Gm and Inv Antigenic Character of Serum and Cerebrospinal Fluid IgG in Multiple Sclerosis.* (31189)

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An immune response perhaps mediated by 7S γ-globulin (IgG) may be involved in the pathogenesis of multiple sclerosis. This possibility is supported by the observation that IgG is relatively increased in the cerebrospinal fluid (CSF) but not the serum of many patients with the disease(1-4). If the majority of IgG in CSF of patients with multiple sclerosis represented an isolated, homogenous antibody rather than an heterogeneous increment, it is conceivable that one or another of the genetically determined characteristics of IgG would differ in serum and CSF. In addition, if certain serotypes of IgG were associated with a predilection for multiple sclerosis, then patients and controls might be expected to show differences in IgG antigenic characteristics. The antigenic characteristics of the IgG immunoglobulins are, therefore, of considerable interest in relation to multiple sclerosis.

The present study was designed to compare Gm and Inv antigenic properties of IgG in serum and CSF of patients with multiple sclerosis and controls. The techniques involved hemagglutination inhibition, immunoelectrophoresis and immunodiffusion and therefore did not measure the γc(5,6,7) or γ trace(8-14) components which have a γ-mobility(5-14) but are not related to the immunoglobulins IgG, IgA or IgM of serum(6, 9,10,11,22).

Materials and methods. Serum and CSF were obtained from 39 patients at the University Hospitals, Minneapolis, Minn. Fifteen patients had well documented multiple sclerosis and 24 controls were selected comprising patients with a variety of non-demyelinating neurological conditions (Table I).

CSF samples of 10-15 cc were concentrated by lyophilization to a total protein concentration of 1-2 mg/cc and one drop of 10% sodium azide added as preservative. After concentration, the samples were stored at 4°C for periods of 1-2 months. Quantitation of IgG was performed by a modification of the Oudin method(15) using specific rabbit antisera to human γ-chains of IgG. Total proteins of the concentrated samples were determined by the Folin method(16).

The Gm and Inv(a) antigenic test systems were used as an indicator of complete or incomplete antigenic expression of IgG. Serum and CSF concentrates were typed in parallel for Gm(a)(17), Gm(b)(18) and Gm(f)(19) as well as for Inv(a)(20); Gm(a) and Gm-(b) typing used a slide technique(21) and Gm(f) and Inv(a) test systems utilized a tube method(19). Doubling dilutions of CSF used in hemagglutination inhibition tests began at a total protein concentration of 1-2 mg/cc.

Concentrated CSF samples were studied in immunoelectrophoresis using the following antisera: 1. horse anti-whole human serum (Hyland Laboratories), 2. rabbit antisera prepared against isolated human γ-chains from IgG(22), 3. rabbit antisera specific for relatively buried L-chain sites(23,24) and 4. rabbit antisera specific for IgA or IgM.

Results. When the Gm and Inv(a) typing of paired samples of CSF and serum was studied, concordant typing was found to occur for Gm(a) and for Gm(b). In 2 controls (#18 and 19) and 2 multiple sclerosis patients (#10 and 11) sera typed positive for Gm(f) and CSF typed negative. One control serum (#2) showed positive typing for Inv-(a) whereas CSF was negative. Difficulty in typing some concentrated CSF samples was occasionally encountered with those samples in which immunologic quantitation of IgG revealed relatively low levels of γ-globulins but this particular technical difficulty can account for only some of the discordant typing

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results. The reason for discordant results is not known.

A comparison of the per cent positive Gm and Inv(a) serotypes (Table II) gave no support for the possibility that patients with multiple sclerosis belonged to groups different from controls.

Immunoelectrophoretic study of concentrated CSF samples against the various specific antisera revealed no extra components or unusual arcs (Fig. 1). No IgA or IgM was identified by immunoelectrophoresis in the CSF samples studied; however, trace amounts in some samples were detected by Ouchter-

TABLE I. Gm and Inv(a) Typing of Control and Multiple Sclerosis Paired Serum and CSF Samples.

