MULTIPLE SCLEROSIS JOURNAL | MSJ

*Original Research Paper*

# Multiple sclerosis: Serum anti-CNS autoantibodies

**John W Prineas and John D.E Parratt**

*Multiple Sclerosis Journal*
2018, Vol. 24(5) 610–622
DOI: 10.1177/1352458517706037



Correspondence to:
**JW Prineas**
Brain and Mind Centre, Department of Medicine, The University of Sydney, Room 711-MO2F, 94 Mallet Street, Camperdown, NSW 2050, Australia.
**john.prineas@sydney.edu.au**

**John W Prineas**
Brain and Mind Centre, Department of Medicine, The University of Sydney, Camperdown, NSW, Australia

**John DE Parratt**
Brain and Mind Centre, Department of Medicine, The University of Sydney, Camperdown, NSW, Australia and Department of Neurology, Royal North Shore Hospital, St Leonards, NSW, Australia

## *Abstract*

**Background:** It is uncertain whether there are autoantibodies detectable by indirect immunofluorescence in the serum of patients with multiple sclerosis (MS).

**Objective:** To determine whether there are anti-central nervous system (CNS) autoantibodies detectable by indirect immunofluorescence in the serum of MS patients.

**Methods:** Sera and in some cases cerebrospinal fluid from 106 patients with multiple sclerosis, 156 patients with other neurological diseases, and 70 healthy control subjects were examined by indirect immunofluorescence using cryostat sections of rat cerebrum fixed by perfusion with paraformaldehyde.

**Results:** Autoantibodies were detected that recognized more than 30 neuronal, glial, and mesodermal structures in 28 of 106 MS cases. Most were also detected in patients with other related and unrelated neurological diseases and several were also found in healthy controls. Novel anti-CNS autoantibodies recognizing particular sets of interneurons were detected in both normal controls and in subjects with CNS diseases.

**Interpretation:** Serum anti-CNS autoantibodies of diverse specificities are common in MS patients. The same anti-CNS autoantibodies are not uncommon in patients with other neurological diseases. The findings provide no support for the proposition that myelin breakdown in MS is caused by exposure of intact myelin sheaths or oligodendrocytes to a pathogenic serum anti-myelin or anti-oligodendrocyte autoantibody.

***Keywords:*** Multiple sclerosis, neuromyelitis optica, autoimmunity, myelin, oligodendrocytes

Date received: 21 December 2016; revised: 20 March 2017; accepted: 29 March 2017

## Introduction

Anti-central nervous system (CNS) autoantibodies have been detected using different techniques in serum, in cerebrospinal fluid (CSF), and in brain elutes in patients with multiple sclerosis (MS). There is evidence that some may be pathogenic based on immunopathological studies of experimental demyelinating lesions in laboratory models of MS and of developing lesions in MS subjects.[1–10]

Other authors report conflicting results, citing especially, unspecific affinity of normal human IgG to bind to various CNS structures including bulk isolated myelin membranes and oligodendrocytes.[1,2,11–13]

Indirect immunofluorescence (IIF) is the most common technique used in clinical practice to detect disease-specific serum autoantibodies. Previous IIF studies of serum autoantibodies in MS refer to the use of cryostat sections of fresh unfixed tissue or sections fixed in acetone, ethanol, or formalin followed by application of the test serum and a fluorochrome-conjugated anti-human IgG antiserum.[14] With this procedure, anti-CNS autoantibodies have been reported to be absent in the serum of patients with MS.[15–17] In this study of serum autoantibodies in MS, a fixation method is used that instantly fixes still vital tissues by whole-body perfusion with chilled paraformaldehyde, a procedure known to optimally preserve some antigens, as well as improve tissue morphology.

## Methods

### *Patients and control subjects*

Serum and in some cases cerebrospinal fluid was obtained from patients attending a neurology clinic

at the Royal North Shore Hospital, St Leonards, Sydney. There were 106 with MS and 156 with other neurological diseases (ONDs). Controls (70 healthy adults) were blood donors at a local clinic. Many of the MS and OND cases had a second disease as expected in a population attending a specialty neurology clinic. Clinical attributions were restricted to the primary neurological diagnosis based on standard diagnostic procedures and follow-up examination. The sex and age distribution of patients and healthy control subjects was approximately the same. Sera were collected throughout the year both from those with and without CNS disease. Clinical data, sera, and CSF specimens were collected in accordance with ethical guidelines of the Royal North Shore Hospital and with the informed consent when required of participants.

