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Distribution of nominal and latent IgG (Gm) allotypes in plaques of multiple sclerosis brain

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SUMMARY

Concentrations of IgG allotypes G1m(1), G1m(3) and G3m(11) in neutral pH eluates from discrete plaques of multiple sclerosis (MS) brain and from white matter of control brain were determined to obtain information about distribution of B cell clones among MS lesions. Within each MS brain a predominant nominal IgG1 allotype was distributed rather homogeneously in all plaques while quantitatively minor allotypes showed some fluctuation. Latent IgG1 allotypes were detected (7–12% of the corresponding nominal allotype level) in some tissue eluates from both MS and control brains, which were homozygous for either G1m(1) or G1m(3). By contrast, the expression of a latent IgG3 allotype, namely G3m(11), was apparently MS restricted. Large amounts of latent allotypes were detected only in recent plaques with lymphoid cells whereas the distribution of total plaque associated IgGs did not correlate with the presence of lymphoid cells. Latent allotypes in recent MS lesions may mark a transient immunological activity which coincides with the infiltration of lymphoid cells and precedes the appearance in these plaques of oligoclonal IgGs, the distribution of which may parallel that of the predominant nominal allotypes.

Keywords multiple sclerosis plaques IgG (Gm) allotypes

INTRODUCTION

IgG containing lymphoid cells around recent plaques in brains of multiple sclerosis (MS) patients (Esiri, 1980) are thought to secrete the oligoclonal IgGs which can be detected in cerebrospinal fluid (CSF) by isoelectric focusing (IEF) (Tourtellotte *et al.*, 1980). The oligoclonal IgGs, which may comprise 20–40% of the total IgGs in MS CSF samples (Delmotte, 1979), do not react with a range of viruses (Vartdal, Vandvik & Norrby, 1980; Vartdal & Vandvik, 1982) or with neuroantigens (Rostrom, 1982; Newcombe, Glynn & Cuzner, 1982). It has been reported that IgGs eluted from individual plaques within one MS brain show different IEF spectra; this observation was attributed to expansion of different B cell clones non-specifically recruited to these sites, which would continue to secrete IgGs unrelated to the pathogenesis of MS. Furthermore, it was suggested that the oligoclonal IgGs in MS CSF represent the sum of contributions from B cells in the different plaques (Mattson, Roos & Arnason, 1980, 1982). These conclusions present a paradox since, if B cell clones are recruited randomly to the brain then, with time, the IEF spectrum of oligoclonal IgGs in CSF samples from MS patients should change whenever a new plaque is formed; however, a cardinal

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feature of MS CSF is that the IEF spectra of IgGs appear very stable over periods of years despite changes in clinical status (Olsson & Nilsson, 1979). This paradox may arise partly because it is often difficult to interpret complex IEF spectra and particularly to equate one focused band of IgG with the presence of one B cell clone (Williamson, 1978).

Within each IgG subclass the constant part of the heavy chain (IgCH) is encoded by either of a series of codominant allelic genes whose products are the Gm allotypes. Caucasians have IgG1 producing B cell clones which express either G1m(1) or G1m(3) IgG1 when homozygous; both allotype producing clones are present in heterozygous individuals; analogously, G3m(11) and G3m(21) allotypes are the products of IgG3 encoding alleles (Steinberg & Cook, 1981). Allotypes found in an individual are termed 'nominal' or 'latent' according to whether or not they are expected from the assumed genotype (Kindt & Yarmush, 1981). An increase of $Gm^{1;21}/Gm^{1;21}$ homozygous frequency (Pandey *et al.*, 1981) and an imbalance in IgG1 allotype amounts in CSF (Salier *et al.*, 1981) have been reported in MS patients but as yet no characterization of Gm allotypes in plaques has been published. The present study was undertaken to determine the distribution of Gm allotypes and hence of B cell clones among discrete MS plaques.

