Clin. exp. Immunol. (1979) 35, 67–75.

Oligoclonal immunoglobulins in subacute sclerosing panencephalitis and multiple sclerosis: a study of idiotypic determinants

G. C. EBERS,* J. B. ZABRISKIE & H. G. KUNKEL *The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA*

(*Received* 13 *June* 1978)

SUMMARY

Studies have been made of the idiotypic determinants of subacute sclerosing panencephalitis (SSPE) antibodies using rabbit antisera to serum and spinal fluid fractions. Evidence is presented indicating that serum and cerebrospinal fluid (CSF) anti-measles antibodies, as judged by their idiotypes, differ in their relative concentrations in the two compartments. The results indicate that some of these antibody subpopulations originate within the CNS, while others are made largely or entirely outside. In addition to strong idiotypic specificity, a limited cross-idiotypic specificity relating antibodies from three out of fourteen SSPE patients has been identified. In the course of these studies, measles virus was found to agglutinate red cells coated with antibody fraction to high titres. This system has proved useful in demonstrating the competition between anti-idiotypic antibody and antigen for the combining sites of the measles antibody. Two anti-idiotypic antisera have also been obtained against the spinal fluid IgG of multiple sclerosis (MS) patients. The possible use of these marker reagents as well as related methodologies in the search for the antigens involved in MS bands is discussed.

INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a slow virus disorder of the central nervous system following measles infection or, less often, measles vaccine. That the clinical manifestations are related to persistent infection of the nervous system by measles virus seems established (Connolly *et al.*, 1967; Meulen, Katz & Muller, 1972). However, the mechanism of persistence is unclear. A characteristic feature of the disorder is the presence of large amounts of measles-directed antibody both in the serum and cerebrospinal fluid (CSF) of these patients. These serum and CSF antibodies are of restricted heterogeneity as shown by the cathodally migrating oligoclonal bands frequently seen on agarose gel electrophoresis (Vandvik *et al.*, 1976a). These bands can be largely absorbed by measles antigen (Vandvik & Norrby, 1973). Further immunochemical studies have demonstrated the predominance of kappa light chains (Bollengier, Lowenthal & Henrotin, 1975) and of the IgG_1 subclass (Vandvik, Natvig & Norrby, 1977).

Many of these features have also been found to be true of the oligoclonal bands seen in the CSF of patients with multiple sclerosis (MS) (Vandvik, Natvig & Wiger, 1976b). In this instance little of the antibody appeared to have measles specificity. One method that might provide additional information regarding these bands is through the use of anti-idiotypic antisera (Kunkel, Mannik & Williams, 1963) to individual protein bands. Such specific antisera should permit the identification and quantification of these proteins in different tissues and body fluids. In addition, since such antibodies relate to the com-

* Present address: University Hospital, 339 Winderemere Road, London, Ontario, Canada.

Correspondence: Dr G. C. Ebers, University Hospital, 339 Windermere Road, London, Ontario, Canada.

0099-9104/79/0010-0067$02.00

bining site of the Ig bands, they may be of help in the detection of an unknown antigen that gives rise to the band.

In the present study, emphasis has been placed on the bands in SSPE as a model system which to explore the idiotypic approach. Specific antisera were obtained and utilized for a variety of studies. Early results on the application of this approach to multiple sclerosis are also presented. A preliminary report on certain of these findings has already been published (Ebers *et al.*, 1977) and a similar system has been reported by Nordal, Vandvik and Natvig (1977).

MATERIALS AND METHODS

Serum and cerebrospinal fluid (CSF). Samples were collected from two patients with SSPE seen at the Rockefeller University Hospital. Additional serum samples from patients with known SSPE were obtained from Montreal, Canada and Bogota, Colombia.

Serum and CSF samples were obtained from patients with multiple sclerosis attending the multiple sclerosis clinic at the Rockefeller University Hospital. Two multiple sclerosis patients having high levels of CSF IgG underwent serial lumbar punctures for the collections of large amounts of fluid.

