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IgG synthesis within the brain in multiple sclerosis and subacute sclerosing panencephalitis

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SUMMARY

Intracerebral IgG synthesis was measured in patients with multiple sclerosis (MS), subacute sclerosing panencephalitis (SSPE) and in two control groups—patients with other neurological disorders and patients with non-neurological diseases. Significantly increased synthesis was found at all times in SSPE ($P<0{\cdot}001$). Significantly elevated values were also found in MS ($P<0{\cdot}001$), but in this group increased values were found mainly during relapse. These findings emphasize the immunological component in SSPE where there is known to be persistent measles virus infection in the brain, and in MS where the aetiology is unknown. Intracerebral IgG synthesis appears to be of diagnostic value in MS. The cerebrospinal fluid (CSF) IgG/total protein ratio was also measured in MS, and correlated only moderately well with the calculated IgG synthesis, indicating that the ratio provides a cruder estimate of intracerebral IgG synthesis.

INTRODUCTION

Persistent viral infection is now recognised to be involved in the pathogenesis of some chronic neurological disorders and suspected of being so involved in others. It has been established that subacute sclerosing panencephalitis (SSPE), a slowly progressive and fatal disease, is caused by a virus that is identical with, or very closely similar to, the measles virus (Payne, Baublis & Itabashi, 1969). The virus persists within the brain, from which it can be isolated, despite high levels of measles antibody in both serum and cerebrospinal fluid CSF (Connolly *et al.*, 1967). In contrast, the aetiology of multiple sclerosis (MS) remains unknown, although a persistent viral infection is a favoured hypothesis, measles virus being perhaps the strongest candidate. However, the evidence implicating measles virus is indirect. Modestly elevated antibody levels to measles virus have been reported (Adams & Imagawa, 1962; Haire, Fraser & Miller, 1973). Measles virus antigen has not, however, been identified in the central nervous system. A recent claim that measles virus can be identified and cultured from jejunal biopsies from patients with MS (Pertschuk *et al.*, 1977; Prasad *et al.*, 1977) has not so far been repeated by other workers (Woyciechowska, Madden & Sever, 1977). In recent years, there have been reports of the identification of various viruses or virus-like particles in the central nervous system (CNS), but these have either been based on single case reports or have not subsequently been substantiated (ter Meulen *et al.*, 1972; Carp *et al.*, 1972; Tanaka, Iwasaki & Koprowski, 1976).

Both in MS and SSPE, three lines of evidence suggest local production of immunoglobulin within the brain, and have been taken as evidence of persistence of antigen. (1) Oligoclonal bands of IgG in the CSF, shown by electrophoresis, suggested synthesis of IgG of restricted specificity (Link & Muller, 1971; Vandvik & Norrby, 1973). (2) The serum to CSF measles antibody ratio was found to be lower than that for other viruses in all patients with SSPE (Connolly, 1968), and in about 60% of patients with

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MS (Norrby *et al.*, 1974; Vandvik & Degré, 1975). (3) Tourtellotte (1970; 1975) devised a method of calculating intracerebral IgG synthesis from simultaneous measurement of IgG and albumin in serum and CSF. Significant synthesis was found in 86% of cases of MS. Raised intracerebral IgG synthesis has been found in SSPE by other techniques. Cutler, Merler & Hammerstad (1968a) studied two cases by measuring the exchange of ^{125}I-labelled IgG between serum and CSF, and Tourtellotte *et al.* (1968) measured the IgG and albumin content of a brain biopsy in one case.

This study was carried out to repeat the work of Tourtellotte on intracerebral IgG synthesis in MS and to extend these studies to examine the influence of relapse on IgG synthesis. We have also studied patients with SSPE, a disease known to be due to persistent viral infection in the brain, in order to compare these results with MS. Studies in SSPE using this technique have not been reported previously. In order to evaluate a simpler and more widely available measurement, we have compared the value of CSF IgG/total protein ratio and the calculated value for intracerebral IgG synthesis.

