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Antibodies Against Viruses and Structural Brain Components in Oligoclonal IgG Obtained From Multiple Sclerosis Brain

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Summary. Immunoglobulin G (IgG) was isolated from three multiple sclerosis (MS) and two control brains by Protein A Sepharose affinity chromatography and was characterized by thin-layer polyacrylamide gel isoelectric focusing (PAG IEF) and antiserum immunofixation. The three MS brains contained oligoclonal IgG. Immunofixation with measles, herpes simplex, varicella, rubella, mumps and cytomegalovirus as antigens and autoradiography revealed that some of the oligoclonal IgG bands separated by PAG IEF contained antibodies against herpes simplex in one, measles in two and varicella virus in all three MS brains. No antibodies were detected with this technique against structural human (crude saline, lipid-proteolipid, ganglioside, and myelin basic protein extracts of MS and normal human brain) and bovine (purified myelin, myelin basic protein and oligodendrocytes of bovine brain) brain components. The finding of viral antibodies and the absence of antibodies against structural brain proteins in oligoclonal MS brain IgG is similar to that previously recorded in MS cerebrospinal fluid (CSF).

Key words: Multiple sclerosis – Oligoclonal brain IgG – Viral antibody synthesis

Zusammenfassung. Immunoglobulin G, IgG, wurde aus der Gehirnsubstanz von drei Fällen mit multipler Sklerose (MS) und zwei Kontrollen mittels Affinitätschromatographie mit Protein A-Sepharose isoliert. Das IgG wurde mit isoelektrischer Fokussierung in Polyacrylamidgelplatten (PAG IEF) und anschließender Antiserum-Immunofixation charakterisiert. Bei MS wurde oligoklonales IgG gefunden. Mit Hilfe von Antigen-Immunofixation mit Masern, Herpes simplex, Varizellen, Rubella, Parotitis und Zytomegalievirus und nachfolgender Autoradiographie (radioaktive Zweitantikörper gegen die genannten Antigene) konnte gezeigt werden, daß die oligoklonalen Bande in den drei MS-Gehirnen Antikörper gegen Herpes simplex in einem, Masern in zwei und Varizellen-Virus in allen drei Fällen enthielten. Keine solchen Antikörper wurden entdeckt, wenn die Technik für strukturelle Gehirn-

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Komponenten vom Menschen (einfache physiologische Salz-, Lipid-Proteolipid-, Gangliosid- und basische Myelo-Protein-Extrakte von MS und normalem Gehirn) und vom Rind (gereinigtes Myelin, basisches Myelo-Protein und Oligo-Dendrozyten aus Gehirnrinde) verwendet wurde.

Introduction

A well-known phenomenon in cerebrospinal fluid (CSF) from patients with multiple sclerosis (MS) is the presence of oligoclonal immunoglobulin G (IgG), which is also found in extracts of brain tissue from these patients (Link 1972; Vandvik et al. 1976b). The oligoclonal brain IgG in MS has previously been investigated regarding measles antibody activity. Vandvik et al. (1976b) found that a minor part of the oligoclonal brain IgG had measles antibody specificity.

The aim of this study was to investigate further the oligoclonal brain IgG in MS regarding antibody specificity against viral preparations and also against structural brain components. For this reason, IgG isolated from three MS and two control brains was subjected to thin-layer polyacrylamide gel isoelectric focusing (PAG IEF), which was followed by immunofixation with preparations of neurotropic viruses and brain extracts as possible antigens and by autoradiography.

Materials and Methods

Patients

IgG and structural tissue components were isolated from brains of three patients with MS and two control patients. The MS patients had a 20–30 year history of moderate to severe disease. The diagnosis was confirmed at autopsy. Previous CSF studies in two of them had revealed raised IgG index (Tibbling et al. 1977) and oligoclonal bands on agarose gel electrophoresis of the CSF. The two control patients had died of acute myocardial infarction. CSF and serum were obtained from six patients with clinically definite MS. All six had elevated CSF IgG index and oligoclonal bands in CSF examined by PAG IEF.

