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Intracerebral synthesis of antibodies to measles and distemper viruses in patients with subacute sclerosing panencephalitis and multiple sclerosis

N. T. GORMAN, J. HABICHT & P. J. LACHMANN *Division of Immunology, Department of Pathology, University of Cambridge and MRC Group on Mechanisms in Tumour Immunity, Hills Road, Cambridge, England*

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SUMMARY

Serum and cerebrospinal fluid (CSF) measles and distemper antibody levels were quantified in a series of twenty patients (four subacute sclerosing panencephalitis (SSPE); ten multiple sclerosis (MS); six non-MS neurological cases) using independent competitive inhibition radioimmunoassays. These results were used in a Tourtellotte calculation to measure the intracerebral IgG synthesis to each virus. The results confirmed that in SSPE there is a greatly enhanced intracerebral measles antibody synthesis (6·0 mg/day). However, it was found that in SSPE this represents only part of a general systemic measles hyperimmunization as the intracerebral measles antibody synthesis relative to the total body measles synthesis was not significantly different from other groups (8%).

In MS patients there was increased intracerebral immunoglobulin synthesis but the measles antibody levels were neither significantly elevated nor different from the control group.

No evidence was found to support the concept that canine distemper virus is implicated in either MS or SSPE.

INTRODUCTION

An increased production of intracerebral IgG is now a consistent observation in multiple sclerosis (MS). This can be calculated from IgG and albumin levels in serum and CSF by the method of Tourtellotte (1970). Tourtellotte concluded that the upper limit of IgG synthesis in normal subjects was +3·3 mg per day and that increased levels were of diagnostic significance. Whilst there has been some doubt on the accuracy of the numerical results obtained the technique has been shown to give a good comparative estimate of immunoglobulin synthesis (Tourtellotte, 1975; Ewan & Lachmann, 1979).

The significance of increased immunoglobulin levels in the cerebrospinal fluid (CSF) in MS is, however, unresolved. It has been widely assumed that this increase represents a specific response to a defined antigenic stimulus within the central nervous system (CNS). This could be due either to a persistent viral infection or to autoantigens of the nervous system such as myelin basic proteins.

Numerous candidate viral agents have been proposed for MS following the measurement of specific antibodies in both serum and CSF (Haire, 1977; 1979). Since the original report by Adams & Imagawa (1962) measles virus has been implicated in many studies (Fraser, 1975; Haire, 1977, 1979). The evidence for the virus as the aetiological agent in MS is, however, far from convincing. The observed increase in serum measles antibody level is quite modest when compared to that found in chronic active hepatitis and even in systemic lupus erythematosus where measles is not considered to be the aetiological

Correspondence: Dr N. T. Gorman, Division of Immunology, Department of Pathology, Laboratories Block, Addenbrooke's Hospital, Hill Road, Cambridge CB2 2QQ.

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agent. Furthermore, the oligoclonal IgG bands in the CSF cannot be absorbed out by individual measles viral antigens (Vandvik *et al.*, 1976). Attempts to detect measles virus directly either by electron microscopical studies or by culture from affected brain specimens and other tissues in MS have been inconsistent (ter Meulen *et al.*, 1972; Carp *et al.*, 1972; Talaka, Iwasaki & Koprowski, 1976; Prasad *et al.*, 1977; Ebina *et al.*, 1979).

In contrast to the rather vague evidence relating measles to MS the data on measles virus as the aetiological agent in subacute sclerosing panencephalitis (SSPE) is compelling (see Connolly *et al.*, 1967; Valdimarsson, Agnarsdottir & Lachmann, 1979). In this condition the affected children show dramatically increased levels of measles antibody in both serum and CSF. Within the CSF are oligoclonal bands, which in complete contrast to MS can be absorbed out with individual measles antigens (Vandvik & Norrby, 1973; Norrby & Vandvik, 1976). Moreover, the identification of measles virus nucleocapsid by electron microscopy and the isolation of measles virus from affected brains convincingly implicate a persistent measles virus infection within the CNS as the aetiological agent in SSPE. The mechanism whereby the measles virus can persist *in vivo* despite a massive IgG antibody response and a competent cellular response is unknown. However, it has been shown that anti-measles IgG will modulate measles cell surface antigens *in vitro* in the absence of complement (Joseph & Oldstone, 1974). Such infected cells, although containing measles virions inside them, will not re-express the cell surface viral antigen until cultured in the absence of measles antibody (Oldstone & Tishon, 1978). Therefore, SSPE raises the question of whether or not other chronic neurological disorders may be caused by an inappropriate immunological response to a common pathogen.