### *IIF*

Twelve-week-old inbred female Lewis rats were perfused with 2% paraformaldehyde in phosphate-buffered saline (PBS). Coronal slices (1- to 2-mm thick), of the cerebrum and cerebellum plus brain stem were stored in fixative for 2–24 hours. Upper small bowel and kidney tissues were also sampled and fixed as described. Blocks were embedded in optimum cooling temperature compound (O.C.T. Compound) chilled in isopentane to less than −100°C in liquid nitrogen then transferred to the cryostat or −80°C freezer.

Cryostat sections were air dried for 2 hours, hydrated using PBS and treated with 10% bovine serum albumin (BSA) in PBS for 60 minutes at room temperature (RT). After rinsing with PBS, the test serum diluted 1:60 with 1% BSA in PBS was absorbed with rat liver powder (20 mg in 360 μL) (IRT-LP-LR; BioCore) using a rotator for 60 minutes in a cold room (4°C). Following centrifugation at 12,000 r/min for 10 minutes, the supernatant was applied to the slides for 60 minutes at RT. After rinsing with PBS, sections were treated with fluorochrome-conjugated anti-human IgG diluted 1:200 (Alexa Fluor 488 goat anti-human IgG (H + L) (A11013; Invitrogen) for 60 minutes at RT. After rinsing with PBS, the sections were mounted using Prolong Gold Antifade Reagent (P36934; Invitrogen).

Primary antibodies used from different species for double labeling were Alexa Fluor 610 goat anti-mouse IgG (A31550; Invitrogen) and Alexa Fluor 610 goat anti-rabbit IgG (A31551; Invitrogen) diluted 1:200 and added to sections for 60 minutes at RT. Secondary antibodies: neurofilaments (Chemicon; AB1991) diluted 1:500, glial fibrilliary acidic protein (GFAP) (Chemicon; CBL411) diluted 1:500, AQP4 (Chemicon; AB3594) diluted 1:500, synaptophysin (Abcam; ab14692) diluted 1:200, dystrophin 1 (Novocastra; NCL-DYS1) diluted 1:20, Caspr-1 (Abcam; ab34151) diluted 1:500, Kir 4.1 (Abcam; ab80959) diluted 1:75, CNPase (Millipore; MAB326) diluted 1:600. Nuclei were stained using Hoechst 33342 trihydrochloride trihydrate (FluoroPure grade H21492; Invitrogen) diluted 1:500 and incubation for 15 minutes at RT.

Omission of the monoclonal antibody and/or inclusion of an irrelevant antibody run in parallel served as negative controls. Such controls were devoid of staining.

### *Analysis*

The staining pattern exhibited by each test serum was determined by two blinded observers who separately photographed each section using a Zeiss Axio Imager microscope equipped with an AxioCam HRm camera and apochromatic objectives. Staining patterns were classified arbitrarily as an immunohistochemically defined antibody (IDA) while recognizing that patterns with more than one labeled structural determinant could reflect the activity of a single autoantibody, of multiple autoantibodies, or multispecificity mediated by conformational diversity.

## Results

IDAs were detected in sera from 88 of the 332 cases examined. The immunostained determinants selected to define each antibody are indicated by the symbol +! in Tables 1A and 1B. Determinants labeled (+) in the tables signify structures recognized by some but not all sera containing a particular IDA. Tables 2 and 3 list the autoantibodies detected in MS subjects and controls. Figures 1–7 show rat tissues immunostained with different IDAs. A narrative description of each IDA is provided as supplementary material.

Most of the antibodies identified in this study are undescribed. The main exception was IDA-6 which corresponds to neuromyelitis optica (NMO) IgG.[18]

Reactivity against white matter tracts was detected in more than 80% of all sera tested. This “antibody” corresponds to anti-myelin reactivity reported to be detectable in the sera of most children by age 4.

Among the previously unknown autoantibodies were several novel antibodies that recognized particular sets of interneurons. The commonest was IDA-1, an

antibody that recognized a particular set of neurons in the cerebellum and cerebrum. Another was IDA-2, an antibody that recognized the surface of choroid plexus epithelial cells, the neuropil of the molecular layer of the cerebellar cortex, and puncta—possibly bouton—in the Purkinje cell layer of the cerebellar cortex.

### *Healthy controls*

CNS autoantibodies were detected in sera from 10 of 70 subjects with no evidence of CNS disease (Tables 2 and 3). In all, 6 of the 10 immunoreactive sera stained basket cells in the cerebellar cortex, small bipolar interneurons in the cerebrum, and Golgi cells in the cerebellar granular layer, a pattern designated IDA-1 (Figures 1 and 2(a)). Higher magnification showed that the antigen recognized by IDA-1 was associated with a system of cytoplasmic vesicles or particles located within perikarya of affected neurons. This antibody was also common in patients with MS and OND. We are unable to explain why this antibody, IDA-1, was so unevenly represented in each of the clinical subgroups.