Electrophoresis. Agarose gel electrophoresis was performed using pre-formed agarose slides (Millipore–Panagel) (Johnson *et al.*, 1977). Cellulose acetate electrophoresis was performed on a 'Microzone' apparatus (Beckman Instruments Inc., Fullerton, California) (Braun, Eichmann & Krause, 1969). Isoelectric focusing was carried out in 5% polyacrylamide gel containing 2% (w/v) ampholines pH 3–10 (LKB Produkter, Bromma, Sweden). Anode and cathode solutions were 1·0 M H_2SO_4 and 1·0 M NaOH in deionized water. 20 µl samples containing 2–10 mg protein per ml of 1% glycine (w/v) in deionized water were applied on 5 × 10 mm squares of Whatman 3 mm paper. Samples were run at 1200 V for 22 hr using an LKB Multiphor. Gels were fixed in 10% TCA for 6 hr and stained with 0·02% Coommassie Blue R (Sigma Chemical Co., St. Louis, Missouri) in acetic acid, methanol and water in a ratio of 2 : 3 : 5 for 20 min followed by de-staining overnight in the acetic acid : methanol : water solution.

Viral antigens. Rubella and measles virus preparations were obtained commercially (complement fixing antigens, Microbiological Associates, Bethesda, Maryland). In addition, measles virus purified by banding on potassium tartrate gradients was obtained from the laboratory of Dr P. Choppin at the Rockefeller University.

Gel diffusion reactions. Double diffusion was carried out in 0·8% agarose (Seakem, Marine colloids, Rockland, Maine), in 0·15 M NaCl 0·1 M sodium phosphate buffered saline pH 7·2 (PBS) and 3% polyethelene glycol (British Drug House).

Isolation of IgG. IgG isolations were performed using octanoic acid precipitation (Steinbuch & Audran, 1969) followed by chromatography on DEAE cellulose (Whatman 52, Whatman Ltd., UK) equilibrated with 0·01 M phosphate buffer pH 7·2 (pH 6·95 for rabbit IgG). Zone electrophoresis in Pevikon (Mercer Chem. Corp. NY, USA) was used for the purification of CSF IgG in some instances (Kunkel, 1954). The purity of the IgG preparations was checked by cellulose acetate electrophoresis and by immunoelectrophoresis.

Further purification of IgG fractions was obtained by liquid column isoelectric focusing in sucrose density gradients. Generally, 2–3% (w/v) ampholine solutions were used over the relevant pH range. A glass suction coil was used for the removal of isoelectric precipitation bands from the columns (Rathnam & Saxena, 1970). Initial runs of serum IgG were made in a 440 ml column (LKB 8100). Peaks of absorption at 280 mm were pooled and refocused in a 110 ml column (LKB 8101) using additional ampholines of the appropriate pH range.

Focusing columns were emptied by pumping in distilled water from the top at a rate of some 60–120 ml/hr. The pH of eluted fractions was measured at 4°C. Fractions were then pooled and sucrose and ampholines were removed by passage through a G-50 Sephadex (Pharmacia Fine Chemicals, Piscataway, New Jersey) column equilibrated with PBS or by dialysis against PBS. CSF IgG fractions from two patients with multiple sclerosis were isolated using isoelectric focusing in Pevikon as a second step procedure following octanoic acid precipitation.

Enzymatic digestion. $F(ab)_2$ fragments were prepared by digestion with 2·0 mg pepsin/100 mg IgG in 0·1 M acetate buffer pH 4·1 at 37°C for 18 hr. F(ab) fragments were obtained either by reduction (0·1 M mercaptoethanol) and alkylation (iodoacetamide) of $F(ab)_2$ or by papain digestion (Porter, 1959). Digests were absorbed with Sepharose 4B, staphylococcal protein A (Pharmacia), as necessary to remove Fc and undigested IgG.

Rabbit immunization. Rabbits were injected with approximately 100–200 µg of whole or digested IgG in Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) in multiple intracutaneous sites. The schedule was weekly for 3 weeks with a final injection following a 3 week rest period. The animals were bled 7 days after the final injection. In some instances, a boost was given subsequently.

Haemagglutination. Haemagglutinating titres of unabsorbed and absorbed antisera were determined in a microtitre system using passive haemagglutination and bis-diazobenzidine (BDB) as the coupling agent (Gordon, Rose & Sehon, 1958). PBS 2% bovine serum albumin (BSA) was used as diluent. Sera were heat-inactivated at 56°C for 30 min and absorbed with human type O RBC.