Canine distemper virus along with rhinderpest and measles form the closely related morbilliviruses within the paramyxoviridae (Andrews, Pereira & Wildy, 1978). In a susceptible, unvaccinated dog, an acute encephalitis may be a sequela to the initial viraemia leaving the animal severely debilitated but in many cases the disease is fatal (Appel, 1969; Appel & Gillespie, 1972). The development of canine distemper encephalitis has been shown to be related to the degree of virally induced lymphopenia in the primary viraemia (McCullough, Krakowa & Koestner, 1974a). Demyelination is a feature of the encephalitis and this has been considered to be due both to a direct viral damage and an active immune response within the CNS (McCullough, Krakowa & Koestner, 1974b). Persistent distemper virus infection has been implicated in certain chronic neurological disorders (Lincoln *et al.*, 1971) although definite evidence is inconclusive (Palmer, 1978). To date, no comparable condition to MS has been defined in canine neuropathology.

Recently, canine distemper virus has been proposed as a candidate agent in MS. Cook, Dowling & Russell (1978) inferred that the sudden increase in the incidence of multiple sclerosis during the twenty years following the last war in the Faeroe Islands was possibly due to the distemper outbreak that occurred when British troops occupied the Island during the war years. A tenuous suggestion has been made on the association of MS with household dogs and even those dogs with apparent neurological disorders (Kurland & Briar, 1978; Jotkowitz, 1977; Cook & Dowling, 1977; Postkanzer, Prenncy & Sheridan, 1977). However, the majority of evidence presented in these reports has been based upon subjective interpretation of questionnaire answers and anecdotal data. The relatively detailed study by Nathanson, Palsson & Gudmundsson (1978) of MS in Iceland provided no comparable supportive epidemiological data to suggest that either dogs or distemper virus were implicated in MS.

The Tourtellotte calculation depends upon the simultaneous measurement of albumin (Alb) and immunoglobulin (Ig) levels in serum and CSF. From the albumin levels an expected value of the IgG can be calculated, this being made up of the Ig that is transudated across a normal blood–brain barrier plus a quantity that is exuding across a leaky blood–brain barrier. A reasonable estimate of the intracerebral IgG synthesis may be obtained by subtracting from the observed CSF IgG level the calculated IgG derived from the plasma. This principle is equally applicable to the measurement and calculation of intracerebral synthesis of individual specific antibodies providing that these quantities can be determined accurately. The purpose of this paper is to report the application of this method to measles and distemper antibodies in the serum and CSF of SSPE, MS and non-MS neurological patients.

MATERIALS AND METHODS

Patients. Serum and CSF samples were collected from twenty patients at the Department of Neurology, Addenbrooke's Hospital, Cambridge and the Hammersmith Hospital, London. The patients were classified clinically only at the completion of the study into (a) four SSPE patients, (b) ten MS patients (three clinically definite MS and seven clinically probable MS as according to McAlpine's classification) and (c) six neurological, non-MS patients. The non-MS patients comprised: (i) cerebral atrophy, (ii) cerebral infarct, (iii) retinal detachment; (iv) familial spastic paraparesis; (v) bilateral hemiplegia and (vi) left hemifacial spasm.

Intracerebral IgG synthesis. The principal of the calculation is covered by Tourtellotte (1970) and Ewan & Lachmann (1979).

Method. Serum and CSF IgG and albumin levels were determined by rocket immunoelectrophoresis. Samples were appropriately diluted (serum 1/400, 1/800; CSF 1/2, 1/4) in veronal buffer pH 8·6 and 3 μl placed in 2 mm wells cut in agarose plates containing optimal concentrations of either anti-human serum albumin and anti-human IgG. Included on each plate was a series of standard dilutions of known IgG or albumin concentration (dilution range 1/200–1/1600). To ensure IgG moved towards the positive electrode the IgG samples were carbamylated with 2 M potassium cyanate. The electrophoresis was run until completion in veronal buffer pH 8·6. The plates were subsequently dried, stained and the height of each rocket measured. The concentration of IgG and albumin were calculated using a linear regression programme.