Patient #	Gm(a) S*	Gm(a) CSF†	Gm(b) S	Gm(b) CSF	Gm(f) S	Gm(f) CSF	Inv(a) S	Inv(a) CSF	Total IgG (mg % of conc CSF)	Total protein (mg % of conc CSF)	Discharge diagnosis
Control group											
1. W.J.	+	+	—	—	—	—	—	—	17	144	Thrombosis vertebral artery
2. S.H.	—	—	+	+	+	+	+	—	9	140	Parkinsons' disease
3. N.B.	—	—	+	+	+	+	—	—	13	114	Basilar artery insufficiency
4. P.A.	—	—	+	+	+	+	—	—	10	118	Cerebral atrophy
5. O.S.	+	+	+	+	+	+	—	—	19	140	Arachnoiditis
6. M.B.	+	+	+	+	+	+	—	—	11	126	Brachial plexus palsy
7. R.P.	+	+	+	+	+	+	—	—	28	176	Basilar insufficiency
8. H.B.	—	—	+	+	+	+	—	—	NT	78	Encephalomalacia
9. J.P.	—	—	+	+	+	+	—	—	12	126	Occlusion left calcarine artery
10. K.S.	—	—	+	+	+	+	—	—	14	104	Cryptococcosis
11. J.W.	—	—	+	+	+	+	—	—	NT	130	Schizophrenia
12. E.K.	+	+	+	+	+	+	+	+	14	116	Middle cerebral artery occlusion
13. O.N.	+	+	+	+	+	+	—	—	5	98	*Idem*
14. R.T.	—	—	+	+	+	+	+	+	9	110	Epilepsy
15. W.M.	+	+	+	+	+	+	+	+	14	104	Occlusion middle cerebral artery
16. M.B.	+	+	+	+	+	+	—	—	10	104	Amyotrophic lat. sclerosis
17. V.F.	—	—	+	+	+	+	—	NT	13	108	Cervical arthritis
18. P.S.	—	—	+	+	+	—	+	+	8	114	Basilar artery insufficiency
19. M.M.	+	+	+	+	+	—	—	—	17	132	Glossophayngeal neuralgia
20. H.S.	+	+	—	—	—	—	—	—	10	136	Cerebral metastases
21. T.B.	+	+	—	—	—	—	—	—	7	154	Intracranial aneurysm
22. W.N.	—	—	+	+	+	+	—	—	8	224	Meniere's syndrome
23. W.S.	—	—	+	+	+	+	+	+	9.0	100	Anxiety state
24. M.O'C.	—	—	+	+	+	+	+	+	19.5	540	Meningioma
Demyelinating group											
1. G.F.	+	+	—	—	—	—	—	—	9	104	
2. W.K.	+	+	+	+	+	+	—	—	13	138	
3. G.M.	+	+	+	+	+	+	—	—	25	212	
4. A.V.	+	+	+	+	+	+	+	+	20	114	
5. C.H.	—	—	+	+	+	+	—	—	24	126	
6. M.G.	—	—	+	+	+	+	+	NT	NT	134	
7. M.E.	—	—	+	+	+	+	+	+	8	100	
8. J.K.	—	—	+	+	+	+	—	—	16	148	
9. H.M.	—	—	+	+	+	+	—	—	85	222	
10. R.B.	—	—	+	+	+	—	—	—	43	190	
11. G.P.	+	+	+	+	+	—	—	—	11	126	
12. N.F.	—	—	+	+	+	+	+	+	50	202	
13. C.B.	—	—	+	+	+	+	+	+	13	162	
14. M.R.	—	—	+	+	+	+	—	—	34	162	
15. L.K.	+	+	+	+	+	+	—	—	33	106	

* S indicates typing of serum for Gm and Inv(a) character.
† CSF indicates typing of cerebrospinal fluid concentrates.

TABLE II. Number and Per Cent Positive Gm and Inv(a) Serotypes in Patients with Multiple Sclerosis and Controls.

	Multiple sclerosis			
	Patients		Controls	
Serotype*	No.	%	No.	%
Gm a(+)	6/15	40	12/24	50
Gm b(+)	14/15	93	22/24	92
Gm f (+)	14/15	93	22/24	92
Inv(a)(+)	5/15	33	7/24	29

* As determined by typing of whole serum.

lony analysis using specific antisera. Concentrated CSF samples stored at 1-2 mg/cc for periods up to 4 months at −20°C showed essentially the same findings.