Absorption of antisera. Antisera were made specific for idiotypic determinants by absorption with pooled human gammaglobulin (FR II) which had been covalently bound to Sepharose 4B (Pharmacia) in a ratio of 4·0 mg IgG/ml of packed

beads by the method of Cuatrecasas (1970). In general, a serum was absorbed twice with an equal volume of FR II beads to render it weakly or non-reactive with an FR II coat.

Anti-measles reactivity. Isolated antibody fractions from SSPE sera were tested for reactivity with measles using indirect fluorescent staining of suspensions of HeLa cells persistently infected with measles virus. $F(ab)_2$ fragments of rhodamine-conjugated rabbit anti-human IgG with $F(ab)_2$ specificity were used as counter-stain.

In addition, antibody fractions were also tested in a viral haemagglutination inhibition system using monkey RBC as the indicator cell (Sever, 1962) (Rhesus monkey RBC were kindly supplied by Dr A. Brinton of the Rockefeller University).

RESULTS

Idiotypic systems defined by antisera to serum and CSF fractions

Band purification. The data from a typical column electro-focusing experiment with the serum IgG from patient AM are shown in Fig. 1. (a) Represents the first focusing run while (b), (c) and (d) represent refocusing of pooled fractions. It can be seen that peaks 1 and 2 are contained within a narrow pH range after refocusing.

Fig. 2 shows the appearance of serum IgG, CSF IgG and peaks 1 and 2 after isoelectric focusing in polyacrylamide gel. From the bottom to the top of Fig. 2, a roughly linear pH gradient of 7–9 is present.

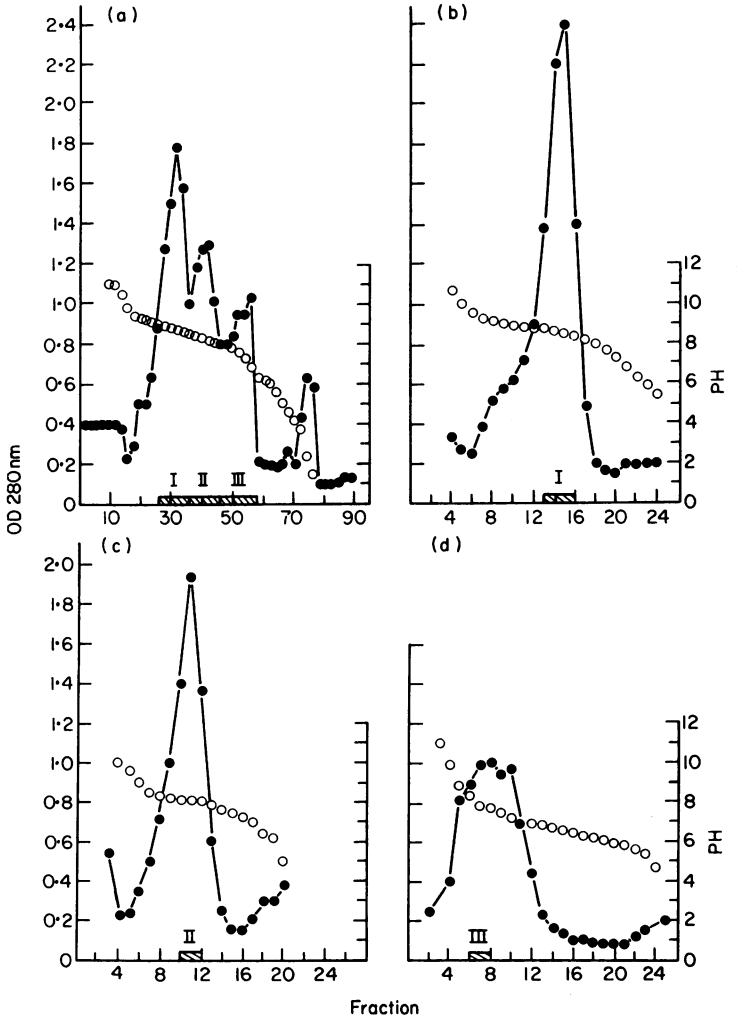

FIG. 1. Liquid column electrofocusing of IgG from SSPE patient AM in 440 ml column. Fractions (5·0 ml) from three peaks (I, II, III) pooled as indicated (⊠). (b), (c) and (d) Represent refocusing of I, II, and III respectively in 110 ml column (5·0 ml fractions).