MATERIALS AND METHODS

Clinical and pathological data. Discrete samples of plaque or control white matter were dissected from the brains of 10 clinically and histologically confirmed cases of MS (six male/four female 45 ± 15 years) and five controls (three male/two female, 54 ± 3 years) with no neurological involvement. All patients were Caucasians. Post-mortem times of brain dissection varied from 2 to 48 h with interim storage at 4°C. With a limited number of plaque samples (see Table 3), a representative piece of the dissected tissue was fixed in 10% formalin; the presence of lymphoid cells in these (coded) samples was assessed after haematoxylin & eosin staining by Professor Ingrid Allen and colleagues, Queens University, Belfast.

Gm genotype was inferred from the Gm phenotype in serum samples or from the Gm allotypes found in CSF samples. In the great majority of cases (>95%), the latter reflect the actual Gm phenotype of the individual (Salier *et al.*, 1983).

When neither serum nor CSF were available (four of 10 MS, five of five control), Gm homozygosity or heterozygosity was assigned on the basis of Gm allotypes detected in brain exudate samples; these were obtained by thawing a piece of intact brain tissue and collecting the fluid which oozed from it which was then clarified by centrifugation.

Preparation of brain tissue eluates. Discrete samples of MS plaques or control white matter were finely minced, washed several times with phosphate-buffered saline (PBS) pH 7·4 and homogenized in 10 mM Tris-HCl-1mM EDTA-0·1 mM phenylmethylsulphonylfluoride. Homogenates were centrifuged (38,000 *g*; 60 min) and the supernatant fractions were taken as the neutral eluate samples (Glynn *et al.*, 1982). Eluates were freeze dried and coded prior to shipment for determination of allotype concentrations. They were then reconstituted at a concentration of 5–20 μg/ml of IgG in PBS and centrifuged (2,000 *g*; 30 min). Insolubilized materials were discarded and Gm allotype and total IgG concentrations were quantified as described below.

In a limited number of samples (see Table 3) total IgGs were determined in the washed particulate fraction of brain tissue homogenates by a ^{125}I-labelled protein A binding assay (Glynn *et al.*, 1982).

Determinations of Gm allotype and total IgG concentrations in neutral eluates. The concentrations of G1m(1), G1m(3) and G3m(11) in coded samples were determined by radioimmunoassay (Salier *et al.*, 1979); the detection limit was about 0·1 μg/ml for each allotype. Total IgGs were quantified by either radioimmunodiffusion (Goust, Hogan & Arnaud, 1982) or by solid phase radioimmunoassay; the latter used a purified myeloma IgG3 as iodinated tracer, rabbit and anti-human gamma chain (Immunobeads RAH-3, BioRad) as solid phase antibodies, and a normal human serum pool with a known IgG content as standard. For the data in Tables 1 and 2 the concentration of each Gm allotype had been recalculated on the basis of a total IgG content of 10 μg/ml in the sample to allow a direct comparison of change in allotype levels from sample to sample,

regardless of the total IgG fluctuations. The code was broken after all measurements and calculations were completed.

RESULTS

Distribution of nominal Gm allotypes in MS plaques and control white matter

The distribution of Gm allotypes in eluates of white matter and plaque samples from individuals whose genotypes were most likely heterozygous is shown in Table 1. The mean G1m(1)/G1m(3) ratio in the MS plaques was significantly greater than in control white matter ($4{\cdot}20 \pm 1{\cdot}71$ *vs* $1{\cdot}67 \pm 0{\cdot}29$; $P < 0{\cdot}01$, Mann & Whitney's non-parametric test), a difference similar to that described previously for CSF IgG1 from heterozygous individuals comparing MS with other neurological diseases (Salier *et al.*, 1981). In addition, the G1m(1)/G1m(3) ratio varied by up to two-fold between different plaques from a single MS brain while more constant values were observed for the control