Viral Antigen Preparations

Preparations of six different neurotropic viruses (measles, herpes simplex (HSV) type 1, mumps, rubella, varicella and cytomegalovirus) were prepared and used at immunofixation as previously described (Roström 1981).

Crude Preparations of Saline, Lipid-proteolipid, Ganglioside, and Myelin Basic Protein Extracts from Human Brain

For the preparation of the *crude saline extracts,* 50 g of brain tissue including grey and white matter was homogenized in the cold at 75 W with the 150-W Ultrasonic Disintegrator in 0.15 *M* NaCl at a ratio of 1:2 (weight/vol). The homogenate was centrifuged at 4° C at 20,000 *g* for 45 min. The supernatant was collected and the pellet was resuspended in 0.15 *M* NaCl, sonicated and centrifuged as described. This was done three times in all. The collected supernatants were concentrated 200 times by ultrafiltration at 4° C in collodion bags (Sartorius Membranfilter, Göttingen, West Germany). The protein content was measured according to Lowry (1951) before use in antigen immunofixation (*extract 1*). The *crude lipid-proteolipid extracts* and *ganglioside*

extracts were prepared according to a modification of the Folch-Pi method (Folch-Pi 1972). One volume of the pellet of brain homogenate obtained after saline extraction was mixed with five volumes of chloroform-methanol (1 : 1; vol/vol) and 0.5 of aqueous 2 *M* KCl. This was done three times. The collected extracts separated into a lower chloroform phase and an upper water-methanol phase. After its dry weight had been estimated, the chloroform phase, which is claimed to contain brain tissue lipid-proteolipids (Folch-Pi 1972), was stored at – 20° C of until used as antigen at immunofixation (*extract 2*). The water-methanol phase, which is claimed to contain gangliosides and non-proteolipid proteins (Folch-Pi 1972), was evaporated to remove methanol, and the remaining aqueous solution was dialyzed overnight against distilled water in tubings with a mean pore diameter of 24 Å (Union Carbide, Chicago, Ill., USA) to remove most of the KCl. After its protein content had been measured, the solution was stored at – 20° C until used as antigen at immunofixation (*extract 3*). In order to obtain a *crude myelin basic protein extract,* the tissue homogenate remaining after chloroform-methanol-KCl extraction was first evaporated and lyophilized to remove chloroform-methanol and then treated with 0.15 *M* NaCl in the proportion 1 : 1 (weight/vol). The pH was adjusted to 2.4 with 1 *M* acetic acid. After centrifugation, pH in the supernatant was adjusted to 7 with 1 *M* NaOH and dialyzed (see above) against 0.05 *M* sodium phosphate buffered saline, pH 7.0. After measuring the protein content, this solution was used as a crude human myelin basic protein extract for antigen immunofixation (*extract 4*).

Preparation of Purified Bovine Myelin, Bovine Myelin Basic Protein and Bovine Oligodendrocytes

Bovine myelin was purified from calf CNS according to Norton and Poduslo (1973). Purified bovine myelin basic protein was prepared from bovine spinal cord by delipidation, acid extraction and column chromatography on sulphoethyl-Sephadex according to Hirschfeld et al. (1970). Oligodendrocytes were isolated from calf cerebellar white matter according to Abramsky et al. (1977). All preparations were kindly supplied by Dr. J. Shorr, Department of Neurology, Haddassah Medical Center, Jerusalem, Israel.

Isolation of IgG from Human Brain

Brain IgG was isolated from the crude saline brain extracts by affinity chromatography using a 1 × 5 cm column with 2 ml Protein A Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.1 *M* phosphate buffered saline (PBS), pH 7.0, according to the manufacturer's instructions. The IgG concentration was measured by nephelometry.