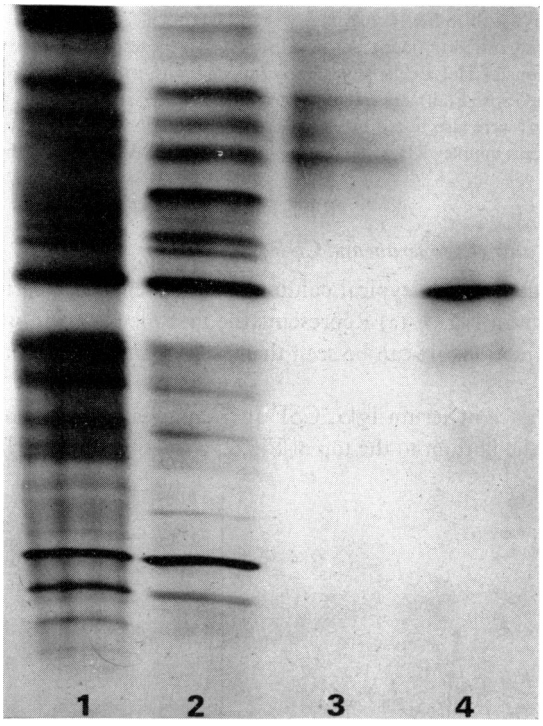

FIG. 2. Isoelectric focusing in polyacrylamide gel of: (1) whole AM serum IgG; (2) whole AM CSF IgG; (3) AM peak I (Fig. 1); and (4) AM peak II (Fig. 1).

It can be seen that there is a close correlation between serum and CSF bands, although some bands are present in the CSF but are weak or not present in the serum. The peaks again show restricted mobility, particularly peak 2.

It was possible to show, using anti-light chain antisera in immunoelectrophoresis, that peak 1 reacted with both anti-kappa and anti-lambda. Peak 2 reacted mainly with anti-lambda, but a weak reaction with anti-kappa was also seen. This antibody population was therefore not homogeneous, despite restricted mobility comparable to that seen in isoelectric focusing with homogeneous antibodies (Awdeh, Williamson & Askonas, 1976).

Peaks 1 and 2 from the above experiment, at a concentration of 1·0 mg/ml produced inhibition of haemagglutination of monkey RBC by measles virus in a dilution of 1/128 and 1/256, respectively. Similarly, both produced strongly positive fluorescence of measles-infected HeLa cells in an indirect system. Peak 3 could not be shown to have measles specificity in haemagglutination by fluorescence.

SSPE sera KEL and DUP were separated and analysed in a similar manner to serum AM. Idiotypic antisera were raised against the purified IgG fractions thus isolated and against an additional preparation from SSPE serum FOS. Similarly, idiotypic antisera were raised against CSF IgG from the CSF of patient AM. Table 1 lists the different antisera obtained which showed idiotypic specificity along with the corresponding immunogen used.

Definition of antisera. All antisera were absorbed with FR II gammaglobulin covalently bound to Sepharose as described in the Materials and Methods section. Table 1 lists the reactivity of idiotypic antisera used in this study after two absorptions. Strong haemagglutination reactions remained only against the autologous coats. Weak reactions were seen with Fr II coats with some of the antisera but, for the antisera utilized, the specific reaction was always considerably greater. Antisera that showed specific titres of 1/16 or below were not studied further.

Dilutions of antibody two to four times less than the haemagglutination titre were used in inhibition

TABLE 1. Haemagglutination titres of idiotypic antisera using pooled IgG fraction II and autologous SSPE IgG coats

Antiserum	Immunogen	HA titre *vs* Fr II coat	HA titre *vs* whole autologous IgG coat
88	Whole serum IgG (AM)	1/2	1/128
91	Whole CSF IgG (AM)	1/2	1/64
249	PK1 (AM)	0	1/128
271	PK2 (AM)	1/2	1/256
278	PK3 (AM)	1/2	1/64
273	PK1 (KEL)	1/2	1/64
317	PK2 (KEL)	1/2	1/64
320	PK1 (DUP)	1/4	1/32
321	PK2 (DUP)	1/2	1/32
251	Whole serum IgG (FOS)	0	1/256

experiments. Idiotypic specificities were defined by the ability of autologous serum or CSF IgG (or $F(ab)_2$ fragments thereof) to inhibit haemagglutination, together with the inability of the following inhibitors to produce inhibition: normal serum, fresh plasma, pooled CSF from patients with negative neurological investigation and Fr II in a concentration of 20 mg/ml, and free kappa and lambda light chains. Table 2 shows a typical inhibition experiment with serum 271 raised against AM peak 2. It can be seen that the system is inhibited only by serum, IgG or CSF from patient AM.

