ods **29**, 133 (1979)]. Lymphocyte viability tested by dye exclusion is > 95 percent.
13. The variances of the home-cage and apparatus control groups were considerably larger than the variances of the shock groups (Fig. 1). Since the estimate of error variance used in making multiple contrasts is derived by averaging the variances in all groups, the larger variance of the control groups would inflate the actual error variance related to the shock groups. This problem was overcome by computing separate analyses of variance contrasting the control groups or the shock groups. The differences in lymphocyte stimulation between the control groups approached significance [$F(1, 22) = 3.94$, $P < .06$], and the low-shock group differed significantly from the high-shock group [$F(1, 22) = 7.27$, $P < .01$].
14. In further studies conducted with the isolated lymphocyte assay, the stressful conditions also suppressed lymphocyte stimulation in PHA-treated cultures incubated for 4 days.
15. In addition to the effects seen with the 3-day incubation, shock also suppressed the unstimulated lymphocyte cultures in the isolated cell assay with a 4-day incubation period [$F(3, 44) = 7.47$, $P < .01$]. Incorporation of ^{125}IdUrd by these cultures was (count/min) 241.3 ± 26.5 for home-cage controls, 149.9 ± 26.5 for apparatus controls, 57.7 ± 5.6 for low-shock animals, and 99.4 ± 18.3 for high-shock animals.
16. G. J. Johnson and P. S. Russell, *Nature (London)* **208**, 343 (1965); D. S. Gregerson, B. Kelly, J. G. Levy, *Immunology* **29**, 237 (1975).
17. A. J. Strelkauskas, V. Schauf, B. S. Wilson, L. Chess, S. F. Schlossman, *J. Immunol.* **120**, 1278 (1978); E. L. Reinherz, R. Parkman, R. Rappeport, F. S. Rosen, S. F. Schlossman, *N. Engl. J. Med.* **300**, 1061 (1979); E. L. Reinherz *et al.*, *ibid.* **303**, 125 (1980).
18. C. M. Harlow and H. Selye, *Proc. Soc. Exp. Biol. Med.* **36**, 141 (1937); F. Elmadjian and G. Pincus, *Endocrinology* **37**, 47 (1945); T. F. Dougherty, *Physiol. Rev.* **32**, 379 (1952); ——— and J. A. Frank, *Am. J. Physiol.* **171**, 721 (1952); A. Ahmed, C. M. Herman, R. C. Knudsen, J. Sode, D. M. Strong, K. W. Sell, *J. Surg. Res.* **16**, 172 (1974).
19. M. C. Berenbaum, P. A. Fluch, N. P. Hurst, *Br. J. Exp. Pathol.* **54**, 607 (1973); A. S. Fauci, *Transplant. Proc.* **7**, 37 (1975); J. S. Goodwin, R. P. Messner, R. C. Williams, Jr., *Cell. Immunol.* **45**, 303 (1979); D. H. Heilman, M. R. Gambrill, J. P. Leichner, *Clin. Exp. Immunol.* **15**, 203 (1973); J. Mendelsohn, M. M. Multer, J. L. Bernheim, *ibid.* **27**, 127 (1977); P. C. Nowell, *Cancer Res.* **21**, 1518 (1961); D. C. Tormey, H. H. Fudenberg, R. M. Kamin, *Nature (London)* **213**, 281 (1967).
20. H. B. Tavadia, K. A. Fleming, P. D. Hume, H. W. Simpson, *Clin. Exp. Immunol.* **22**, 190 (1975).
21. J. Mendelsohn, M. M. Multer, J. L. Bernheim, *ibid.* **27**, 127 (1977); F. A. Wyle and J. R. Kent, *ibid.*, p. 407.
22. J. S. Goodwin, R. P. Messner, R. C. Williams, Jr., *Cell. Immunol.* **45**, 303 (1979); P. C. Nowell, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **21**, 73 (1962); J. W. Smith, A. L. Steiner, C. W. Parker, *J. Clin. Invest.* **50**, 442 (1971).
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Preferential Synthesis of the G1m(1) Allotype of IgG1 in the Central Nervous System of Multiple Sclerosis Patients