### *NMO*

A total of 20 sera were examined from patients with a clinical diagnosis of NMO. Anti-CNS autoantibodies were detected in 12 (Tables 2 and 3). The most common, designated IDA-6, present in eight, was an antibody that recognized glial-limiting membranes of small blood vessels in a pattern similar to that described in NMO by Lennon et al[18] (Figure 3(e)). The same antibody also recognized perivascular astrocytes located around blood vessels with prominent perivascular spaces (Figure 3(d)), small bowel villous epithelium, and the surface of Purkinje cells. Serum AQP4 antibodies were measured in two of the eight cases with the IDA-6 pattern of immunostaining and both were found to be positive.

### *Multiple sclerosis*

Of the 106 sera examined, there were 28 that reacted with rat tissue. The latter comprised 12 different autoantibodies (Tables 2 and 3). As in the healthy control group, the most common was IDA-1, present in nine. Notably, no immunoreactivity against oligodendrocytes was detected in MS sera. Regarding anti-myelin autoantibodies in MS, an assessment of the relative frequency of immunoreactivity directed at tracts of myelinated fibers in a separate blinded analysis of a consecutive series of sera (n = 102) showed the percentage of positive sera in MS cases (n = 37) as 78.38%, in normal subjects (n = 27) 81.5%, and in patients with ONDs including eight subjects with NMO (n = 38) 78.95%. Anti-myelin antibody activity appearing as tramlines in longitudinally sectioned nerve fibers or in the form of rings in large diameter fibers cut in cross section similar to that seen in sections stained for myelin oligodendrocyte glycoprotein (MOG) was very rarely observed in this study.

### *ONDs*

Sera from 156 OND cases were tested. In all, 38 were positive for antibodies that reacted with rat CNS tissue. The different autoantibodies and determinants recognized by these sera are shown in Tables 2 and 3.

A group of 40 sera from cases with diagnoses of optic neuritis (ON) (n = 5), transverse myelitis (n = 18), longitudinally extensive transverse myelopathy (LETM) (n = 8), and clinically isolated syndrome (CIS) (n = 9), most variant forms of MS, showed eight positive for autoantibodies. The serum antibody profile was very similar to that seen in MS including the occurrence of cases with IDA-1. None had anti-oligodendrocyte activity.

In a large group with acute or subacute diseases of the CNS, a group that included cases with ischemic infarction, cerebral tumors, psychiatric illnesses, other inflammatory diseases, and organic CNS diseases of uncertain cause (n = 96), there were 30 cases with sera that reacted with rat tissue (Tables 2 and 3). Anti-myelin and anti-oligodendrocyte activities for the group were similar to that seen in the MS group.

## Discussion

The study provides a catalogue of anti-CNS antibodies that are sometimes present in the serum of patients with MS. It is likely that many of the antibodies are previously undescribed natural autoantibodies of the sort known to be highly seropositive in health and disease. The study says nothing of the specificity of MS CSF IgG or serum antibodies that recognize determinants that are expressed or accessible only in MS tissue. Other autoantibodies that may be present in MS serum but are undetectable using the present IIF procedure are serum antibodies that react with human CNS tissue but not rodent CNS tissue. Examples of such antibodies are antibodies that recognize conformationally intact human anti-myelin–associated glycoprotein (MOG) and that are common in childhood inflammatory demyelinating