Table 1. Gm allotypes in brain eluates from $Gm^{1;21}/Gm^{3;11}$ heterozygous individuals

Patient	Tissue sample (location)	G1m(1)	G1m(3)	G3m(11)	G1m(1)/G1m(3) Ratio
B120 (MS)	P (lat-vent)	4·61	0·69	0·12	6·68
	P(FL)	4.00	0·90	0·21	4·44
	P (TL)	3·47	1·20	0·15	2·89
B126 (MS)	P (BS)	8.0	1.72	0·20	4·65
	P (TL-vent)	5·55	2·39	0·44	2·32
B125 (control)	WM (FL)	3.54	1.89	0·39	1·87
	WM (TL)	3·03	1·49	0·13	2·03
	WM (OL)	3·61	1·93	0·09	1·87
B133 (control)	WM (FL)	5·26	3·37	0·17	1·56
	WM (TL)	4·07	3·14	0·30	1·30
	WM (OL)	4·58	3·22	0·30	1·42

Each Gm allotype concentration has been recalculated on the basis of a total IgG content of 10 μg/ml in the sample to allow a direct comparison of change in allotype levels from sample to sample, regardless of the total IgG fluctuations. All values are expressed as μg/ml. P = plaque; WM = white matter; FL = frontal lobe; TL = temporal lobe; OL = occipital lobe; BS = brain stem; Lat-vent = lateral ventricle; SC = spinal cord; GL.WM = gliosed white matter.

white matter samples. Within normal or MS brains the content of the G3m(11) allotype of IgG3 displayed greater fluctuations between samples than the quantitatively predominant IgG1 allotypes G1m(1) and G1m(3) (Table 1). In samples from individuals who were most probably homozygous $Gm^{1;21}/Gm^{1;21}$ or $Gm^{3;11}/Gm^{3;11}$ (Table 2) the levels of the nominal G1m(1) or G1m(3) allotypes were stable in controls and did not vary by more than 1·6-fold within each MS brain. A greater variation (up to 2·4-fold) was seen in the plaque to plaque concentrations of the minor nominal allotype G3m(11) in MS patients B115 and B123 and even in B66 where the distribution of the G1m(3) allotype was very homogenous. Thus the overall impression of plaque to plaque variations in nominal Gm allotype levels was of an underlying common distribution of a predominant IgG1 allotype associated with fluctuations in a quantitatively minor IgG3 allotype.

Latent Gm allotypes in MS plaques and control white matter

In eluates from some brain tissue samples homozygous individuals unexpected or 'latent' Gm

Table 2. Gm allotypes in brain eluate from homozygous individuals

Patient No. and probable Gm genotype	Tissue sample (location)	G1m(1)	G1m(3)	G3m(11)
B42 (MS)	P (OL)	—	2·09	nd
$Gm^{3;11}/Gm^{3;11}$	P (FL)	—	2·28	1·77
	P(FL†)	—	2.29	nd
B66 (MS)	P (FL)	—	3·19	2·69
$Gm^{3;11}/Gm^{3;11}$	P (OL)	—	3·24	nd
	P (OL†)	—	3·33	1·11
B111 (MS)	GL WM (FL)	—	8·19	0·17
$Gm^{3;11}/Gm^{3;11}$	P(TL-Vent)	—	7.74	nd
	P (Medulla)	—	5·54	0·38
B123 (MS)	P (TL)	0·25*	7·69	0·59
$Gm^{3;11}/Gm^{3;11}$	P (TL†)	0·24*	4·72	0·51
	P (SC)	—	5.38	0·28
B124 (MS)	P (TL-Vent)	—	9·55	0·11
$Gm^{3;11}/Gm^{3;11}$	P (OL)	—	6·32	0·10
B109 (control)	WM (FL)	0·53*	3·73	nd
$Gm^{3;11}/Gm^{3;11}$	WM (TL)	—	3·37	0·59
	WM (OL)	—	3·75	0·60
B56 (MS)	P (OL)	3·80	—	0·97*
$Gm^{1;21}/Gm^{1;21}$	P (TL)	5·64	1·09*	0·81*
B90 (MS)	P (BS)	2·19	3·25*	0·72*
$Gm^{1;21}/Gm^{1;21}$	P (BS†)	2.61	—	nd
B116 (MS)	P (FL)	9·53	—	—
$Gm^{1;21}/Gm^{1;21}$	P (TL)	7·47	0·53*	—
B132 (control)	WM (FL)	8·96	—	—
$Gm^{1;21}/Gm^{1;21}$	WM (TL)	7·32	0·80*	—
	WM (OL)	9·13	0·74*	—
B134 (control)	WM (FL)	5·71	0·45*	—
$Gm^{1;21}/Gm^{1;21}$	WM (TL)	5·64	0·51*	—
	WM (OL)	7·24	0·88*	—