Abstract. *Quantitations of the G1m(1) and G1m(3) allotypic determinants of human immunoglobulin G were performed by radioimmunoassay on cerebrospinal fluid and serum samples from patients with multiple sclerosis and from patients with other neurological disorders. In multiple sclerosis patients that were heterozygous for these determinants, G1m(1) concentration in the cerebrospinal fluid was greatly increased—reflected by an increased ratio of G1m(1) to G1m(3)—in comparison with that of patients with other neurological disorders. These results suggest that in the heterozygous multiple sclerosis patients, most of the plasma cells in the central nervous system that secrete oligoclonal immunoglobulin G preferentially synthesize G1m(1) IgG1 molecules.*

The *Gm* genes constitute a polycistronic system encoding for either of two allelic γ heavy chains within each immunoglobulin G (IgG) subclass in humans (*1*). Different alleles are present in different ethnic groups. In Caucasians, G1m(1) and G1m(3) are antigenic determinants located on the allelic forms of IgG1 molecules (*2*); homozygotes have either G1m(1) or G1m(3) γ1 chains, whereas heterozygotes have both G1m(1) and G1m(3) γ1 chains. G3m(5) and G3m(21) represent the pseudoallelic forms of IgG3. The Gm^1 and Gm^{21} genes are almost always inherited simultaneously, that is, as a haplotype, as are the Gm^3 and Gm^5 genes. Caucasians are thus either homozygous $Gm^{1;21}/Gm^{1;21}$, homozygous $Gm^{3;5}/Gm^{3;5}$, or heterozygous $Gm^{1;21}/Gm^{3;5}$. In serum from heterozygous individuals, the amounts of the G1m(1) and G1m(3) allotypes of IgG1 are approximately equal (*3*), and each represents about half of the amount of IgG1—either G1m(1) or G1m(3)—present in the corresponding homozygous population. Quantitative variations in G3m(5) and G3m(21) in the different genotypes are more complex (*3*, *4*).

Multiple sclerosis (MS) is a chronic, relapsing, and remitting disease characterized by demyelination in the central nervous system. A well-known feature of MS is an increase of IgG (*5*), particularly IgG1 (*6*, *7*), in the cerebrospinal fluid, resulting from intrathecal synthesis by lymphoplasmocytes in the central nervous system (*8*). The oligoclonal electrophoretic patterns of immunoglobulins from the cerebrospinal fluid or brain tissue of MS patients (*9*) suggest their production by a few B cell clones (*10*). They may originate from a limited number of B cell clones in the central nervous system (*10*). We now report a preferential increase in G1m(1) compared to G1m(3) in the cerebrospinal fluid of MS patients who are heterozygous $Gm^{1;21}/Gm^{3;5}$. This finding is evidence for (i) the oligoclonal character of IgG1 in the cerebrospinal fluid, (ii) the persistent presence of a limited number of (nonsuppressed) B cell clones in the central nervous system, and (iii) the involvement of two unlinked genetic systems in the susceptibility to this disease.

Measurements of G1m(1) and G1m(3) were performed in paired samples of cerebrospinal fluid and serum from patients with MS or other neurological disorders (OND) without demyelinating or infectious disease of the central nervous system (seizure disorder, dementia, ethanol abuse, syncope, cerebrovascular accident, and Parkinson's disease). Probable Gm genotypes were inferred from the Gm phenotypes detected in serum by hemagglutination inhibition on microtiter plates (*11*). Concentrations of G1m(1) and G1m(3) were measured by radioimmunoassay (*4*); the normal values in serum have been published (*4*). Samples of cerebrospinal fluid from healthy donors were not available. The mean content of G1m(1) and G1m(3) and the ratio G1m(1)/G1m(3), if any, were calculated in serum and cerebrospinal fluid for each group (Table 1). The statistically significant differences observed between groups are shown in Table 2.