**Table 1A.** Immunohistochemically defined autoantibodies (IDAs) numbered 1–14.

| IDA | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Choroid plexus | | | | | | | | | | | | | |
| Epithelial cell cytoplasm | | | | | | | | | | (+) | | | |
| Epithelial cell surface | | +! | | | | | | | | | | | |
| Blood vessels | | | | | | | +! | | | | | | (+) |
| Small blood vessels | | | | | | +! | +! | | | | (+) | | |
| Nuclei | | | | | | | | | | | | | |
| CNS only | | | +! | | | | | | | | | | |
| CNS + small intestine | | | | +! | | | | | | | | | |
| Ependyma only | | | | | +! | | | | | | | | |
| Selected neurons | | | | | | | | | | | | | |
| White matter | | | | | | | | | | | | | |
| Myelin-intense staining | | | | | | | | | | | | | |
| Nodes of Ranvier | | | | | | | | +! | | | | | |
| Oligodendrocytes | | (+) | | | | | | | | | | | |
| Cerebrum | | | | | | | | | | | | | |
| Neurons | | (+) | | | | | | | +! | | +! | | +! |
| Bipolar interneurons | +! | | | | | | | | | | | | |
| Perivascular astrocytes | | | | | | +! | | | | | | | |
| Microglia | | | | | | | | | | | | | |
| Processes | | | | | | | | | | | | | |
| Cerebellum—Purkinje cell layer | | | | | | | | | | | | | |
| Purkinje cell cytoplasm | | | | | | | | | | (+) | | | +! |
| Purkinje cell surface | | | | | | | | | | | | +! | |
| Purkinje cell “pad” | (+) | | | | | | | | | | | | |
| Puncta (? bouton) | | +! | | | | | | | | (+) | +! | | |
| Processes (axons, dendrites) | | (+) | | | | | | | | | +! | | |
| Other cells | | | | | | | | | +! | | | | |
| Cerebellum—molecular layer | | | | | | | | | | | | | |
| Basket cells | +! | | | | | | | | | | | | |
| Parallel processes (Bergmann) | | | | | | | | | | +! | | | |
| Other cells | | | | | | (+) | | | | | | | |
| Neuropil | | +! | | | | | | | | | | +! | |
| Processes (axons, dendrites) | | | | (+) | | | | | | | | | +! |
| Puncta (bouton) | | | | | | | | | | | | | |
| Granular layer | | | | | | | | | | | | | |
| Granule cells | | | | | | | | | | | | +! | (+) |
| Golgi neurons | +! | | | | | | | | | | | | +! |
| Glomeruli (mossy fibers) | | | | | | | | | | | | | |
| Puncta (? bouton) | | (+) | | | | | | | | | | | |
| Processes (axons, dendrites) | | | | | | | | | | | | | +! |
| Autonomic plexuses—small intestine | | | | | | | | | | | | | |
| Myenteric plexus | (+) | (+) | (+) | | | | (+) | | | | | +! | +! |
| Submucosal plexus | | (+) | | | | | | | | | | | |

CNS: central nervous system.
Determinants in rat CNS tissue recognized by IDA-1–IDA-14. Positively stained determinants are indicated by two symbols: +! = obligatory (defining) and (+) = variable; ?bouton: resemble bouton.

**Table 1B.** Immunohistochemically defined autoantibodies (IDAs) numbered IDA-15–IDA-35.

| IDA | 15 | 16 | 17 | 18 | 21 | 22 | 25 | 27 | 28 | 29 | 30 | 31 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Choroid plexus | | | | | | | | | | | | | |
| Epithelial cell cytoplasm | | | | | | | | | +! | | | | |
| Epithelial cell surface | | | | | | | | | | | | | |
| Blood vessels | | | | | | | | | | | | | |
| Small blood vessels | | | | | | | | | | | | | |
| Nuclei | | | | | | | | | | | | | |
| CNS only | | | | | | | | | | | | | |
| CNS + small intestine | | | | | | | | | | | | | |
| Ependyma only | | | | | | | | | | | | | |
| Selected neurons | | | | | | +! | | +! | | | | | |
| White matter | | | | | | | | | | | | | |
| Myelin-intense staining | | | | | | | | | | +! | | | +! |
| Nodes of Ranvier | | | | | | | | | | | | | |
| Oligodendrocytes | | | (+) | | | | +! | | +! | | +! | | |
| Cerebrum | | | | | | | | | | | | | |
| Neurons | | | +! | | | | | | +! | | | | |
| Bipolar interneurons | | | | | | | | | | | | | |
| Perivascular astrocytes | | | | | | | | | | | | | |
| Microglia | | | | | | | | | | | | | |
| Processes | | | | | | | | | | | | | |
| Cerebellum—Purkinje cell layer | | | | | | | | | | | | | |
| Purkinje cell cytoplasm | | | +! | | | | | | +! | | | | |
| Purkinje cell surface | | | | | | | | | | | | | |
| Purkinje cell “pad” | | | | | | | | | | | | | |
| Puncta (? bouton) | | | | | | | | | | | | | |
| Processes (axons, dendrites) | +! | | | | | | | | | | | +! | |
| Other cells | | | | | | | | | +! | | | | |
| Cerebellum—molecular layer | | | | | | | | | | | | | |
| Basket cells | | | | | | | | | +! | | | | |
| Parallel processes (Bergmann) | | | | | | | | | | | | | |
| Other cells | | | | | | | | | | | | | |
| Neuropil | (+) | +! | (+) | | | | | | | | | | |
| Processes (axons, dendrites) | +! | | | | | | | | | | | | |
| Puncta (bouton) | | | | | +! | | | | | | | +! | |
| Granular layer | | | | | | | | | | | | | |
| Granular neurons | | | (+) | | | +! | | | +! | | +! | | |
| Golgi neurons | | | (+) | | | | | | +! | | | +! | +! |
| Glomeruli (mossy fibers) | | | | | +! | | | | | | | | |
| Puncta (? bouton) | | | | | +! | | | | | | | | |
| Processes (axons, dendrites) | | | | | | | | | | | | | |
| Autonomic plexuses—intestine | | | | | | | | | | | | | |
| Myenteric plexus | | | | +! | | | | | +! | | | | |
| Submucosal plexus | | | | (+) | | | | | | | | | |