Preparation of IgG fraction for use in radioimmunoassay. (a) *Anti-measles IgG.* Serum from a patient with SSPE (S.W.) was used as a reference source of anti-measles antibody. The IgG fraction was obtained by DEAE-cellulose chromatography equilibrated with 10 mM phosphate buffer pH 7·0. Under the conditions of the assay used it has been calculated that 10% of the S.W. IgG fraction was directed specifically against measles virus. This reference sera has been shown to have activity against all the seven polypeptides of the measles virus (Ewan & Lachmann, 1979, in preparation). There is not an absence of anti-matrix protein as suggested by Hall, Lamb & Choppin (1979) in this reference serum.

(b) *Anti-distemper IgG.* A series of four dogs were hyperimmunized with attenuated distemper virus to produce high titre anti-distemper antibody. The IgG fraction was obtained from the pooled canine serum by DEAE cellulose chromatography equilibrated with 10 mM phosphate buffer pH 7·5. Under the conditions of the assay used it has been calculated that 2% of the canine IgG fraction was specifically directed against distemper virus.

(c) *Trace labelling.* The IgG fraction was labelled with ^{125}I by a modified chloramine T method.

Competitive radioimmunoassay for anti-measles and anti-distemper viral antibodies. (a) *Anti-measles antibodies.* A radioimmunoassay has been developed to detect antibodies to those measles antigens expressed on the surface of HeLa cells persistently infected with Edmonston strain of measles virus fixed with 0·06% gluteraldehyde. Uninfected HeLa cells were used as control antigen. In each assay a standard curve was prepared where unlabelled reference IgG was used to inhibit the uptake of the radiolabelled IgG on the solid phase antigen (Fig. 1).

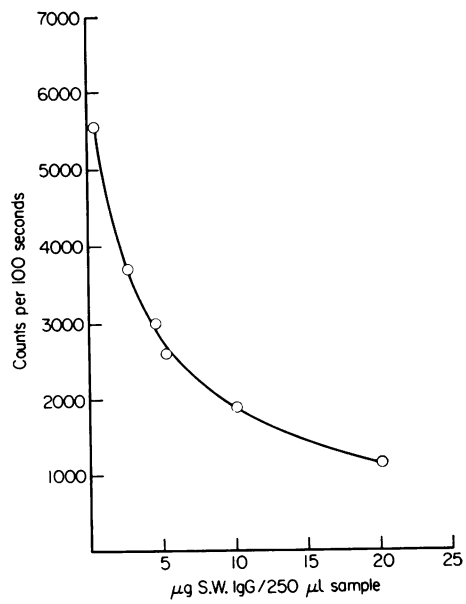

FIG. 1. A radioimmunoassay was developed to detect antibodies to those measles antigens expressed on the surface of HeLa cells persistently infected with Edmonston strain of measles. A standard curve has been prepared where unlabelled reference IgG was used to inhibit the uptake of the radiolabelled IgG on the solid phase antigen.

(b) *Anti-distemper antibodies.* Vero cells persistently infected with canine distemper virus (isolated from a field case of canine distemper) were fixed similarly to provide the solid phase antigen. Uninfected vero cells were used as control antigen. The standard curve was prepared with unlabelled and radiolabelled anti-distemper IgG (Fig. 2).

(c) *Method.* Standard solutions containing known quantities of either reference anti-measles IgG or reference anti-distemper IgG (0·4 μg–200 μg/ml) were made in PBS-azide containing 10% heated normal rabbit serum.

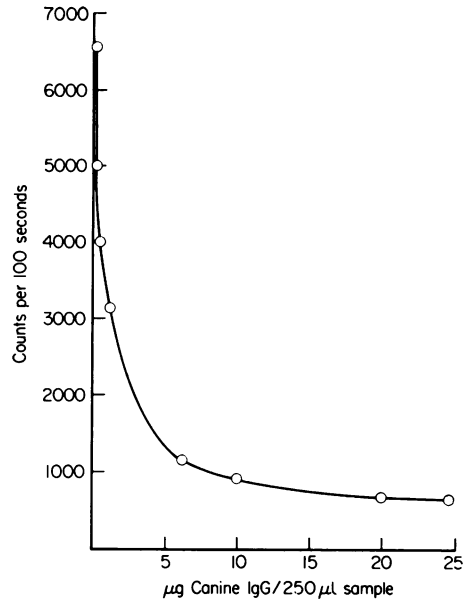

FIG. 2. Vero cells persistently infected with canine distemper virus were fixed (see Materials and Methods section) to provide the solid phase antigen. The standard curve was prepared with unlabelled and radiolabelled anti-distemper IgG.