To show that the specificity defined by each antibody system was indeed present in IgG molecules, an anti-IgG immunoabsorbent was made. The IgG from the rabbit anti-human γ-chain antisera was isolated and bound covalently to Sepharose 4B (Cuatrecasas, 1970) in a ratio of 0·5 mg IgG/ml of packed beads. Approximately 0·5 mg of the IgG fraction used as immunogen, or whole IgG was passed down a 0·5 × 15 cm column (Econo-column, Bio-Rad Laboratories, Rockville Centre, New York) filled with anti-IgG beads. The column was then washed with PBS and eluted with recrystallized 3·0 M ammonium thiocyanate. After slow dialysis of the eluate with PBS, it was shown that only the thiocyanate eluate would inhibit the specific haemagglutination system. Inhibition was not seen with the concentrated column washings.

Serum–CSF relationships

The inhibition by serum and CSF of the idiotypic system raised against a serum IgG fraction shows

TABLE 2. Haemagglutination inhibition patterns obtained for serum 271 made against PK 2 from the serum of patient AM

Inhibitors	Reciprocal Log_2 of dilutions: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	16
Fr II 40 mg/ml	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fresh plasma	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Normal serum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
'Normal' CSF pool (5)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SSPE sera (13)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MS CSF pool (50)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MS sera (10)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serum AM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
IgG AM 2·0 mg/ml*	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+
CSF AM	0	0	0	0	0	0	0	0	0	+	+	+	+	+	+	+

* Red cell coat = AM IgG.

the identity of serum and CSF antibody defined by the idiotypic system. This was also shown to be true for idiotypic systems in which CSF IgG had been the immunogen. These results are consistent with the identical electrophoretic mobility of many of the bands observed in the isolated fractions with those in both serum and CSF. This is illustrated especially well for the major band of PK II shown in Fig. 2 which is also visualized in both the serum and CSF IgG.

A standard ratio was compiled using as a model, serum and CSF from a patient with an IgG myeloma in whom an anti-idiotypic serum had been raised against the monoclonal protein. A serum/spinal fluid ratio of 512 was obtained, comparing favourably with reported serum/CSF ratios of IgG (Felgenhauer, Schliep & Rapic, 1976). Table 3 shows the ratios obtained for three antisera raised against serum IgG

TABLE 3. Serum/CSF ratios of idiotypic determinants

Antiserum	Immunogen	Inhibition titre: Serum	CSF	Serum/CSF ratio
88	AM CSF IgG	1/1024	1/32	32
271	AM PK 2	1/64,000	1/128	512
249	AM PK 1	1/32,000	1/64	512
252	FOS IgG	1/16,000	1/64	256

fractions from patients AM and one against AM CSF IgG. It can be seen that only the system defined by serum 88 differs significantly from the ratio seen in the myeloma system where we have assumed there was no local synthesis of the IgG in the nervous system. The ratio of 1/32 suggests local CNS synthesis of the idiotype defined by this antiserum. The ratios for idiotypic systems defined by sera 271, 250 and 252 would indicate that these idiotypes were synthesized primarily outside the nervous system.

Cross-idiotypic specificity

Once defined, the SSPE antibody systems were also tested for inhibition by other SSPE sera, multiple sclerosis sera, and both individual and pooled multiple sclerosis CSF concentrated to 10 mg/ml. In addition, IgG fractions from other SSPE sera and multiple sclerosis sera and CSF were also used for RBC coats. Each of these coats were then tested for agglutination by carefully absorbed homologous idiotypic antisera. Where this was found, inhibition of agglutination of an homologous IgG coat was then sought using each of the above inhibitors. This has previously been shown to be a sensitive method of testing for cross-idiotypy (Kunkel *et al.*, 1973).