CNS: central nervous system.
Determinants in rat CNS tissue recognized by IDA-15–IDA-35. Positively stained determinants are indicated by two symbols: +! = obligatory (defining) and (+) = variable; ?bouton: resemble bouton.

**Table 2.** Specificity of serum anti-CNS autoantibodies.

| | No CNS disease (n = 10) | NMO (n = 12) | MS (n = 28) | ON, LETM, TM, CIS (n = 12) | OND (n = 28) |
|---|---|---|---|---|---|
| Choroid plexus | | | | | |
| Epithelial cell cytoplasm | | | 1 | | |
| Epithelial cell surface | | | 3 | | |
| Small blood vessels | 1 | 8 | 2 | 3 | 5 |
| Nuclei | | | | | |
| CNS only | | | 2 | 2 | 2 |
| CNS + small intestine | | | 4 | | 4 |
| Selected neurons | | | | | |
| White matter | | | | | |
| Myelin[a] | | | | | |
| Nodes of Ranvier | | 1 | | | |
| Oligodendrocytes | | 1 | | | 4 |
| Cerebrum | | | | | |
| Neurons | | | 5 | | 2 |
| Bipolar interneurons | 6 | | 9 | 2 | 2 |
| Perivascular astrocytes | | 7 | | | |
| Microglia | | | | | |
| Processes | | | | | |
| Cerebellum—Purkinje cell layer | | | | | |
| Purkinje cell cytoplasm | | 1 | 5 | | |
| Purkinje cell surface | | 3 | | | 3 |
| Purkinje cell “pad” | 6 | | 9 | 2 | 1 |
| Puncta (? bouton) | 1 | | 6 | 2 | 4 |
| Processes (axons, dendrites) | | 1 | 1 | 2 | |
| Other cells | | 1 | 1 | | 1 |
| Cerebellum—molecular layer | | | | | |
| Basket cells | 6 | | 10 | 2 | 4 |
| Parallel processes (Bergmann) | 1 | | 3 | | |
| Other cells | 1 | 1 | | | 1 |
| Neuropil | 2 | 1 | 4 | | 1 |
| Processes (axons, dendrites) | | 1 | | | 1 |
| Puncta (bouton) | | | 3 | 1 | |
| Cerebellum—granular layer | | | | | |
| Granule cells | | | | | |
| Golgi neurons | 6 | | 10 | 2 | 4 |
| Glomeruli (mossy fibers) | | 1 | | | |
| Puncta (? bouton) | 1 | | | | |
| Processes (axons, dendrites) | 1 | | 1 | | |
| Autonomic plexuses—intestine | | | | | |
| Myenteric plexus | 5 | | 4 | 3 | 7 |

NMO: neuromyelitis optica; MS: multiple sclerosis; ON: optic neuritis; LETM: longitudinally extensive transverse myelopathy; TM: transverse myelitis; CIS: clinically isolated syndrome; OND: other neurological diseases; CNS: central nervous system; ?bouton: resemble bouton.
Determinants in rat CNS tissue recognized by autoantibodies detected in sera of patients with neurological diseases and adult control subjects.
[a]Myelin: reactivity directed at white matter tracts was detected in 80% of sera tested in normal controls and in each of the clinical subgroups.

diseases as well as some cases of aquaporin-4 (AQP4)–negative NMO.[19,20]

This study is the first report showing that anti-CNS autoantibodies of different specificities are frequently present in the serum in patients with MS. Why such antibodies have not been detected in previous large IIF studies[15–17] relates chiefly to differences in the methods used to fix the tissue. Traditionally, cryostat sections of snap-frozen rodent tissue are exposed to