PATIENTS AND METHODS

Patients. Twelve patients with multiple sclerosis were studied (Table 1). The clinical state at the time of study varied. One patient (no. 8) was studied twice, during the initial episode, and then 4 months later during her first relapse. In nine out of thirteen studies, CSF IgG as a percentage of total CSF protein was also measured. One of the patients was on steroids at the time of study.

Twenty studies were performed on seven children, (two males and five females, aged 5–14 years) with SSPE. Three were receiving intermittent therapy with 'transfer factor' (dialysable leucocyte extract from donors with positive delayed-hypersensitivity skin tests to measles antigen).

Two control groups were studied. The first consisted of thirty-nine patients (twenty-four male and fifteen female) with a variety of other neurological disorders, subgrouped as follows: myelopathy (drug- or radiation-induced (4), motor neurone disease (5), cervical spondylosis (2), Guillaine–Barré syndrome (3) (six studies), peripheral neuropathy (5), cerebro-vascular disease (ischaemic) (4), transient loss of consciousness (1), epilepsy (3), Parkinson's disease (1), cortical atropy (4), cerebellar degeneration (2), neurosyphilis (2), viral meningitis (1), viral encephalitis (1) and febrile convulsions (1). The second control group consisted of eight patients (six males and two females) with a variety of non-neurological diseases.

Intracerebral IgG synthesis. Principle. IgG in the cerebrospinal fluid can be derived from the blood plasma and from local synthesis within the CNS. Albumin, on the other hand, is derived entirely from the blood plasma.

The amount of plasma-derived IgG can be calculated from the albumin concentrations in the blood and CSF, and the IgG concentration in the blood, if it is assumed that relative values of the blood brain barrier for albumin and IgG remain the same when the absolute level of the barrier and the plasma concentrations of albumin and IgG vary. The difference between the measured IgG level in the CSF and the amount calculated as coming from the plasma represents the amount synthesised in the CNS.

This is the basis of the method of Tourtellotte (1970). From studies with iodinated proteins given to normal subjects, he found the normal plasma/CSF molar concentration ratio to be 230 for albumin and 369 for IgG. A normal volume of CSF formed of 500 ml per day (Culter *et al.*, 1968b) is also assumed.

The following formula is then used to calculate the IgG synthesis in the CNS:

$$\text{IgG synthesis} = \left\{\left[\text{IgG}_{\text{CSF}} - \frac{\text{IgG}_{\text{serum}}}{369}\right] - \left[\text{Alb}_{\text{CSF}} - \frac{\text{Alb}_{\text{serum}}}{230}\right] \times \left[\frac{\text{IgG}_{\text{serum}}}{\text{Alb}_{\text{serum}}}\right] \times 0{\cdot}43\right\} \times 5$$

where IgG_{CSF} = CSF IgG (mg/100 ml), $\text{IgG}_{\text{serum}}$ = serum IgG (mg/100 ml), Alb_{CSF} = CSF albumin (mg/100 ml), and $\text{Alb}_{\text{serum}}$ = serum albumin (mg/100 ml).

Method. IgG and albumin levels were measured by rocket electrophoresis. Samples were diluted and placed in wells cut in agarose plates containing either antibody to human serum albumin (anti-HSA), or antibody to IgG (anti-IgG). A series of standards were included on each plate. For IgG estimations, samples were carbamylated, so that the IgG moved towards the positive electrode. Only 3·0 μl is required to fill each of the 2·0 mm wells. The electrophoresis was run overnight in veronal buffer pH 8·6. The plates were dried and stained, and the height of rockets measured using a photo enlarger. The concentrations of IgG and albumin were calculated from a linear regression programme.

CSF IgG/total protein ratio. This estimation was performed in Professor Wootton's Department of Chemical Pathology, Royal Postgraduate Medical School. The CSF IgG level used in this ratio was measured by nephelometry, after the addition of standard amounts of antibody to IgG. The total protein in the CSF was measured by trichloracetic acid precipitation.