* Latent allotype; † different from previous sample. All other abbreviations and methods as described in Table 1. nd = not done.

allotypes were detected (Table 3). Of the $Gm^{3;11}/Gm^{3;11}$ homozygotes, one control patient (B109) and one MS patient (B123) showed low levels of latent G1m(1) in eluates of one of three and two of three brain tissue samples, respectively. Latent Gm allotypes [i.e. G1m(3) and G3m(11)] were expressed more strongly in samples from $Gm^{1;21}/Gm^{1;21}$ homozygous individuals. In control patients, B132 and B134 and MS patient B116 G1m(3) was present at levels between 7–12% of the nominal G1m(1) allotype in eluates from two of three, three of three and one of two brain tissue samples, respectively. By contrast, the latent allotype G1m(3) was expressed strongly in one plaque from B56, and in one plaque from B90 its concentration exceeded that of the nominal G1m(1) allotype. In both these last MS brains another latent allotype, G3m(11), normally absent in $Gm^{1;21}/Gm^{1;21}$ homozygous individuals was represented at levels which were above most of the values found for its nominal counterpart in $Gm^{3;11}/Gm^{3;11}$ patients.

Perivascular infiltrates of lymphoid cells are generally observed in relatively recent, rather than old plaques (Esiri, 1980). A comparison was made of the distribution of latent Gm allotypes, total

Table 3. Total IgG and latent Gm allotypes in multiple sclerosis plaques: correlation with presence of lymphoid cells

MS patient and probable Gm genotype	Tissue sample (location)	Total IgGs*	Lymphoid cells	Latent allotypes
B120 ($Gm^{1;21}/Gm^{3;11}$)	P (lat-vent)	3·5	+	not applicable
	P (FL)	3·6	+	not applicable
	P (TL)	2·0	+	not applicable
B66 ($Gm^{3;11}/Gm^{3;11}$)	P (FL)	12·7	−	—
	P (OL)	7·1	−	—
	P (OL†)	8·1	−	—
B115 ($Gm^{3;11}/Gm^{3;11}$)	GL WM (FL)	2·4	−	—
	P (TL-vent)	3·4	−	—
	P (medulla)	3·9	−	—
B56 ($Gm^{1;21}/Gm^{1;21}$)	P (OL)	10·6	+	G3m(11)
	P (TL)	9·9	+	G1m(3), G3m(11)
B90 ($Gm^{1;21}/Gm^{1;21}$)	P (BS)	3·5	+	G1m(3), G3m(11)
	P (BS†)	4·4	−	—

* Total IgGs values are expressed relative to a mean value for control brain samples of 63 femtomol ^{125}I-protein A bound per mg protein (see Materials and Methods). Abbreviations as in Table 1.

IgGs, and histologically identified lymphoid cells among the limited number of plaque samples for which all three sets of data were available (Table 3). Total levels of plaque associated IgGs showed no correlation with the presence of lymphoid cells whereas latent allotypes were found only in those samples in which such cells were identified.