In occasional concentrated CSF samples immune diffusion against antisera specific for buried L-chain sites(23,24) revealed the presence of trace amounts of free L-chains in cerebrospinal fluids from both normal controls and multiple sclerosis patients. In no instances did the amounts of free L-chains detected by this method approach the 4.5% of total CSF protein as reported for the γc-globulin component studied by MacPherson (25).

Discussion. The concordant Gm and Inv-(a) typing in paired samples of serum and CSF from controls as well as multiple sclerosis patients indicates no gross antigenic deficiency in the IgG of the cerebrospinal fluids studied. It is entirely possible, however, that the cerebrospinal fluid IgG antigenic character studied by this procedure reflects not only the γ-globulins produced in the central nervous system but also the heterogenous population of serum γ-globulins reaching the spinal fluid across the blood-brain barrier(26, 27). If the majority of γ-globulin present in CSF of patients with multiple sclerosis represented an isolated, homogenous antibody rather than an heterogeneous increment either produced in the central nervous system or transferred from serum across the blood-brain barrier, it is conceivable that one or another genetic IgG characteristic could be lacking (28). During this study, CSF samples showing sharp monoclonal γ-globulin components were not encountered.

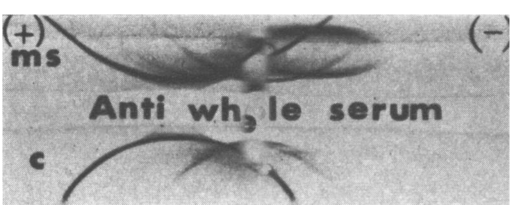

FIG. 1. Immunoelectrophoresis of concentrated spinal fluid from patient with multiple sclerosis above and a normal CSF control below developed with rabbit antiserum to whole human serum. A heavier line is noted with the γ-globulin arc from multiple sclerosis CSF than is present with control. Since the antiserum used was made against whole human serum, no extra arcs of γ-mobility are apparent (6-14).

Steinberg(29) has stated that the percent of Gm(a) positive individuals in European populations decreased from Northern Europe to the South. As multiple sclerosis also shows a gradient of decreasing prevalence toward the equator(30) it was conceivable that individuals susceptible to multiple sclerosis might show a Gm or Inv serotype different from the general population. This possibility was not supported by the present study.

Many other workers have detected γ-globulin or γ-mobility components using immunoelectrophoresis and antiserum to CSF absorbed with normal serum. It seemed plausible that some of these components might represent free light chains unattached to whole γG, γA or γM molecules. However, the lack of distinct antigenic relationship to what is defined as immunoglobulin by specific antisera makes this unlikely. Although trace amounts of free L-chains could be detected by us in some concentrated CSF samples, the amount was too small to account for 4-5% γc component which MacPherson(25) measured in total CSF protein.

Summary. Antigenic analysis of γ-globulins in the CSF of controls and patients with multiple sclerosis showed no gross deficiency in Gm or Inv typing. Concordant typing for Gm and Inv factors from serum and CSF identifies the antigenic completeness of IgG in both control and multiple sclerosis CSF. No support was obtained for the possibility that individuals with multiple sclerosis differ from controls in Gm or Inv(a) serotypes.

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Challenge Virus Resistance and Interferon Produced in BS-C-1 Cells By Dengue Virus. (31190)

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Dengue viruses infect mammalian cell cultures without producing obvious cytopathic effect(1). Halstead showed that continuous cultures of African green monkey kidney cells (BS-C-1) or rhesus kidney cells (LLC-MK2) infected with dengue viruses resist superinfection with yet another virus(2). This challenge virus resistance (CVR) is superficially similar to that induced by rubella virus in BS-C-1 cells(3) and by tick-borne encephalitis viruses in chick embryo cell cultures(4), and has been useful for the recovery of dengue viruses from patients' blood. This technique is more sensitive and efficient than is the use of the suckling mouse for isolation and characterization of some strains of dengue viruses(5). The present experiments show that dengue virus-induced CVR is accompanied by the elaboration of an interferon-like substance. The characterization of this interferon, its behavior in BS-C-1 cells, and the circumstances of its production are the subject of this report.

Materials and methods. Viruses. The dengue virus (PR-38) was isolated from a patient during the epidemic in Puerto Rico in 1963(5). The strain was recovered originally in BS-C-1 cell monolayers; third and fourth