TABLE 1. Multiple sclerosis patients

Patient number	Age	Sex	Clinical classification*	Duration of disease	Disease activity	Intracerebral IgG synthesis (mg/day) normal: up to +10 mg/day	CSF IgG/ total protein (%) normal: up to 13%	Blood brain barrier (serum/CSF albumin)
1	35	F	Clinically definite	2 yr	Relapse	+54	n.d.	108
2	24	F	Early probable	6 months	Relapse	+10	n.d.	171
3	22	F	Early probable	7 weeks	Relapse	+12	12	228
4	21	F	Early probable	4 months	Relapse	+10	25	372
5	54	F	Early probable	5 months	Relapse	+36	31	189
6	26	F	Early probable	7 weeks	Initial episode	+9	24	306
7	28	F	Clinically definite	10 yr	Relapse	+38	n.d.	79
8	45	F	Early probable	4 weeks	Initial episode	+3	7	284
			Early probable	5 months	Relapse	+20	20	366
9	31	F	Clinically definite	9 yr	Relapse	+26	23	170
10	46	M	Clinically definite	15 yr	2 year remission	+4	n.d.	272
11	52	M	Clinically definite	3 yr	Relapse and progressive paraplegia	+21	16	183
12	30	M	Early probable	4 weeks	Initial episode	+2	14	270

* According to criteria of McDonald & Halliday (1977).
n.d. = Not done.

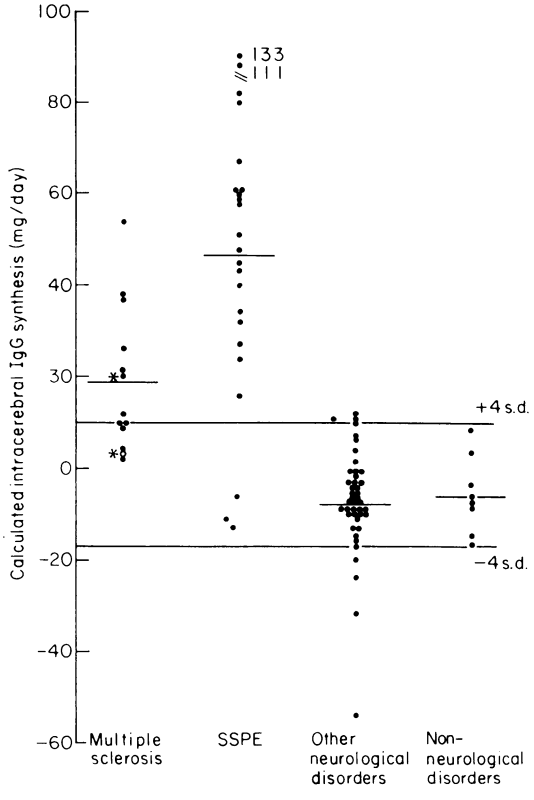

FIG. 1. Intracerebral IgG synthesis in MS, SSPE, and two control groups. * Indicates results in one MS patient studied on two occasions. The normal range indicated (mean ± 4 s.d.) is taken from Tourtellotte's data obtained in normal subjects (see the Discussion). Horizontal bars indicate mean for each group. The differences between MS and the neurological control group, and SSPE and the neurological control group are statistically significant ($P < 0{\cdot}001$ for both by Student's t-test).

RESULTS

These are shown in Tables 1 and 2 and Fig. 1. Significantly elevated intracerebral IgG synthesis was found in MS (mean ± 1 s.d. = 18·9 ± 15·8 mg/day, $P < 0{\cdot}001$, Student's t-test) and SSPE (mean ± 1 s.d. = 46·6 ± 20·2 mg/day, $P < 0{\cdot}001$) compared to the neurological control group (mean ± 1 s.d. = −7·7 ± 11·5 mg/day) and the non-neurological controls (mean ± 1 s.d. = −6 ± 8·4 mg/day). In Tourtellotte's series, the mean value for intracerebral IgG synthesis, derived from seventy normal studies, was −3·3 mg/day with an s.d. of ± 3·3 mg/day.

