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The elution of IgG from subacute sclerosing panencephalitis and multiple sclerosis brains

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SUMMARY

IgG was extracted from sections of a multiple sclerosis brain by repeated washing with phosphate-buffered saline. After this extraction, no IgG was recovered by further washing at pH 2·2. This indicates that the IgG of the plaques has no strong affinity for insoluble constituents of the brain. Sections of a subacute sclerosing panencephalitis brain were used as positive controls: after repeated extraction with phosphate-buffered saline, IgG with anti-measles antibody activity was eluted at acid pH.

INTRODUCTION

Weil *et al.* (1975) have extracted, at acid pH, IgG from the brain of a patient with subacute sclerosing panencephalitis (SSPE) and demonstrated in the eluate an antibody activity against measles virus. As IgG is also present in the plaques of multiple sclerosis (MS) and their peripheries, we applied the same experimental procedure to see whether acid pH released IgG from MS brain. We recovered IgG from certain sections of a MS brain but, after washing with a neutral solution, found no IgG in an acid extract. As a positive control we used a SSPE brain which gave us acid-extractable IgG with anti-measles antibody activity. These observations will be discussed in the light of our recent finding that aggregated IgG or immune complexes tend to attach loosely to myelin basic protein (MBP) (Sindic *et al.*, 1980).

MATERIALS AND METHODS

Brains were resected within 12 hr after death and kept at −20°C. The MS brain was from a 44-year-old man. Paresthesia in the lower limbs appeared in 1973, followed in 1976 by ataxic gait. In 1977 the patient was urgently hospitalized for pneumonia due to food inhalation. He was dysarthric and polypneic with paresis of the four limbs and the lower cranial nerves. Agar gel electrophoresis of the cerebrospinal fluid showed an oligoclonal pattern of IgG. The patient's condition deteriorated quickly and despite respiratory assistance he died 3 months later. Histology (Unité de Neuropathologie, Professor J. M. Brucher, Université Catholique de Louvain) confirmed the diagnosis. A few plaques were visible macroscopically but many microscopic plaques were found in the centrum semiovale and near the ventricles. A large paramedian plaque, clearly of recent origin, was present

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in the medulla oblongata. The samples that we studied were from the white matter of the left hemisphere and, with one exception, were devoid of macroscopically visible plaques.

The SSPE brain was from an 11-year-old patient who died 3 months after the first symptoms of the disease, i.e. intellectual impairment, gait disturbances and seizures. The titres of anti-measles antibodies, determined by complement fixation, reached 1/64 in the serum and 1/8 in the cerebrospinal fluid. Agar gel electrophoresis of the cerebrospinal fluid showed numerous oligoclonal IgG bands. Histology confirmed the diagnosis of SSPE (Laboratoire de Neurologie du Développement, Professor G. Lyon, Université Catholique de Louvain).

As a control we studied the brain of a 50-year-old man who died from intestinal haemorrhage. No neurological signs had been found in this patient.

Methods. The sections (2 g of wet tissue) of normal, SSPE and MS brains were treated simultaneously as described by Weil *et al.* (1975) with slight modifications. The brain samples were homogenized in a glass homogenizer (Kontes Glass, Vineland, New Jersey) in a $\times 10$ sample volume of saline buffered by 0·01 M phosphate, pH 7·2 (PBS), containing 0·2% sodium azide and, to inhibit proteases, 100,000 u/l Trasylol (Bayer, FRG). The homogenate was stirred with a bar magnet for 20 min and ultracentrifuged at 100,000 ***g*** for 1 hr. After two washings the pellets were resuspended in 20 ml of 0·1 M glycine–HCl buffer, pH 2·2, containing 0·05 M ε-aminocaproic acid and 0·2% sodium azide. The suspension was stirred for 90 min and ultracentrifuged at 140,000 ***g*** for 1 hr. Supernatants were immediately neutralized by adding 0·1 N NaOH, and then concentrated by ultrafiltration to 1 ml.