DISCUSSION

A recent report from our laboratory noted the problems in interpreting complex IgG IEF spectra and laid emphasis on the quantitative rather than qualitative differences between spectra from plaques within each MS brain (Glynn *et al.*, 1982). Subsequent more extensive studies have reinforced the view that, in discrete plaque eluates, certain focused IgG bands differ in intensity producing quantitative variations superimposed on a common pattern for each MS patient. In the present study the plaque to plaque distribution of B cell clones was investigated by determining Gm allotype concentrations in discrete tissue eluates, an approach which may avoid some of the problems incurred with IEF analysis.

The overall impression from our survey of some nominal Gm allotypes in plaque eluates was of an underlying common distribution of a predominant IgG1 allotype associated with fluctuations in level of a quantitatively minor IgG3 allotype. This picture seems to concur with the conclusions from our IEF studies and suggests a parallel distribution between plaques of the predominant nominal allotypes and the oligoclonal IgGs. Furthermore, all nominal Gm allotypes investigated were found in each plaque within brains from MS patients; this argues against a qualitative heterogeneity of B cell clones from plaque to plaque, at least as far as nominal IgCH encoding genes are concerned.

In some immune perturbations an immunoglobulin allotype may be detected in an individual whose genotype theoretically permitted no such antigenic expression; some characteristics of these latent allotypes have been reviewed recently (Kindt & Yarmush, 1981). Latent Gm allotypes have been found in a small percentage of CSF samples from MS patients (Salier *et al.*, 1983). In the

present study, quite variable amounts of latent Gm allotypes were detected in tissue eluates from both plaques and control white matter. Thus, expression of latent allotypes in brain tissue is not an MS specific phenomenon but it may be enhanced in this disease since for example, latent G3m(11) IgG3 molecules were detected only in plaque samples. It is noteworthy that those plaques containing the greatest amounts of latent allotypes were characterized by the presence of lymphoid cells suggesting recent immunological activity. Although the presence of IgG containing lymphoid cells in MS brain is well established (Esiri, 1980), large amounts of IgGs which display oligoclonal IEF spectra can be eluted from plaques in which such cells are absent (Glynn *et al.*, 1982). Additionally in an immunofluorescent study of MS brain, the highest levels of IgGs were observed in old rather than recent plaques (Tavolato, 1975). Thus, it appears that IgGs can be detected in the vicinity of a plaque for longer than lymphoid cells. The disparity between the distributions of total IgGs and latent Gm allotypes make it unlikely that the latter themselves comprise the oligoclonal IgGs in MS. Indeed, the latent allotypes in recent MS lesions may be products of an initial transient event which coincides with infiltration of lymphoid cells and precedes the appearance in these plaques of oligoclonal IgGs.

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REFERENCES

DELMOTTE, P. (1979) Measurement of blood/CSF permeability coefficients and intrathecal synthesis of IgG by capillary isotachophoresis. In *Humoral Immunity in Neurological Diseases.* NATO Advanced Study Series, Series A. Vol. 24. (ed. by D. Karcher, A. Lowenthal & A.D. Strosberg) pp. 221–228. Plenum Press, New York.

ESIRI, M. (1980) Multiple Sclerosis: a qualitative and quantitative study of immunoglobulin containing cells in the central nervous system. *Neuropathol. Neurobiol.* **6,** 9.

GLYNN, P., GILBERT, H.M., NEWCOMBE, J. & CUZNER, M.L. (1982) Analysis of immunoglobulin G in multiple sclerosis brain: quantitative and isoelectric focusing studies. *Clin. exp. Immunol.* **48,** 102.

GOUST, J.M., HOGAN, E.L. & ARNAUD, P. (1982) Abnormal regulation of IgG production in multiple sclerosis. *Neurology*, **32,** 228.

KINDT, T.J. & YARMUSH, M. (1981) Expression of latent immunoglobulin allotypes and alien histocompatibility antigens: relevance to models of eukaryotic gene regulation. *Critic. Rev. Immunol.* **2,** 297.