The serum concentrations of G1m(1) and G1m(3) and the G1m(1)/G1m(3) ratio in Gm homozygous and heterozygous OND patients were not different from those in healthy donors; consequently, the OND group can be considered as a reference population for normal values in the cerebrospinal fluid. The concentrations of G1m(1) or G1m(3) in homozygotes, or of G1m(1) + G1m(3) in heterozygotes, were markedly increased in the cerebrospinal fluid of MS patients as compared with the OND group, in agreement with the previously reported increase of IgG1 in the cerebrospinal fluid of MS patients (*6*, *7*). A modest, not statistically significant, increase in G1m(1) in serum was observed in the $Gm^{1;21}$ homozygous MS population, whereas the $Gm^{3;5}$ homozygous MS population exhibited a mean value of G1m(3) close to that for OND patients and healthy donors.

The most striking quantitative results were obtained in the Gm heterozygous MS population, in which a greatly increased G1m(1) concentration was found in the cerebrospinal fluid, whereas the G1m(3) content was identical to that observed in the OND group. This shift of

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Table 1. Glm(1) and Glm(3) concentrations in serum and cerebrospinal fluid from healthy donors and patients with MS or other neurological disorders. Gm concentrations were determined by radioimmunoassay. Samples were diluted 1:1000 (serum) or 1:10 (cerebrospinal fluid) for the assays. Values are means ± standard deviation, with number of samples given in parentheses.

Genotype*	Serum			Cerebrospinal fluid		
	Glm(1) (mg/ml)	Glm(3) (mg/ml)	Glm(1)/ Glm(3)†	Glm(1) (μg/ml)	Glm(3) (μg/ml)	Glm(1)/ Glm(3)†
			Healthy donors			
$Gm^{1;21}/Gm^{1;21}$	7.38 ± 2.73 (39)					
$Gm^{3;5}/Gm^{3;5}$		7.12 ± 2.32 (41)				
$Gm^{1;21}/Gm^{3;5}$	3.14 ± 1.28 (38)	3.80 ± 1.96 (38)	0.89 ± 0.39 (38)			
			Other neurological disorders (OND)			
$Gm^{1;21}/Gm^{1;21}$	7.16 ± 3.02 (15)			24.1 ± 12.6 (4)		
$Gm^{3;5}/Gm^{3;5}$		8.14 ± 3.74 (15)			23.2 ± 20.0 (15)	
$Gm^{1;21}/Gm^{3;5}$	3.50 ± 2.14 (18)	3.62 ± 1.71 (18)	1.16 ± 0.88 (18)	15.3 ± 10.0 (14)	16.2 ± 10.9 (14)	1.52 ± 1.64 (14)
			Multiple sclerosis (MS)			
$Gm^{1;21}/Gm^{1;21}$	9.19 ± 3.33 (11)			140.8 ± 113.9 (3)		
$Gm^{3;5}/Gm^{3;5}$		8.08 ± 3.19 (17)			125.3 ± 155.9 (16)	
$Gm^{1;21}/Gm^{3;5}$	3.30 ± 1.88 (22)	2.87 ± 1.63 (22)	1.49 ± 1.07 (22)	82.6 ± 83.7 (10)	18.4 ± 14.1 (10)	4.76 ± 3.78 (10)

*Genotype inferred from phenotypes in serum. †Mean calculated from each individual ratio.

one allotype content was also reflected in the G1m(1)/G1m(3) ratio in the cerebrospinal fluid, which was significantly greater (4.76) than that for the OND group (1.52). The G1m(1)/G1m(3) ratio was also increased in the serum of heterozygous MS patients because of a decreased G1m(3) concentration; the G1m(1) concentration was equivalent to that in serum from heterozygous healthy donors and OND patients.