The IgG and albumin (Alb) contents of the three washes and the acid eluate were determined by immunonephelometry in the Technicon AIP system (Tarrytown, New York). To test the anti-measles antibody activity we used a latex agglutination technique called PACIA for particle counting immunoassay (Cambiaso *et al.*, 1977). Polystyrene particles (latex) of 0·8 μm from Rhône-Poulenc (Courbevoie, France) were coated with IgG from the acid eluate by adsorption (72 μg IgG for 50 μl of 10% latex suspension). For agglutination, 25 μl of the $\times 200$ diluted latex in 0·1 M glycine–NaOH buffer, pH 9·2, containing 0·17 M NaCl and 1% (w/v) bovine serum albumin was mixed with 25 μl of serial dilutions of measles antigen or control antigen (Behring Institute, Marburg/Lahn, FRG, or Flow Laboratories, Rockville, Maryland) in the same buffer. After incubation for 45 min at room temperature on a vortex mixer and a 100-fold dilution with the glycine buffer (without BSA), the suspension was aspirated into a Technicon Autoanalyser, where it was diluted again ten times before passing through the Technicon Autocounter which measures the agglutination by counting the residual non-agglutinated particles.

RESULTS

From the normal brain, a mean of 178 μg (s.d. 63) of Alb and 45 μg (s.d. 19) of IgG were recovered per g of wet tissue in the first PBS washing (Fig. 1). The IgG/Alb ratio (0·24) corresponded to that found in serum (Table 1). Negligible amounts of Alb and IgG were found in the second and third washings (Fig. 1). Neither IgG or Alb was detected in the acid extract.

From the SSPE brain, three times more Alb (519 μg/g; s.d. 185) and ten times more IgG (458 μg/g; s.d. 346) than from the normal brain were obtained by the first PBS washing. IgG/Alb ratios calculated for the material extracted from each piece of the SSPE brain exceeded those of the normal brain or serum (Table 1). After the third PBS washing, which recovered only traces of IgG, the pH 2·2 buffer extracted 58·5 μg/g (s.d. 26) of IgG and 5·1 μg/g of Alb (Fig. 1). This amount of IgG represented 12·8% of the IgG of the first PBS washing. When latex was coated with this material, after neutralization of pH, the particles were strongly agglutinated by measles antigens (Fig. 2).

From the MS brain, the mean amounts of Alb (194 μg/g; s.d. 110) and IgG (60 μg/g; s.d. 30) recovered did not differ significantly from the normal brain values (Fig. 1). The mean IgG/Alb ratios did not differ significantly either (Table 1). However, as the samples of MS brain had been taken in areas which were differently affected the material recovered from each section had to be considered individually. In two MS samples the first PBS washing was characterized by an IgG/Alb

Table 1. IgG/Alb ratios in the first PBS washing of pieces from normal, MS and SSPE brains

Normal ($\bar{x}=0{\cdot}24$; s.d. $=0{\cdot}05$)	MS ($\bar{x}=0{\cdot}33$; s.d. $=0{\cdot}1$)	SSPE ($\bar{x}=0{\cdot}99$; s.d. $=0{\cdot}52$)
0·33	0·45*	0·46*
0·25	0·21	0·53*
0·22	0·43*	0·43*
0·23	0·25	0·44*
0·27	0·32	1·16*
0·26		1·79*
0·26		1·17*
0·15		0·98*
		1·32*
		0·97*
		0·69*
		1·98*

* Significantly different ($P<0{\cdot}001$) from the mean IgG/Alb ratio in the normal brain.