Thin-layer Polyacrylamide Gel IEF

IEF was carried out on LKB Ampholine thin-layer PAGs (PAG plates; LKB, Stockholm, Sweden), pH range 3.5–9.5 as described (Laurenzi et al. 1980). CSF concentrated to an IgG concentration of 3–4 g/l, serum diluted to the same IgG concentration with 0.05 *M* NaCl and separated brain IgG with a concentration of 3–4 g/l were applied to the gel at pH 6.2–6.4. Ten microliters of the sample were applied on 5 × 5 mm filter papers. Diluted pooled donor serum was run as reference, and carboxyhemoglobin as marker.

Antiserum Immunofixation for the Demonstration of IgG Separated by IEF

Brain IgG separated by PAG IEF was identified by immunofixation using rabbit antihuman γ-chain antiserum as described (Laurenzi et al. 1980).

Radio-labelling of Anti-human Ig Antibodies

Rabbit antihuman Ig antibodies (code A-107; Dako Immunoglobulins, Copenhagen, Denmark) was iodinated with ^{125}I (code IMS 30; The Radiochemical Center, Amersham, England) using sodium hypochlorite (Nordal et al. 1978).

Table 1. Concentrations of various antigens incorporated into agarose plates for antigen immunofixation

Antigen	Amount per ml agarose
Viral preparations	
Measles	30 µl
Herpes simplex	2 µl
Mumps	10 µl
Rubella	10 µl
Varicella	10 µl
Cytomegalovirus	40 µl
Crude human brain extracts	
Extract I (water soluble proteins)	4, 10 and 20 mg
Extract II (lipid-proteolipids)	0.1 mg only and with 5, 10 and 15 mg of cholesterol
Extract III (gangliosides)	0.6, 0.2 mg
Extract IV (myelin basic protein)	0.1 mg
Purified bovine nervous tissue preparations	
Myelin basic protein	200 pg
Myelin	400 pg
Oligodendrocytes	200 pg

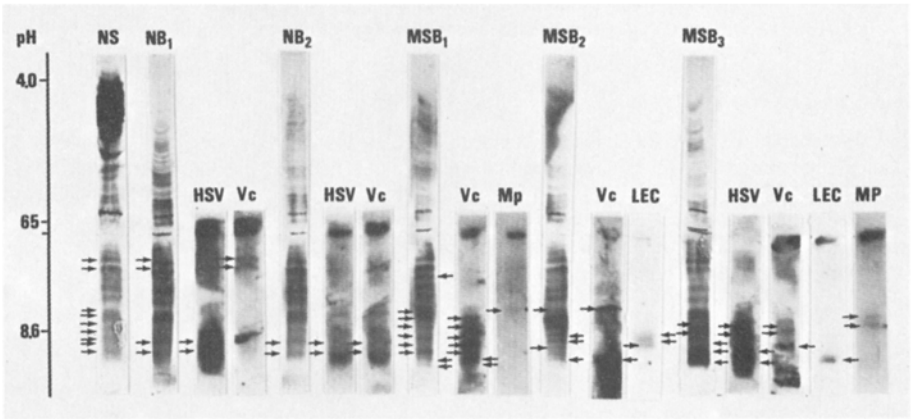

Fig. 1. Pattern from thin-layer PAG IEF of serum from a normal control *(NS)* and IgG isolated from brains of two controls *(NB* 1, 2) and three patients with MS (*MSB* 1, 2, 3), and autoradiograms of the corresponding IgG preparations from the five brains after immunofixation with measles *(LEC)*, herpes simplex *(HSV)*, varicella *(Vc)* and mumps virus *(Mp)*. Arrows to the left of the autoradiograms indicate bands on PAG IEF and arrows to the right bands with correspondence in oligoclonal IgG bands on PAG IEF

Preparation of Antigen-containing Agarose Gel Plates

The viral and brain preparations were mixed with 1.5% agarose (Litex, Glostrup, Denmark) in 0.05 *M* Tris-barbital buffer, pH 8.6, at 56°C in concentrations given in Table 1. The amounts of antigens per milliliter agarose gel were determined in calibration experiments (Roström 1981). The lipid-proteolipid extracts were used separately and together with three different concentrations of cholesterol (Merck, Darmstadt, West Germany) which is claimed to increase the antigenicity of these compounds (Ryberg 1978). Purified bovine myelin basic protein were used and coupled to cyanide bromide activated Sepharose beads (Sepharose 4B; Pharmacia) at a concentration of 0.4 mg protein per milliliter agarose according to the manufacturer's instructions. Bovine oligodendrocytes were suspended in 0.25 *M* NaCl and mixed with agarose.