In assessing our findings we have divided the values found into three groups: (i) those with a synthesis of below 3 mg/day (mean + 2 s.d.) which we regard as normal; (ii) those with synthesis of between 3·3 and 10 mg/day (mean + 2 s.d. to mean + 4 s.d.) which we regard as a marginal increase; and (iii) those with synthesis above 10 mg/day (mean + s.d. 4) which we regard as a definite increase.

These s.d. apply only when the blood brain barrier is near to normal (see the Discussion) and for this reason, the value of the blood brain barrier is also given in the results. When this is markedly abnormal (e.g. less than 100), the synthesis results must be viewed with suspicion.

Increased IgG synthesis was found in all seven SSPE patients, but fluctuations were observed where serial studies were performed. Measurements made before and during transfer factor therapy in three patients showed that institution of this treatment did not alter the results.

Whereas all of the SSPE patients showed abnormally high values, the MS patients showed more variation. Definitely increased synthesis was found in seven patients, all of whom were studied during a relapse. Four patients had marginally increased synthesis: two of these were studied in relapse, one in

the initial episode and one in remission. Only two out of thirteen MS patients had normal values, and both were studied during the initial episode. Thus all nine patients studied during a relapse showed increased synthesis. The duration of disease in these patients varied, the shortest history being only 7 weeks. One patient (no. 8), who had a normal synthesis on presentation, was restudied 4 months later during her first relapse when she had an abnormal IgG synthesis of +20 mg/day.

In nine patients with MS, both the CSF IgG/total protein ratio and the intracerebral IgG synthesis were measured. The results correlated in seven patients (taking +3·3 mg/day as abnormal synthesis), or in five patients (taking +10 mg/day as abnormal synthesis).

TABLE 2. SSPE patients

Patient	Date (day, month)	CSF IgG (mg/100 ml)	Blood brain barrier (serum/CSF albumin) (normal = 230)	Intracerebral IgG synthesis mg/day (normal: up to +10)
1	12·6	13	779	61
	15·7	14	507	60*
	27·10	7·5	378	27*
	19·11	15	138	51* (mean = 50)
2	29·4	18	305	80
	12·6	14	207	58*
	28·6	10	90	40*
	15·7	12	226	45*
	23·10	14	179	59*
	27·10	10	145	34*
	19·11	33	155	133* (mean = 64)
3	22·3	16	483	67
	28·6	11	196	48*
	5·8	19	270	82*
	14·10	25	182	111*
	27·10	11	155	32* (mean = 68)
4	17·11	4·9	460	16
5	19·8	6·7	556	24
6	25·11	12·2	285	43
7	18·7	16	419	61

* Receiving transfer factor.

DISCUSSION

The Tourtellotte formula for calculating IgG synthesis

IgG in the CNS comes from three sources. The first is a 'transudate' from the blood across the normal blood brain barrier. Tourtellotte assumes this to occur in everyone to the same extent (whether or not the blood brain barrier is normal). The amount of IgG derived from this transudate (which one may call IgG_t) is given by the IgG_{serum} divided by 369. Similarly, the albumin derived in this way ($Albumin_t$) is given by the $albumin_{serum}$ divided by 230. These values are derived from studies in normal subjects with radio-iodinated proteins. The ratio of $albumin_{serum}$ to $albumin_{CSF}$ is taken as a measure to the blood brain barrier. This is normally 230 therefore and values below 230 indicate increased leakiness.

The second source of CSF IgG is by what may be described as 'exudation' from the blood across a leaky blood brain barrier. The amount of this exuded IgG is calculated from the albumin levels in the serum and CSF. First, exuded albumin ($albumin_e$) is calculated by subtracting the $albumin_t$ from the $albumin_{CSF}$. It is, however, then necessary to make a further assumption in calculating a value for the exuded IgG (IgG_e) from the $albumin_e$ value.