A difference in quantitative expression of the G1m(3) antigen has been related to the κ or λ type of the light chains present in the G1m(3) IgG molecules, κ chains leading to weaker G1m(3) antigenicity than λ chains (*12*). An increase in the κ/λ ratio in the cerebrospinal fluid has been described in about 50 percent of MS patients (*13*), and it might be suggested that the finding of normal G1m(3) but increased G1m(1) in the cerebrospinal fluid of Gm heterozygous MS patients could be due to a concomitant increase in κ chain IgG that masks an actual increase in G1m(3) IgG1. This is unlikely for several reasons, including evidence reported by others that the increase in κ chains in the cerebrospinal fluid of MS patients is probably due to free κ chains (*14*, *15*).

Neither the normal exchange of IgG from cerebrospinal fluid to serum nor altered permeability of the blood-brain barrier can account for the moderate serum abnormalities observed in the Gm heterozygous MS population, whose serum G1m(3) content was decreased and G1m(1) content was unchanged, in contrast with changes in both the G1m(1) and G1m(3) levels in the cerebrospinal fluid of the same group. The shift in the G1m(1)/G1m(3) ratio in cerebrospinal fluid as compared to serum is best explained by de novo IgG synthesis within the central nervous system, which occurs both in MS and, to a much lesser extent, in OND patients.

The IgG synthesized de novo within the central nervous system of MS patients corresponds to the oligoclonal IgG described in this disease (*8*). This finding leads to the assumption that two series of immunoglobulin-producing cells are present in the central nervous system: (i) those reflecting the antigenic exposure of the patient (for example, common viruses), and (ii) a second group with uncontrolled growth, possibly arising from a lack of suppressor T cells (*16*, *17*) or an intrinsic inability of the B cell clones to respond to suppressor signals (*18*). Our results strongly suggest that, in the Gm heterozygous MS population, most of these plasma cells synthesize G1m(1) IgG1. This may be due to a relationship between IgG structure and function, assuming that a given immunoglobulin allotype favors the antibody activity (*19*) or the Fc biological functions of the immunoglobulin molecule, or both (*20*).

A Gm^3 gene deficiency at a regulatory level could also explain the unexpected

Table 2. Statistically significant differences observed for Glm(1) and Glm(3) concentrations and Glm(1)/Glm(3) ratios in healthy donors (HD), other neurological disorders (OND), and multiple sclerosis (MS) patients. Statistical comparisons were first performed with Student's *t*-test, after log transformation of the data when a skewed frequency occurred in a population. When a significant difference was observed, Mann and Whitney's nonparametric *U* test was further used because of the small number of samples in most populations.

Item	*T'**	*P* value*
Differences in serum		
Glm(3) concentration in heterozygous MS and HD†	1.94	~ .05
Glm(1)/Glm(3) ratio in heterozygous MS and HD†	2.65	< .01
Differences in cerebrospinal fluid‡		
Glm(3) concentration in MS and OND homozygous for Gm^3	2.51	< .02
Glm(1) concentration in heterozygous MS and OND	2.75	< .01
Glm(1)/Glm(3) ratio in heterozygous MS and OND	3.045	< .01

*Mann and Whitney's *U* test. †There were no significant differences between MS and OND populations. ‡Too few values for Glm(1) in CSF from Gm^1 homozygous patients (MS and OND) were available for a statistical comparison of these two groups.

absence of an increase in G1m(3) content in the cerebrospinal fluid of Gm heterozygous MS patients. However, the marked increase of G1m(3) in the cerebrospinal fluid of Gm^3 homozygous MS patients makes this unlikely. Studies of *Gm* gene and haplotype frequencies in MS compared to OND patients and healthy people should provide evidence for or against this hypothesis. Susceptibility to MS is most likely polygenic and might depend not only on genes for histocompatibility antigens (*HLA*) (*21*), but also on *Gm* genes, which are on a different chromosome (*22*). In man, immune responsiveness to flagellin (*23*) and susceptibility to autoimmune chronic active hepatitis (*24*) involve interaction between histocompatibility antigens and Gm. Non–antigen-specific binding of IgG molecules to cell structures such as Fcγ receptors could occur in MS (*9*). Preferential interaction between Fcγ receptors and a given immunoglobulin subclass (*25*) or G1m allotype has been observed (*26*), and our finding of increased levels of G1m(1) (located on the Fc portion of IgG1) may indicate a role of such interactions in the demyelination that characterizes MS.