Five milliliters of the antigen-containing agarose gel was poured onto 5 × 12.5 cm plastic sheets (LKB 2117-407 Multiphor Plastic Plates). The solidified antigen-gel was stored for up to 24 h at 4°C in a humid chamber before use. Plates containing agarose gel only and agarose with 30 mg cholesterol per milliliter gel were used as controls.

Antigen Immunofixation and Autoradiography for the Detection of Antibodies in IgG Separated by IEF

Antigen immunofixation was performed as described (Roström et al. 1981).

Results

Viral Antibodies in PAG IEF Separated IgG from Control Brain

The IgG isolated from the two control brains was separated by PAG IEF and identified by antiserum immunofixation. The IgG bands demonstrable had the same mobility as the IgG bands of pooled donor serum run in parallel (Fig. 1).

When the IgG from the two control brains separated on PAG IEF was submitted to immunofixation using the various viral preparations as antigens, bands of antibody activity against HSV and varicella were found on the autoradiograms of both subjects (Table 2). All bands on the autoradiograms had their counterparts in polyclonal IgG seen on PAG IEF on the corresponding brain IgG preparations.

Demonstration of Oligoclonal IgG Bands in MS Brains

IgG isolated from the brain tissue of the three patients with MS displayed three additional IgG bands on PAG IEF (Table 2), when compared with the band patterns of control brain IgG and of normal serum IgG. These bands were considered to represent oligoclonal IgG.

Viral Antibodies in PAG IEF Separated MS Brain IgG

Immunofixation with the viral preparations as antigens and autoradiography revealed bands of antibodies against varicella in all three MS brain IgG preparations, against measles in two, mumps in two and HSV in one (Table 2).

Bands on the autoradiograms that corresponded to oligoclonal bands on the PAG IEF separated brain IgG were considered to represent antibodies synthesized in the brain. Thus, locally synthesized antibodies against varicella were found in all

Table 2. Relation between viral antibodies demonstrated by autoradiography and IgG bands on PAG IEF of saline extracts from two control brains and three MS brains

Brain	No. of oligoclonal IgG bands detected by PAG IEF	Viral antibody specificity detected by autoradiography	No. of IgG bands corresponding to bands of viral antibodies on the autoradiogram	
			Oligoclonal	Polyclonal
Control 1	0	Herpes simplex (2)[a]	0	2
		Varicella (2)	0	2
Control 2	0	Herpes simplex (2)	0	2
		Varicella (2)	0	2
MS 1	3	Varicella (7)	2	5
		Mumps (1)	0	1
MS 2	3	Varicella (2)	1	1
		Measles (3)	2	1
MS 3	3	Herpes simplex (4)	2	2
		Varicella (3)	1	2
		Measles (1)	1	0
		Mumps (2)	0	2

[a] Numbers within brackets denote bands on the autoradiograms

three MS brains, against measles in MS brain Nos. 2 and 3, and against HSV in MS brain No. 3 (Table 2). At least one viral antibody specificity could be traced to all oligoclonal bands except one in MS brain No. 1. Two different viral antibody specificities could be traced to one single oligoclonal band of MS brain No. 3 (Fig. 1).

The bands of viral antibodies on the autoradiograms with correspondence in polyclonal IgG fractions on PAG IEF (Table 2) were considered to represent antibodies synthesized outside the CNS-CSF compartment.