Using the immunogen as a coat, no inhibition was found with any homologous sera, spinal fluid or IgG fraction: only autologous reactivity was observed as indicated in Table 2 with serum 271. However, when other coats were screened with absorbed serum 271, that obtained from SSPE serum DUP was agglutinated to a titre of 1/16. Coats made using IgG obtained from twelve other SSPE and five MS sera were not agglutinated. The 271-DUP system was then screened for inhibition by other SSPE and MS sera and CSF. One additional serum (AV) inhibited this system in low dilution. The results of these experiments are summarized in Table 4. Sera AM, DUP and AV all inhibited this system in decreasing dilution. Serum AV had not inhibited the 271-immunogen system. These results indicate a cross-idiotypic specificity relating the antibodies from these three patients.

Several other systems using the idiotypic antisera in Table 1 were similarly investigated without further evidence for additional cross-idiotypic systems.

Inhibition by antigen

Initially, attempts were made to block the haemagglutination of the immunogen-coated RBC by its anti-idiotypic serum with a measles antigen. However, this was not feasible since it was found that the measles virus preparation itself specifically agglutinated the immunogen coat. (Agglutination was not

TABLE 4. Haemagglutination inhibition titres in a cross-idiotypic system—271 *vs* DUP coat

Inhibitors	1	2	3	4	5	6	7	8	9
Fr II 40 mg/ml	+	+	+	+	+	+	+	+	+
Normal serum	+	+	+	+	+	+	+	+	+
Fresh plasma	+	+	+	+	+	+	+	+	+
AM serum	0	0	0	0	0	0	0	0	+
DUP serum	0	0	0	0	0	+	+	+	+
AV serum	0	0	+	+	+	+	+	+	+
Other SSPE sera (11)	+	+	+	+	+	+	+	+	+

seen with a commercial rubella virus preparation.) This reverse agglutination was studied in some detail. Serial dilutions of purified and briefly sonicated measles virus preparations containing approximately 0·5 mg protein per ml were tested for their ability to agglutinate RBC coated with SSPE IgG fractions in a microtitre system. For AM Peak 2 as coat, a reverse agglutination titre of 1/512 was obtained (faint agglutination by measles of 1/2–1/4 dilution was noted against uncoated human RBC). This system was then utilized to study the competition between measles virus and idiotypic antibody. A dilution of the virus two to four times less dilute than the reverse haemagglutination titre was used. F(ab) fragments of idiotypic antiserum 271 were then tested for their ability to inhibit reverse agglutination by incubation with RBC coated with either immunogen or whole IgG, prior to the addition of the haemagglutinating virus. F(ab) fragments of the rabbit IgG with specificity for the coat idiotype inhibited the measles agglutination. The same F(ab) fragments, however, would not block when the whole IgG coat was used, probably because of the known presence of measles antibodies with different idiotypes. F(ab) fragments of anti-idiotypic sera with specificities for another band from the same patient (249-AM Pk 1) or for a band from a different SSPE patient known not to show cross-idiotypy, failed to inhibit.

Idiotypic antisera to MS CSF fractions

Using CSF collected from serial lumbar punctures from two patients with MS it was possible to raise idiotypic antisera to IgG fractions from the two spinal fluids. This was demonstrated by methods similar to that described above for the SSPE material. Evidence for selective concentration of these idiotypes in spinal fluid was obtained. Cross-idiotypy, however, was not demonstrated. In these experiments with the two antisera, IgG from a concentrated pool of CSF from fifty patients with MS and, additionally, the IgG from five individual MS CSFs were used as coats in haemagglutination. No cross-haemagglutination was seen with either antiserum in these circumstances.

DISCUSSION

In this study it has proved possible to markedly purify certain monoclonal bands from both the serum and spinal fluid of SSPE and MS patients and to produce anti-idiotypic antibodies against them in rabbits. Following absorption with homologous pooled IgGs, these antisera were shown to be specific for the immunizing fractions and the respective serum or spinal fluid from which it was obtained. It was thus possible to obtain a useful marker for a specific band to study its site and mode of production.