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References and Notes

1. J. B. Natvig and H. G. Kunkel, *Adv. Immunol.* **16**, 1 (1973).
2. World Health Organization, *Eur. J. Immunol.* **6**, 599 (1976).
3. S. D. Litwin and S. Balaban, *J. Immunol.* **108**, 991 (1972).
4. J. P. Salier, H. Sarvas, H. M. Reisner, A. C. Wang, H. H. Fudenberg, *Mol. Immunol.* **16**, 217 (1979).
5. H. Link, E. Möller, R. Müller, E. Norrby, J. E. Olsson, L. Stendhal, G. Tibbling, *Acta Neurol. Scand. Suppl.* **63**, 173 (1977).
6. B. Vandvik, J. B. Natvig, D. Wiger, *Scand. J. Immunol.* **5**, 427 (1976).
7. S. Al Kassab, L. Dittmann, H. Olesen, *Acta Neurol. Scand.* **60**, 129 (1979).
8. W. W. Tourtellotte and B. I. Ma, *Neurology* **28** (Suppl.), 76 (1978).
9. D. H. Mattson, R. P. Roos, B. G. W. Arnason, *Nature (London)* **287**, 335 (1980).
10. E. Norrby and B. Vandvik, in *Immunopathology*, P. A. Miescher, Ed. (Grune & Stratton, New York, 1976), pp. 161–172.
11. G. N. Vyas, H. H. Fudenberg, H. M. Pretty, E. R. Gold, *J. Immunol.* **100**, 274 (1968).
12. J. P. Salier *et al.*, *J. Immunogenet.* **7**, 123 (1980).
13. H. Link and O. Zettervall, *Clin. Exp. Immunol.* **6**, 435 (1970).
14. H. Iwashita, F. Grunwald, H. Bauer, *J. Neurol.* **207**, 45 (1974).
15. F. Bollengier, N. Rabinovitch, A. Lowenthal, *J. Clin. Chem. Clin. Biochem.* **16**, 165 (1978).
16. J. P. Antel, B. G. W. Arnason, M. E. Medof, *Ann. Neurol.* **5**, 338 (1979).
17. E. L. Reinherz, H. L. Weiner, S. L. Hauser, J. A. Cohen, J. A. Distaso, S. F. Schlossman, *N. Engl. J. Med.* **303**, 125 (1980).
18. J. M. Goust, P. M. Hoffman, J. Pryjma, E. L. Hogan, H. H. Fudenberg, *Ann. Neurol.* **8**, 526 (1980).
19. C. C. Chien and J. S. Ingraham, *J. Immunol.* **118**, 1896 (1977).
20. J. P. Salier, C. Rivat, L. Rivat, C. Bastard, G. Virella, H. H. Fudenberg, A. C. Wang, *Mol. Immunol.* **17**, 229 (1980).
21. J. L. Tiwari, S. E. Hodge, P. I. Terasaki, M. A. Spence, *Am. J. Hum. Genet.* **32**, 103 (1980).
22. C. M. Croce, M. Shander, J. Martinis, L. Cicurel, G. G. D'Ancona, T. W. Dolby, H. Koprowski, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3416 (1979).
23. S. Whittingham, J. D. Mathews, M. S. Schanfield, J. V. Mathews, B. D. Tait, P. J. Morris, I. R. Mackay, *Clin. Exp. Immunol.* **40**, 8 (1980).
24. S. Whittingham, J. D. Mathews, M. S. Schanfield, D. B. Tait, I. R. Mackay, *ibid.* **43**, 80 (1981).
25. D. Gilbert, M. Daveau, C. Fontaine, *Scand. J. Immunol.*, in press.
26. M. Daveau, unpublished data.
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Development of Stereopsis and Cortical Binocularity in Human Infants: Electrophysiological Evidence