**Table 3.** Serum anti-CNS autoantibodies detected in MS subjects and controls.

| Patients and control subjects | Anti-CNS autoantibodies | IDAs |
|---|---|---|
| No CNS disease (n = 70) | 10/70 (14%) | IDA −1, −2, −11, −16 |
| MS (n = 106) | 28/106 (26%) | IDA −1, −2, −3, −4, −8, −10, −11, −12, −14, −17, −31 |
| NMO (n = 20 | 12/20 (60%) | IDA −6, −7, −8, −15, −17, −21 |
| ON, LETM, CIS, TM (n = 40) | 8/40 (20%) | IDA −1, −3, −4, −6, −7, −15, −18 |
| OND (n = 96) | 30/96 (31.25%) | IDA −1, −3, −4, −7, −13, −15, −17, −18, −22, −25, −27, −30, −31, −35 |

IDAs: immunohistochemically defined autoantibodies; MS: multiple sclerosis; NMO: neuromyelitis optica; ON: optic neuritis; LETM: longitudinally extensive transverse myelitis; CIS: clinically isolated syndrome; TM: transverse myelitis; OND: other neurological diseases; CNS: central nervous system.

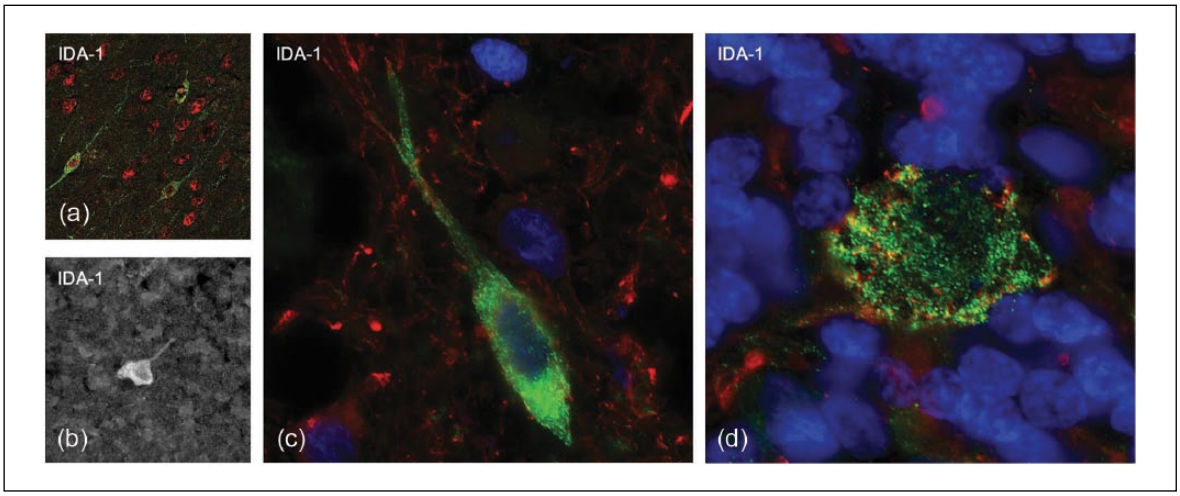


**Figure 1.** IDA-1: (a and c) positively stained small bipolar neurons located in the cerebral cortex and (b and d) positively stained Golgi neurons in the granular layer of the cerebellar cortex. Other granular layer neurons are unreactive. Cytoplasmic staining is particulate in appearance (IDA-1 green, nuclei blue, NF red. (a) ×180, (b) ×360, (c) ×1800, (d) ×2400.

the test serum without fixation or following treatment of the section for 10 minutes with ethanol or acetone or with formalin. While this is a tested method for preserving CNS antigens, morphology is extremely poor compared to that achieved by whole-body perfusion with paraformaldehyde prior to sectioning.[21] McKeon et al,[17] in a study employing formalin-fixed frozen sections prepared according to standard procedures,[18,22] reported an absence of anti-CNS autoantibodies detectable by IIF in sera from 173 healthy controls and 77 patients with MS. The only sera that were positive for anti-neuronal autoantibodies were from patients with NMO where 34% of 177 cases had anti-neuronal autoantibodies in addition to NMO-IgG autoantibody.

Faint staining of tracts of myelinated nerve fibers was detected by IIF at a dilution of 1:60 in approximately 80% of both healthy controls and patients with disease. There are previous reports of anti-myelin antibodies present in the serum of 88%–100% of normal individuals that are acquired between 1 and 4 years of age. Such antibodies are detectable by IIF at dilutions of 1:4 using acetone or ethanol fixed or unfixed frozen sections of human, guinea pig, bovine, monkey, and rat tissue. The affinity appears to be specific for the 7S gamma-globulin fraction and is not seen with other major protein fractions of serum or CSF. Not all investigators are satisfied that such binding of immunoglobulins to myelin represents genuine antibody activity rather than an ionic or some other non-specific interaction.[2,11,15,16,23]

A second antibody detected in this study with some frequency in healthy controls, MS, and OND cases was IDA-1, an antibody interesting as it recognized a defined set of interneurons in the cerebellum and cerebral hemispheres.