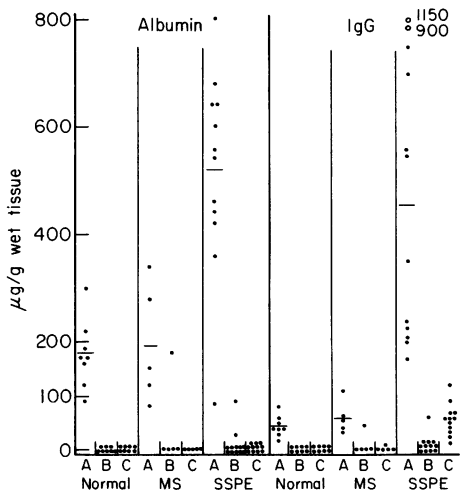

Fig. 1. Quantities of Alb and IgG extracted by successive washings from normal, MS and SSPE brains. A = first PBS washing; B = second and third PBS washings; C = washing at pH 2·2.

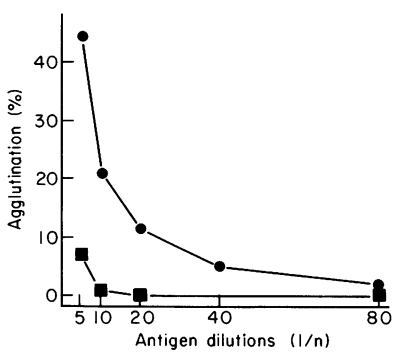

Fig. 2. Agglutination by measles antigen (●——●) of particles coated with IgG eluted at pH 2·2 from the SSPE brain. The agglutination was measured by the PACIA system. The control antigen (■——■) consisted of an extract of a non-contaminated cell line used for virus replication.

ratio about twice the mean value in the normal brain and reaching the lowest values observed in the SSPE brain (Table 1). Further extraction at pH 2·2 did not provide detectable amounts of IgG.

DISCUSSION

We found, as did Tourtellotte & Parker (1967), that certain areas of MS brain contain larger amounts of IgG than would be expected from the Alb content, confirming that IgG is locally produced or concentrated because of its antibody activity. The possibility that changes in the Alb values could explain the differences in IgG/Alb ratios is unlikely because, in the normal brain, the wide variations observed in the recovered amounts of Alb were related to those of IgG, as shown by the relatively small standard deviation (s.d. 0·05) compared to those of the MS brain (s.d. 0·1) and the SSPE brain (s.d. 0·52). In addition, we showed the possibility of extracting IgG from MS brain without the use of dissociating agents, indicating that IgG is not combined with insoluble structures.

By immunofluorescence, several authors (Simpson *et al.*, 1969; Tavolato, 1975; Woyciechowska & Brzosko, 1977) have observed that extracellular IgG from MS brain could be washed out with saline. This contrasted with the tissue-binding of IgG in SSPE where significant amounts of IgG were recovered at acid pH.

The apparent absence of IgG in the acid eluate of the MS brain could be due to the lack of sensitivity of our immunoassay. In the SSPE data a further 12·8% of the IgG was recovered at acid pH. If we assume that the same proportion of IgG should have been eluted at acid pH from the MS brain, this quantity of IgG would have been detected by our method which was sensitive to 0·5 μg/g brain.

We do not know whether some of the IgG extracted from certain pieces of the MS brain was free or in the form of soluble immune complexes. The latter could contain antigens from infectious agents, soluble brain autoantigens, or more common autoantigens such as deoxyribonucleic acid or IgG itself. IgG with rheumatoid factor activity (anti-IgG autoantibody) tends to form complexes with itself. Self-associated IgG are quite common as they have been detected in the serum of healthy individuals (Hay *et al.*, 1976). Our recent finding that MBP binds loosely to the Fc region of IgG which has been aggregated by heating or by association with antigen (Sindic *et al.*, 1980) could explain the deposits of IgG. Such deposits are not necessarily due to antibody activities against brain antigens or viral antigens associated with this tissue. Immune complexes could be entrapped by MBP becoming accessible during the demyelinization process. For example, complexes consisting of self-associated IgG could be produced locally by B lymphocytes stimulated non-specifically by various mitogens from infectious or brain origins (Izui, Eisenberg & Dixon, 1979; Möller, 1976).

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