Abstract. *Dynamic random-dot stereograms and correlograms were used to elicit visually evoked brain potentials from human infants, and these potentials were compared with potentials evoked by classical checkerboard pattern reversal. The results indicate that infants begin to produce stereoscopically evoked potentials at the age of 10 to 19 weeks, several weeks after showing classical checkerboard-evoked potentials, and suggest that the onset of cortical binocularity precedes stereopsis.*

There has been increasing interest in assessing binocular visual function in human infants, since early detection of defects could aid in the treatment of strabismus and in the prevention of amblyopia and loss of depth perception. Behavioral responses to random-dot stereograms have been used to demonstrate stereoscopic discrimination in infants 3 to 6 months of age (*1*). An electrophysiological method (*2, 3*) has revealed that infants as young as 2 months can detect changes in binocular spatial correlation between random-dot patterns presented to both eyes (*4*). We report electrophysiological evidence indicating that the visual system begins to process random-dot stereograms when the infant is 10 to 19 weeks of age, several weeks after the processing of luminance patterns begins.

Dynamic random-dot correlograms and dynamic random-dot stereograms contain stereoscopic cues that can only be perceived by subjects with functional stereopsis (*5–7*). When viewed monocularly or by subjects without stereopsis, these correlograms and stereograms appear similar to the "snowstorm" on an untuned television set. Dynamic random-dot correlograms and stereograms, unlike static ones, are devoid of monocular cues caused by binocular correlation changes or by binocular disparity changes (*8*). This is important in measuring visually evoked potentials (VEP's), where repeated presentation of the same stimulus and averaging of the stimulus-locked brain potentials are required.

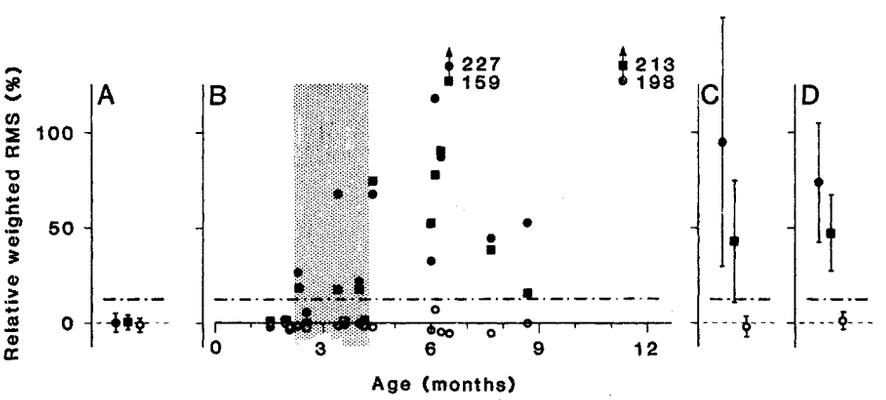

Fig. 1. Relative weighted root-mean-square (RMS) values of the two stereoscopic test stimuli NP (●) and DF (■) and of the control stimulus SS (○). Data are given for (A) stereoblind adults and children (N = 15), (B) infants (N = 17), (C) stereonormal children (N = 20), and (D) stereonormal adults (N = 20). Criterion responses (relative weighted RMS ≥ 12 percent) are above the dashed line. The shaded zone (2 to 4.3 months) is the age range where cyclopean VEP's developed in our sample of infants. Data in (A), (C), and (D) represent means ± 1 standard deviation.

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