Failure to Demonstrate Antibodies Against Structural Brain Components in Brain IgG and CSF from MS Patients

The IgG isolated from the three MS and two control brains was separated on PAG IEF and submitted to immunofixation, using the various structural brain components as antigens (Table 1). The corresponding autoradiograms gave no evidence of antibody synthesis against any of these components. CSF and serum from six patients with MS were studied for these antibodies, also with negative results.

In addition, CSF and serum from four of the patients with MS were studied by immunofixation, using as antigen the preparation of bovine myelin basic protein coupled to Sepharose beads. No antibodies were detectable on the corresponding autoradiograms.

Discussion

By isolating brain IgG, its separation and identification on PAG IEF was improved. Protein A used for isolation binds predominantly IgG subclasses 1, 2 and 4; IgG 3 had therefore not been examined. This is, however, of minor importance, since there is accentuated predominance of IgG 1 in MS CSF, while the proportion of IgG 3 is very low (Palmer et al. 1973; Vandvik et al. 1976a). The extraction of tissue-bound IgG has been carried out at neutral pH, which recently has been found to be adequate for extraction of most IgG from MS brain, in contrast to the brain of subacute sclerosing panencephalitis, where extraction at acidic pH is more efficient (Sindic 1980b).

Oligoclonal IgG bands were found in all three MS brains, when the IgG band patterns were compared with those of the two control brains and of normal serum. The presence of oligoclonal IgG bands was confirmed by antiserum immunofixation. It was not possible to compare the band patterns from brain IgG with the patients' CSF and serum, since such samples were not available.

The heterogeneous viral antibody response detectable in oligoclonal brain IgG was of a type similar to that previously observed in MS CSF (Roström et al. 1981). The finding of more than one antibody specificity in one brain IgG band or CSF IgG band (Roström et al. 1981) separated by PAG IEF indicates that the high separation capacity of PAG IEF is still inadequate for the separation of antibodies with individual specificities. This, together with the observation that one or more oligoclonal CSF and brain IgG bands remained unidentified regarding antibody specificity, indicates that the detectable antibody specificities most probably represent only a minority of the oligoclonal IgG, and the major antibody specificity of the oligoclonal IgG in MS is still unknown. The method of antigen immunofixation and autoradiography is mainly qualitative, and absorption studies, or preferably the development of other techniques, are necessary to resolve the problem of antibody specificities in oligoclonal IgG bands.

The occurrence in CSF and serum from patients with MS of antibodies against a variety of nervous tissue components—which would indicate an autoimmune response—has previously been reported inter alia in serum to bovine oligodendrocytes (Abramsky et al. 1977), in CSF to human myelin basic protein (Panitch et al. 1980) and to saline and lipid extracts of human brain (Ryberg 1978). The significance of these findings is, however, difficult to interpret, since the IgG molecule has been found to react unspecifically to a variety of nervous tissue components, inter alia myelin basic protein (Aarli et al. 1975; Sindic et al. 1980a), oligodendrocytes (Traugott et al. 1978) and lipophilic membranes through electrostatic or hydrophobic bonds (Tagesson et al. 1977). The antibody response to brain tissue components could also be a phenomenon secondary to a viral infection or to a breakdown of nervous tissue components, as previously shown to occur regarding the cellular immune response (Field et al. 1973; Youngchaiyud et al. 1974).

The failure in the present study to demonstrate antibodies in oligoclonal CSF IgG against any of the nervous tissue preparations may indicate that the technique used, although sensitive enough for viral antibody detection, might not enable the demonstration of antibodies against structural brain components. Alternatively,

no such antibodies against structural brain components occur in the brain or CSF from patients with MS.

The conclusion of this study is that there is a similar heterogeneous viral antibody response present in oligoclonal MS brain IgG as previously demonstrated in CSF from such patients (Nordal et al. 1978; Roström et al. 1981). However, further studies are warranted to establish the cause of this viral antibody response. The failure to demonstrate antibodies against structural brain components might indicate that—if MS is caused by autoimmune mechanisms—these are mainly dependent on a cellular immune response.

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