The origin of the serum and CSF bands has been in some doubt. Perhaps because the prominent clinical manifestations of SSPE relate to the nervous system, attention has been directed towards the CSF antibody bands. Reduced ratios of total serum/CSF IgG have been given as evidence for local CNS synthesis of IgG. This has been confirmed by similar ratios using assays of anti-measles antibody in serum and CSF (Salmi, Norrby & Panelius, 1972). It has been suggested that the serum bands represent reabsorbed CSF antibody (Meulen *et al.*, 1972; Vandvik & Norrby, 1973). In evaluating this

question, we have used the haemagglutination inhibition titre ratios of serum/CSF with different anti-idiotype antisera. Although only semi-quantitative observations were made, they have provided certain information. For a number of prominent SSPE serum bands selected here for study, the calculated ratios indicate that the antibodies were made largely or entirely outside the nervous system. This finding strongly suggests that viral antigens persist outside the nervous system. It is possible that these antigens might differ from those present inside the CNS eliciting the production of antibodies with different idiotypes. In relation to this it is important to note that measles virus has been isolated from the lymph nodes of SSPE patients (Horta-Barbosa *et al.*, 1971).

On the other hand, the demonstration of a lowered ratio, as seen with an anti-CSF IgG antiserum, provides evidence of local synthesis of a specific idiotype confirming previous findings with the measurement of the total IgG and antiviral antibody activity. It is to be expected that more extended studies would show a spectrum of serum/CSF antibody ratios. The idiotype approach thus provides a method for evaluating the origin of specific serum and CSF antibodies. Furthermore, it provides a basis for comparing serial samples for elevation or depression of idiotype levels. Although we have primarily used a haemagglutination system in these studies, preliminary evidence with a solid phase radioimmunoassay indicates the value of more precise quantification.

Previous studies have shown shared idiotypic determinants (cross-idiotypy) among antibodies of the same specificity from unrelated patients (Kunkel *et al.*, 1973; Williams, Kunkel & Capra, 1968; Førre, Natvig & Michaelson, 1977). In man, these antibodies have included anti-Rh antibodies, IgM cold agglutinins and rheumatoid factors. Using the idiotypic antisera as described, we were able to find evidence of cross-idiotypy with one antiserum. A specificity shared by three out of fourteen SSPE patients was defined. With analysis of more antisera it may prove possible to obtain more broadly reactive cross idiotypic antisera. These could prove of special value in relating measles antibodies produced by different clones at different sites in these patients, as well as relating them to measles antibodies produced during the usual measles infection.

One of the main objectives of the present study was to develop idiotypic systems that would be sensitive to competition by antigen for combining sites on serum or spinal fluid antibodies. Such a system might prove of special use in MS studies in a search for unknown antigens that would inhibit such idiotypic systems. Partial success was achieved in the model SSPE studies described above. Competition between anti-idiotypic antibody and measles antigen was most readily demonstrated in the situation in which red cells coated with measles antibody were agglutinated by measles virus. The coating of red cells with the homogeneous band fractions from MS spinal fluid offers a promising reagent with which to screen for viral or CNS antigens that would agglutinate the cells, as was the case with the measles virus and the SSPE band fractions.

Sharon Neulinger provided excellent technical assistance. Some SSPE sera used in this study were provided by Drs A. Sazant, M. Patarroyo and H. Hauffe. Light chain antisera were a gift from Dr S. M. Fu. Drs I. van de Rijn and B. Fraser provided help and advice on isoelectric focusing.

Dr G. C. Ebers is a Fellow of the Multiple Sclerosis Society of Canada.

REFERENCES

Awdeh, Z.L., Williamson, A.R. & Askonas, B. (1976) One cell–one immunoglobulin: origin of limited heterogeneity of myeloma proteins. *Biochem. J.* **116**, 241.

Bollengier, F., Lowenthal, A. & Henrotin, W. (1975) Bound and free light chains in subacute sclerosing panencephalitis and multiple sclerosis serum and cerebrospinal fluid. *Z. Klin, Chem. Klin. Biochem.* **13**, 305.

Braun, D.G., Eichmann, K. & Krause, R.M. (1969) Rabbit antibodies to streptoccccal carbohydrates. *J. exp. Med.* **129**, 809.

Connolky, J.H., Allen, I.V., Hurwitz, L.J. & Millar, J.H.D. (1967) Measles virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet*, **i**, 542.

Cuatrecasas, P. (1970) Protein purification by affinity chromatography. *J. Biol. Chem.* **245**, 3059.

Ebers, G.C., Fraser, B.F., van de Rijn, I., Zabriskie, J.B. & Kunkel, H.G. (1977) Idiotypic determinants in SSPE. *Clin. Res.* **25**, 482A.

Felgenhauer, K., Schliep, G. & Rapic, N. (1976) Evaluation of the blood-CSF barrier by protein gradients and the humoral response within the cerebral nervous sytem. *J. Neurol. Sci.* **30**, 113.