MATTSON, D.H., ROOS, R.P. & ARNASON, B.G.W. (1980) Isoelectric focusing of IgG eluted from multiple sclerosis and subacute sclerosing panencephalitis brains. *Nature*, **287,** 335.

MATTSON, D.H., ROOS, R.P. & ARNASON, B.G.W. (1982) Oligoclonal IgG in multiple sclerosis and subacute sclerosing panencephalitis brains. *J. Neuroimmunol.* **2,** 261.

NEWCOMBE, J., GLYNN, P. & CUZNER, M.L. (1982) Analysis by transfer electrophoresis of reactivity of IgG with brain proteins in multiple sclerosis. *J. Neurochem.* **39,** 1192.

OLSSON, J.E. & NILSSON, K. (1979) Gamma globulins of CSF and serum in multiple sclerosis: isoelectric focusing on polyacrylamide gel and agar gel electrophoresis. *Neurology*, **29,** 1383.

PANDEY, J.P., GOUST, J.M., SALIER, J.P. & FUNDEBERG, H.H. (1981) Immunoglobulin G heavy chain (Gm) allotypes in multiple sclerosis. *J. clin. Invest.* **67,** 1797.

ROSTROM, B. (1982) Antibodies against viruses and structural brain components in oligoclonal IgG obtained from multiple sclerosis brain. *J. Neurol.* **226,** 255.

SALIER, J.P., SARVAS, H., REISNER, H.M., WANG, A.L. & FUDENBERG, H.H. (1979) Quantitative studies on Gm allotypes. III. Some effects of IgG aggregation in radioimmunoassays using human IgM and rabbit IgG anti-Gm antibodies. *Mol. Immunol.* **16,** 217.

SALIER, J.P., GOUST, J.M., PANDEY, J.P. & FUDENBERG, H.H. (1981) Preferential synthesis of the G1m(1) allotype of IgG1 in the central nervous system of multiple sclerosis patients. *Science*, **213,** 1400.

SALIER, J.P., GOUST, J.M., LINK, H., PANDEY, J.P., DAVEAU, M. & FUDENBERG, H.H. (1983) Latent immunoglobulin G (Gm) allotypes: occurrence in the cerebrospinal fluid in some neuropathological states. *J. Immunogenet.* **10,** 311.

STEINBERG, A.G. & COOK, G.E. (1981) *The Distribution of Human Immunoglobulin Allotypes.* Oxford University Press, Oxford.

TAVOLATO, B.E. (1975) Immunoglobulin G-distribution in multiple sclerosis brain: an immunofluorescence study. *J. Neurol. Sci.* **24,** 1.

TOURTELLOTTE, W.W., POTVIN, A.R., POTVIN, J.H., BAUMHEFNER, R.W. & SYNDULKO, A. (1980) Multiple sclerosis *de novo* central nervous system IgG synthesis: measurement, antibody profile, significance, eradication and problems. In *Progress in Multiple Sclerosis Research* (ed. by H. J. Bauer, S.

Posner & G. Ritter) pp. 106–116. Springer-Verlag, Berlin.

VARTDAL, F. & VANDVIK, B. (1982) Multiple sclerosis: electrofocused bands of oligoclonal CSF IgG do not carry antibody activity against measles, varicella-zoster or rotaviruses. *J. Neurol. Sci.* **54,** 99.

VARTDAL, F., VANDVIK, B. & NORRBY, E. (1980) Viral and bacterial antibody responses in multiple sclerosis. *Ann. Neurol.* **8,** 248.

WILLIAMSON, A.R. (1978) Isoelectric focusing of immunoglobulins. In *Handbook of Experimental Immunology* Vol. 1, 3rd Edn. (ed. by D. M. Weir) Chap. 9. Blackwell Scientific Publications, Oxford.