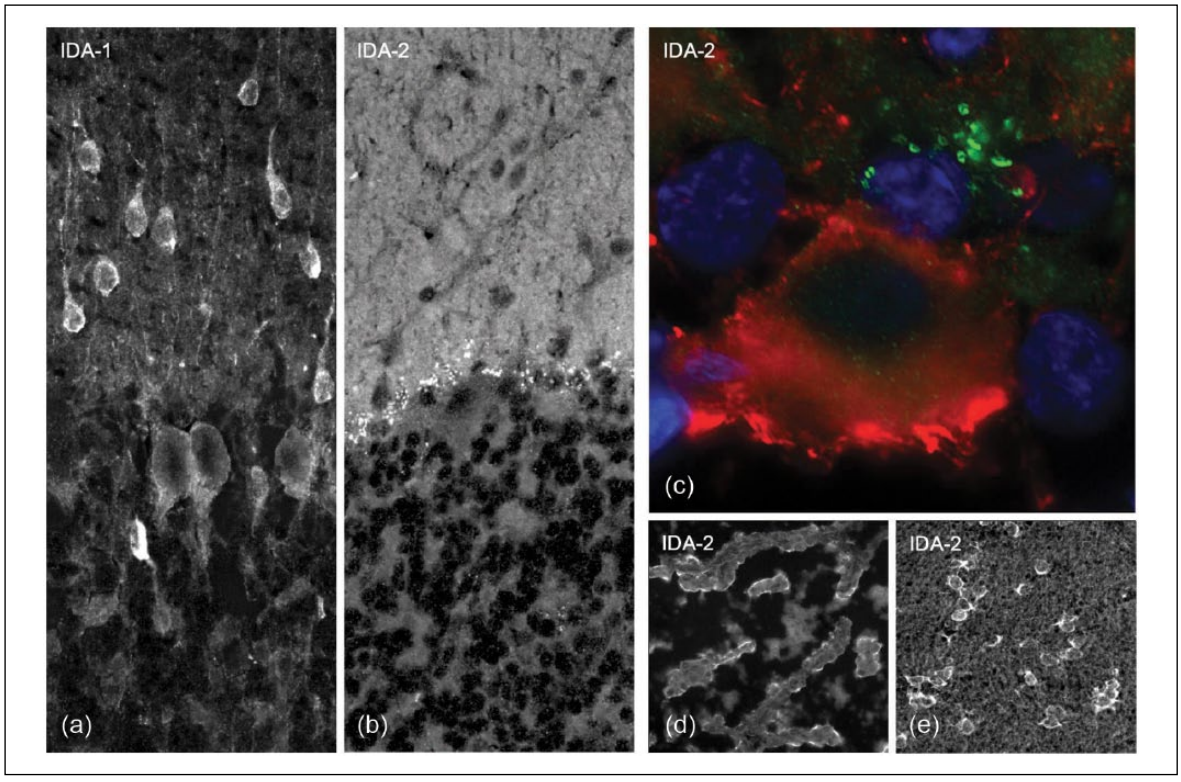


**Figure 2.** (a and b) IDA-1 and IDA-2: cortex of cerebellum comparing IDA-1 and IDA-2 immunoreactivity. In sections exposed to IDA-1 serum, positive staining is restricted to basket cells in the molecular layer, Purkinje cell "pads," and a single cell (a Golgi cell) located in the granular layer. In sections exposed to IDA-2 serum, positive staining is restricted to diffuse staining of the neuropil of the molecular layer together with small puncta located in the Purkinje cell layer. (c) Higher magnification shows that the puncta are located extracellularly. (d) A section of a choroid plexus treated with IDA-2 serum showing staining of the surface of choroid plexus epithelial cells. (e) Oligodendrocytes in a white matter tract in the cerebrum react positively in a section treated with IDA-2 serum (IDA-2 serum immunoreactivity green, nuclei blue, NF red. (a and b) ×480, (c) ×2200, (d) ×200, (e) ×160.