(a) The assumption made by Tourtellotte (1970) is that the exudation across the blood brain barrier occurs to an extent proportional to the molar concentrations of the two proteins.

$$\text{Then, } IgG_e = Alb_e \times \frac{IgG_{serum}}{150{,}000} \times \frac{69{,}000}{Alb_{serum}}, \text{ i.e., } IgG_e = \frac{IgG_{serum}}{Alb_{serum}} \times 0{\cdot}43.$$

This is the formula used by Tourtellotte and has been used in calculating our results.

(b) Alternatively, it is possible to assume that the 'exudation' across the leaky blood brain barrier occurs as if the exudate were whole diluted serum.

$$\text{Then, } IgG_e = Alb_e \times \frac{IgG_{serum}}{Alb_{serum}}.$$

This would give a value for IgG_e that is almost $2\frac{1}{2}$ times that given by assumption (a).

(c) Thirdly, it is possible to assume that the exudation across the leaky blood brain barrier shows the same discrimination between albumin and IgG as does the transudation across the normal blood brain barrier.

$$\text{Then, } IgG_e = Alb_e \times \frac{IgG_{serum}}{369} \times \frac{230}{Alb_{serum}}, \text{ i.e., } IgG_e = Alb_e \times \frac{IgG_{serum}}{Alb_{serum}} \times 0{\cdot}62.$$

In this case the value for IgG_e would be roughly $1\frac{1}{2}$ times as large as that given by assumption (a).

(d) Finally, it may be assumed (and indeed would seem not at all unlikely) that the leak across the blood brain barrier may, at least in some cases, be selective allowing relatively more albumin across. A parallel might be drawn here with the leak of protein across the glomerular basement membrane in some forms of renal disease. In those circumstances IgG_e will be less than the IgG_e value as calculated in (a), but to what extent cannot be calculated.

The third source of the CSF IgG is that synthesised in the CNS. IgG synthesis = $IgG_{CSF} - IgG_t - IgG_e$.

In cases where the blood brain barrier is normal the value for IgG_e becomes zero because $albumin_e$ becomes zero, and all the problems about the assumptions referred to above disappear. Fortunately, in most cases with MS and in SSPE, a fairly normal blood brain barrier is found (as in this series) and the problems raised about the true value for IgG_e do not disturb the calculation. However, in cases where the blood brain barrier is significantly leaky, the value for IgG_e becomes significant and the value for IgG synthesis therefore becomes liable to uncertainty and should be given wide confidence intervals. In practice the errors seem to become significant only if the barrier value is below 100, and dramatic if it is below 50.

Discussion of results

In this study we have shown persistent and gross elevation of intracerebral IgG synthesis in SSPE, a condition known to be related to persistent infection of the CNS by measles or a closely related virus. Abnormal intracerebral IgG synthesis was also found in MS, but only in patients studied during a relapse (including the first relapse). In contrast, all MS patients studied in remission or during the initial episode had normal values. A small number (two out of eight) of patients in relapse had values at the upper limit of normal. This test may therefore be of diagnostic value in MS, provided it is performed when there is clinical evidence of disease activity.

Because of the limited studies on measurements of intracerebral IgG synthesis, it is important to discuss the normal range and variations in neurological disease. From our results it can be seen that an upper limit of +10 mg/day is perhaps the best value to take for diagnostic purposes. Only two of our controls had values above this. Our normal range of −17 to +10 mg/day is wider than that quoted by Tourtellotte (mean ± 2 s.d. = −9·9 to +3·3 mg/day). His normal range was derived from normal subjects with intact blood brain barriers, while all our controls were patients with acute illnesses and variable blood brain barriers. Our controls give a correspondingly wider scatter of values (Fig. 1), because the confidence limits of Tourtellotte's formula widen sharply as the blood brain barrier becomes more permeable.