To aliquots of 2×10^5 cells (250 μl) was added 250 μl of (a) the standard or (b) 1/10 dilution of test serum or (c) neat CSF containing 10% normal rabbit serum and each sample was tested in triplicate. After rotation for 1 hr at room temperature, 0·1 μg (250 μl) of the ^{125}I-labelled antibody was added to each tube and rotated at room temperature until equilibrated. The cell pellets were washed extensively in PBS-azide and the resultant activity of each sample counted. The root mean square value of each sample was calculated to determine the μg specific IgG in each sample from the standard curve.

Estimation of whole body anti-measles response. This may be estimated roughly as follows: assume for an adult a plasma volume of 5 litres and a further extracellular fluid volume of 10 litres whose IgG concentration is about half that in plasma (in children with SSPE 80% of these volumes were used). The total anti-measles IgG in body = (10× measured concentration in μg/ml) mg. If half life of IgG is taken as 21 days then synthesis rate in mg/day $= \frac{\text{Total Ab in mg.}}{42}$

Canine serum. To standardize the radioimmunoassay for the detection of specific anti-distemper antibodies thirty canine sera were examined (kindly provided by Burroughs Wellcome Ltd., Beckenham, Kent, England). Serum from each 4 m.o. dog was collected just prior to vaccination with canine distemper virus (Epivax, Burroughs Wellcome) and again 21 days post vaccination.

RESULTS

Three groups of patients were examined in this study. A group of four SSPE, ten MS patients and six non-MS neurological patients. The MS group was further sub-divided on the basis of the intracerebral IgG synthesis within the CNS (a) over +3·3 mgm/day (MS1) and (b) below +3·3 mgm/day (MS2).

IgG synthesis within the CNS

It is clear from Table 1 that the intracerebral IgG synthesis in SSPE patients was grossly elevated (31·8±0·6 mgm/day) and this was significantly different from either of the MS groups and the non-MS control (Student's *t*-test, $P<0·01$). In all patients the blood–brain barrier was within the normal limits.

TABLE 1. Serum and cerebrospinal fluid measles and distemper antibody levels quantified in twenty patients

	SSPE	MS1	MS2	Other
Number	4	6	4	6
Blood–brain barrier	292	290	184	273
I/c IgG synthesis	31·8	16·4	−2·3	−8·4
Measles antibody levels				
Serum μg/ml	529	27	15·4	16·5
CSF μg/ml	13·4	0·9	1·1	0·5
I/c synthesis mg/day	6·0	0·4	0·5	0·2
Distemper antibody levels				
Serum μg/ml	3·6	1·6	1·0	0·8
CSF μg/ml	0·2	0·1	0·3	0·2
I/c synthesis mg/day	0·1	0·05	0·1	0·05

I/c = Intracerebral.

TABLE 2. Measles virus antibody synthesis in the CNS

	SSPE	MS1	MS2	Other
Number	4	6	4	6
Blood–brain barrier	292	290	184	273
I/c IgG synthesis mg/day	31·8	16·4	−2·3	−8·4
Measles antibody levels				
Serum μg/ml	529	27	15·4	16·5
CSF μg/ml	13·4	0·9	1·1	0·5
Ratio serum:CSF	39·5	30·3	14·7	33
I/c measles antibody synthesis mg/day	6	0·4	0·5	0·2
I/c measles antibody synthesis as per cent of i/c IgG synthesis	18·9	2·3	—	—
I/c measles antibody synthesis as per cent of total measles antibody synthesis in body	8	5	12	6
Total measles antibody synthesis in body (approx.) mg/day*	101	6	4	4
I/c measles antibody synthesis as per cent of total measles antibody synthesis in body	8	5	12	6

* Calculation assumes plasma volume of 5 litres and further ECF volume of 10 litres (with antibody concentration one half that of plasma) for adults (for children with SSPE 80% of these volumes were assumed); and assumes half life of IgG to be 21 days.

