Arch. Neurol. **14**, 213 (1966).

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

31. Berg, O. and Bergstrand, H., Acta Pathol. Microbiol. Scand. **73**, 195 (1968).

32. Bloch, K. J., Progr. Allergy **10**, 84 (1967).

33. Kolar, O. and Zeman, W., Z. Immunitaetsforsch. Allergie Klin. Immunol. **134**, 267 (1967).

Received March 24, 1969. P.S.E.B.M., 1969, Vol. 131.