Førre, Ø., Natvig, J.B. & Michaelson, T.E. (1977) Cross-idiotypy of anti Rh antibodies. *Scan. J. Immunol.* **6**, 997.

Gordon, J., Rose, B. & Sehon, A.H. (1958) Detection of

non-precipitating antibodies in sera of individuals allergic to ragweed pollen by an *in vitro* method. *J. exp. Med.* **108**, 37.

HORTA-BARBOSA, L., HAMILTON, R., WITTIG, B., FUCILLO, D.A., SEVER, J.L. & VERNON, M.L. (1971) Subacute sclerosing panencephalitis: Isolation of suppressed measles virus from lymph node biopsies. *Science,* **173**, 840.

JOHNSON, K.P., ARRIGO, S.C., NELSON, B.J. & GINSBERG, A. (1977) Agarose electrophoresis of cerebrospinal fluid in multiple sclerosis. *Neurology,* **27**, 273.

KUNKEL, H.G. (1954) Zone electrophoresis. *Methods of Biochemical Analysis* (ed. by D. Glick), Vol. 1, p. 141. Wiley-Interscience, New York.

KUNKEL, H.G., AGNELLO, V., JOSLIN, F.G., WINCHESTER, R.J. & CAPRA, J.D. (1973) Cross-idiotypic specificity among monoclonal IgM proteins with anti-globulin activity. *J. exp. Med.* **137**, 331.

KUNKEL, H.G., MANNIK, M. & WILLIAMS, R.C. (1963) Individual antigenic specificity of isolated antibodies. *Science,* **140**, 1218.

MEULEN, V. TER, KATZ, M. & MULLER, D. (1972) Subacute sclerosing panencephalitis. A review. *Curr. Top. Microbiol. Immunol.* **57**, 1.

NORDAL, H.J., VANDVIK, B. & NATVIG, J.B. (1977) Idiotypy of measles virus nucleocapsid-specific IgG kappa antibody in serum and cerebrospinal fluid in subacute sclerosing panencephalitis. *Scand. J. Immunol.* **6**, 1351.

PORTER, R.R. (1959) The hydrolysis of rabbit gamma-globulin and antibodies with crystalline papain. *Biochem. J.* **73**, 119.

RATHNAM, P. & SAXENA, B.B. (1970) Isolation and physiochemical characterization of luteinizing hormone from human pituitary glands. *J. Biol. Chem.* **245**, 3725.

SALMI, A.A., NORRBY, F. & PANELIUS, M. (1972) Identification of different measles virus-specific antibodies in the serum and CSF of patients with subacute sclerosing panencephalitis and multiple sclerosis. *Infect. Immunity,* **6**, 248.

SEVER, J.L. (1962) Application of microtechnique to viral serological investigations. *J. Immunol.* **88**, 320.

STEINBUCH, M. & AUDRAN, R. (1969) The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch. Biochem. Biophys.* **134**, 279.

WILLIAMS, R.C., KUNKEL, H.G. & CAPRA, J.D. (1968) Antigenic specificities related to the cold agglutin activity of gamma M globulins. *Science,* **161**, 379.

VANDVIK, B., NATVIG, J.B. & NORRBY, E. (1977) IgG, subclass restriction of oligoclonal measles virus-specific IgG antibodies in patients with subacute sclerosing panencephalitis and in a patient with multiple sclerosis. *Scand. J. Immunol.* **6**, 651.

VANDVIK, B., NATVIG, J.B. & WIGER, D. (1976(b)) IgG subclass restriction of oligoclonal IgG from cerebrospinal fluids and brain extracts in patients with multiple sclerosis and subacute encephalitides. *Scand. J. Immunol.* **5**, 427.

VANDVIK, B. & NORRBY, E. (1973) Oligoclonal IgG antibody response in the cerebral nervous system to different measles virus antigens in subacute sclerosing panencephalitis. *Proc. Nat. Acad. Sci. (Wash.),* **70**, 1060.

VANDVIK, B., NORRBY, E., NORDAL, H. & DEGRE, M. (1976a) Oligoclonal measles virus-specific IgG antibodies isolated from cerebrospinal fluids, brain extracts and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis. *Scand. J. Immunol.* **5**, 479.