Total anti-measles IgG (mg) = 10× plasma conc. (μg/ml) and synthesis rate in mg/day

$$\frac{\text{total Ab}}{42} = \frac{\text{plasma conc. } (\mu\text{g/ml})}{4{\cdot}2}.$$

I/c = Intracerebral.

Measles antibody synthesis

The results in Table 1 amply indicate that the intracerebral synthesis of specific anti-measles IgG in SSPE ($6{\cdot}0 \pm 2{\cdot}3$) was significantly elevated over the other groups (Student's t-test, $P < 0{\cdot}001$). However, in contrast to intracerebral IgG, the specific intracerebral anti-measles IgG synthesis was similar in all the three other groups.

In SSPE nearly 20% of the IgG synthesis in the CNS was found to be anti-measles which is grossly in excess of any other group. If, however, the intracerebral anti-measles IgG synthesis was compared to an estimate of the whole body anti-measles IgG synthesis an interesting observation was apparent.

As shown in Table 2 this relationship in SSPE was no different to other groups. Therefore, although there was apparent massive intracerebral anti-measles IgG response, this merely represented an overall enhanced response to the measles virus.

Distemper antibody synthesis

The normal range for serum measles antibody in the specific radioimmunoassay employed has been reported previously at 5–25 μg/ml (Lachmann & Habicht, 1979). However, the efficiency of the radioimmunoassay to detect specific anti-distemper IgG has not as yet been considered. To test the system thirty paired canine serum samples were measured before and 21 days after vaccination with canine distemper virus. It was found in this series that the expected increase following vaccination was observed. (Pre-vaccination $\bar{x} = 0{\cdot}25$ μg/ml (0·03–0·92) $n = 30$, post-vaccination $\bar{x} = 14{\cdot}3$ μg/ml (7·2–26·3) $n = 30$.) Normal healthy human donors in this laboratory were found to have a mean serum anti-distemper IgG level of $0{\cdot}8$ μg/ml serum $\pm 0{\cdot}4$.

In none of the test groups examined were striking levels of anti-distemper IgG found (Table 3) although the level in SSPE was significantly different to the others (Student's t-test, $P < 0{\cdot}02$).

The calculated synthesis of anti-distemper IgG within the CNS failed to show great variation between the groups but it would appear that the quantity of anti-distemper IgG present in the CNS represented

TABLE 3. Distemper virus antibody synthesis in CNS

	SSPE	MS1	MS2	Other
Number	4	6	4	6
Blood–brain barrier	292	290	184	273
IgG synthesis mg/day	31·8	16·4	−2·3	−8·4
Distemper antibody levels				
Serum μg/ml	3·6	1·6	1·0	0·8
CSF μg/ml	0·2	0·1	0·3	0·2
Ratio serum:CSF	18	12·2	3·9	5·4
I/c distemper antibody synthesis mg/day	0·1	0·05	0·1	0·05
I/c distemper antibody synthesis as per cent of i/c IgG synthesis	0·4	0·3	—	—
Total distemper antibody synthesis in body (approx.) mg/day	0·7	0·4	0·2	0·2
I/c distemper antibody synthesis as per cent of total distemper antibody synthesis in body*	25	11	47	23

* As in Table 2.

a considerable portion of the total anti-distemper IgG synthesis in the body (Table 3). This does, however, have to be placed in perspective considering the actual μg/ml amounts used to arrive at the percentages.

Finally, as with anti-measles IgG, there was no correlation between the intracerebral IgG synthesis and specific anti-distemper IgG.

DISCUSSION

The results in this study confirmed that in SSPE there was a marked elevation of total intracerebral IgG synthesis. This supported the findings of the previous report (Ewan & Lachmann, 1979). In MS the IgG synthesis in six patients (MS1) was found to be above the upper limit of the normal range (+3·3 mg/day). Indeed, the mean value of this group compared closely with other reports by Tourtellotte (1970) (16·0 mg/day) and Ewan & Lachmann (1979) (18·9 mg/day). There were four patients (MS2) who failed to show markedly elevated intracerebral IgG synthesis and these were treated as a separate subgroup for the viral antibody studies. Initially it would appear that this finding was contrary to previous data. Retrospective analyses of clinical data, however, indicated that three of these particular cases were in remission and only one in relapse. This would lend support to the findings of Tourtellotte (1970) and Ewan & Lachmann (1979) who found that, generally, only MS patients in relapse showed an increased intracerebral IgG synthesis.