TABLE 3. Patients with present or previous CNS infections (in neurological control group)

Patient	Age	Sex	Diagnosis	Treponemal serology		CSF IgG (mg/100 ml)	Serum/CSF albumin (normal = 230)	Intracerebral IgG synthesis mg/day (normal: −17 to +10)
				Serum	CSF			
JH	48	M	Previous neurosyphilis 6 years ago. Intermittent claudication.	Positive	Negative	3·0	123	−5
KO	52	F	Latent neurosyphilis	Positive	Positive	3·0	279	+1
JB	33	F	Viral meningitis 4 months previously. Migraine	Negative	n.d.	0·7	169	−13
DF	30	F	Viral encephalitis	n.d.	n.d.	5·6	130	−0·9

n.d. = Not done.

The control patients with synthesis greater than +10 mg/day both had neurological disorders (one had nerve root compression and latent syphilis and the other had amyloid neuropathy). Five of our neurological control group and one of the non-neurological control group had values above the +2 s.d. limit of +3·3 mg/day.

Extremely low values, below −17 mg/day, were found in four patients in the neurological control group. All four had greatly increased permeability of the blood brain barrier as assessed by low values for the serum/CSF albumin ratios, *viz* 20, 50, 98, 24 (normal 230). We believe this to be due to the problems in the calculations discussed above, or to the fact that a leaky barrier may be selective, analogous to a leaky glomerular basement membrane.

In view of reports of increased CSF IgG levels in neurosyphilis (Laterre *et al.*, 1970), we have shown our results in four of the neurological control group who had present (two out of four) or previous (two out of four) infection of the CNS (Table 3). All of these had normal values for intracerebral IgG synthesis.

The finding of significant intracerebral IgG synthesis in SSPE and MS, suggests that in both diseases there is a persistent antigenic stimulus within the brain. In SSPE, there is good evidence (very high levels of measles antibody and oligoclonal IgG directed against measles virus in the CSF) to suggest that the stimulus to IgG production in the brain is persistent measles virus. In MS, the nature of the antigenic stimulus is not clear. The two major possibilities are that the antigens are viral (e.g. measles virus antigens), or that they are autoantigens (e.g. some component of nervous tissue, such as myelin basic protein). CSF measles antibody levels are known to be modestly elevated in MS (Haire *et al.*, 1973); however, only a small part of the oligoclonal IgG proteins seem to be associated with measles antibody activity (Vandvik *et al.*, 1976). There is no evidence of antibody to myelin basic protein in MS (Lennon & Mackay, 1972; Lisak *et al.*, 1968; Gutstein & Cohen, 1978), although demyelination factors have been demonstrated in MS sera (Lumsden, 1971). Abramsky *et al.* (1977) have recently demonstrated serum antibodies to oligodendroglia in MS and suggest that oligodendroglia are the primary target sites in this disease, demyelination occurring secondary to this. It is therefore possible that the intracerebral IgG synthesis we have demonstrated represents auto-antibody formation to abnormal membrane antigens (? virus-induced) on oligodendroglia.

The differences demonstrated in intracerebral IgG synthesis between the SSPE and MS groups may reflect either differences in the nature of the antigenic stimulus in the brain, or differences in host response in the two diseases.

The lack of a direct correlation in MS between the CSF IgG/total protein ratio and intracerebral IgG synthesis is not susprising. In this ratio, only a rough correction is made for the component of CSF IgG derived from the blood. In addition, the techniques used to measure the IgG and total protein are rather crude. The main advantage of the IgG/total protein ratio is that it is simple and easy to perform. A previous report showed that 66% of patients with MS have a statistically significant elevation of the CSF IgG/total protein ratio (Tourtellotte, 1975). However, its lack of specificity became apparent in a study by Link & Muller (1975), who found an elevated IgG/total protein ratio in an even higher proportion of MS patients (73%), but also in infections of the CNS (36%) and other neurological disorders (16%). These observations, taken with our findings, suggest that the intracerebral IgG synthesis is a much more valuable diagnostic tool in MS than the CSF IgG/total protein ratio.

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