In SSPE it was clearly evident that there was a marked synthesis of anti-measles antibody which confirmed earlier work although caution was expressed over the previously calculated low intracerebral anti-measles IgG synthesis in SSPE (4%) (Lachmann & Habicht, 1979). The levels in this study were significantly greater (18·9%) and attributable to the addition of heat inactivated normal rabbit serum to the CSF samples in order to avoid non-specific uptake of the radiolabelled IgG.

In both radioimmunoassays the solid phase antigen expressed only two, the haemagglutinin and the fusion factor of the seven polypeptides of the viruses (Woo, 1979; Gorman, unpublished observations). Although this may slightly underestimate the response it represented an improvement over the functional haemagglutination inhibition test and the results are more quantitative than virus neutralization assays. It must be stressed that the mass units defined in the tests (μg/ml) may be inaccurate and a more correct expression of activity may be 'equivalent inhibitory capacity' to a reference serum. This inhibitory capacity will depend upon both the specific antibody affinity and its relative concentration in the serum or CSF.

The fascinating observation in this study was that the enhanced intracerebral synthesis represented the same percentage of the whole body synthesis of anti-measles IgG as the other groups. It is important to conclude from this observation that in SSPE there is a systemic increase in the antibody synthesis to measles virus and not a selective increase in the specific intracerebral anti-measles IgG. This degree of systemic hyperreactivity in SSPE has not been taken generally into account in the evaluation of the pathogenesis of this disease. It would therefore appear that the abnormality is not situated entirely with the brain, although persistent leakage of antigen from the CNS may account for the hyper-immunization.

The level of anti-measles IgG in the MS patients was in stark contrast to that of SSPE. The marginally elevated level in MS1 group was not significantly different to the others and of a very similar order to the previous study (Lachmann & Habicht, 1979). It can be inferred that neither the serum nor CSF measles antibody levels were of any great diagnostic significance in MS which complements the observations of other workers using established techniques for measles antibody determination (Haire, 1977; 1979).

In all patients the ratio between serum and CSF anti-measles IgG was surprising. This was considerably lower than would be expected from the normal distribution of IgG in serum and CSF, and differs from the relatively normal distribution of anti-viral antibodies (Norrby, Link & Olsson, 1974; Norrby *et al.*, 1974). The reason for this is not readily explicable but it may relate to the differing affinities between serum and CSF antibody, i.e. if the anti-measles IgG (CSF) is of higher affinity than the equivalent antibody in serum then this could overestimate the total activity in the CSF IgG. The precise explanation of anti-measles IgG in the CSF of all patients studied is unknown, but may be the result of measles virus invasion during the normal acute clinical infection (Weber, Canton & Dereux, 1969).

The very low amounts of anti-distemper IgG provided no evidence to support the hypothesis that distemper virus was involved in the aetiology of multiple sclerosis. Although serum anti-distemper IgG levels had been measured before in MS patients and found to be insignificant (Imagawa, 1968; Salmi, 1973; Krakowa & Koestner, 1978) the level within the CNS had not been reported previously. Assays designed to measure anti-distemper IgG levels in man are complicated by the cross-reactivity of measles and distemper virus, i.e. measles antibody neutralizes distemper virus but distemper antibody does not neutralize measles virus. In the specific distemper radioimmunoassay employed in this study a certain degree of cross-reactivity has been observed (Gorman & Habicht, unpublished observations) and therefore a percentage of the anti-distemper IgG most probably represents anti-measles IgG. A constant relationship between intracerebral measles and distemper IgG synthesis was not observed. Although a definite conclusion from this cannot be made it could indicate that not all the anti-distemper IgG was simply a function of the measles IgG.

In conclusion, this study revealed that the level of CSF and serum measles and distemper antibody was not of diagnostic significance in a series of ten MS patients. The intracerebral synthesis of antibody against the candidate agents failed to account for any appreciable proportion of the observed abnormal synthesis. It therefore remains to discover what this IgG may be. In contrast, this study reinforced the evidence of measles viruses as the aetiological agent in SSPE. However, the novel finding was that in SSPE there was a general overall increase in the antibody response to measles virus, and the abnormal intracerebral levels were just a function of this response, not a unique, separate event.

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