According to a number of investigators, the most compelling evidence for a demyelinating antibody in MS derives from a series of immunohistochemical studies that report that in about two-thirds of all MS cases, myelin destruction is not secondary to a loss of oligodendrocytes but is mediated by an anti-myelin autoantibody which, according to the authors, is evidenced by the presence in the lesion of IgG together with myelin sheaths and macrophages that stain positively for activated complement (C3d and C9neo).[24] This form of MS has been designated "type 2 MS" to distinguish it from "type 3 MS," a form where myelin breakdown is accompanied by oligodendrocyte apoptosis in the absence of activated complement on myelin or in macrophages.[4,6,7,10] It is further noted that while oligodendrocyte apoptosis does not occur together with complement deposition in developing MS lesions, this is a typical finding in developing lesions in AQP4-positive tumefactive NMO.[25]

Other groups have challenged this interpretation of the presence and distribution in MS lesions of activated complement and IgG as evidence of a demyelinating anti-myelin autoantibody in MS.

First, there are now a number of independent studies that report that there is no evidence of immunopathological heterogeneity in MS based on lesional IgG and complement distribution or oligodendrocyte apoptosis, either in cases of long-standing MS or in acute early cases of the disease.[26–31] Regarding reports that oligodendrocyte apoptosis and complement-positive myelin are not seen together in MS lesions but only in tumefactive NMO,[25] in our own studies of prephagocytic and actively demyelinating MS lesions, we have never observed commencing myelin breakdown by macrophages where myelin sheaths are not opsonized by complement or where macrophages stain negatively for complement. Affected sheaths have been observed to stain positively for IgG and

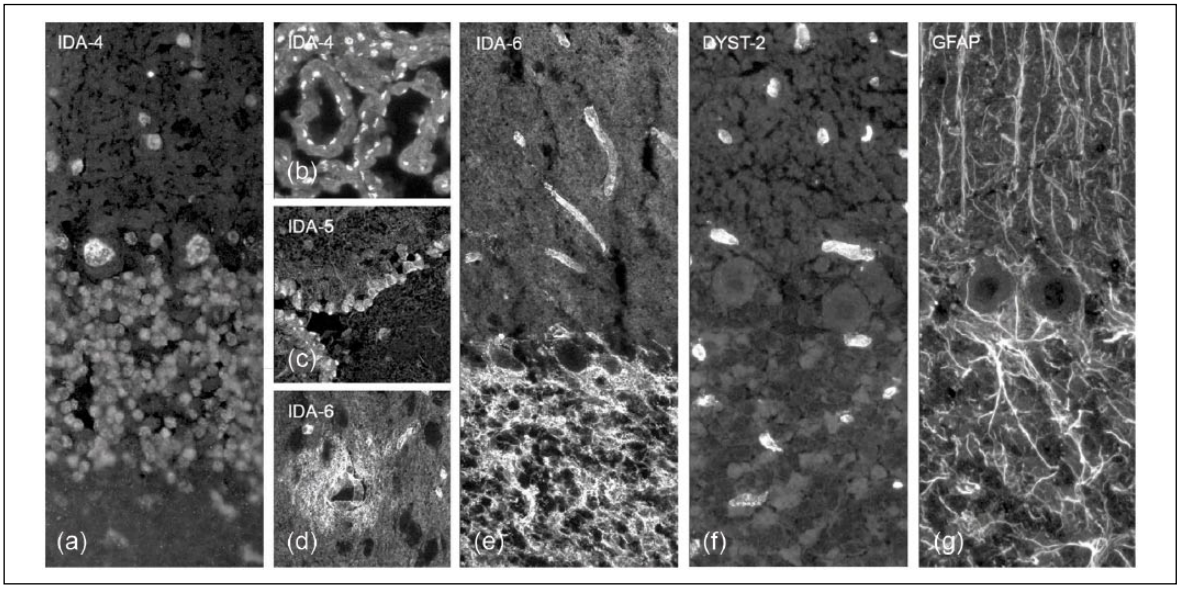


**Figure 3.** IDA-4, -5, and -6. IDA-4: cell nuclei (a) in the cerebellum and (b) in the choroid plexus react positively in sections treated with IDA-4 serum. (c) IDA-5: nuclei in the ependymal epithelium stain positively with IDA-5 serum. (d and e) The two pictures illustrate three of the structures in the cerebrum that react positively with IDA-6 serum (NMO-IgG), namely, astrocytes associated with veins with (d) perivascular spaces, (e) the walls of small blood vessels and an unidentified component of the granular layer of the cerebellum. The latter was unreactive in sections stained for (f) dystrophin-2 and (g) GFAP both of which are present in the granular layer of the cerebellum. (a) ×400, (b–d) ×200, (e–g) ×380.

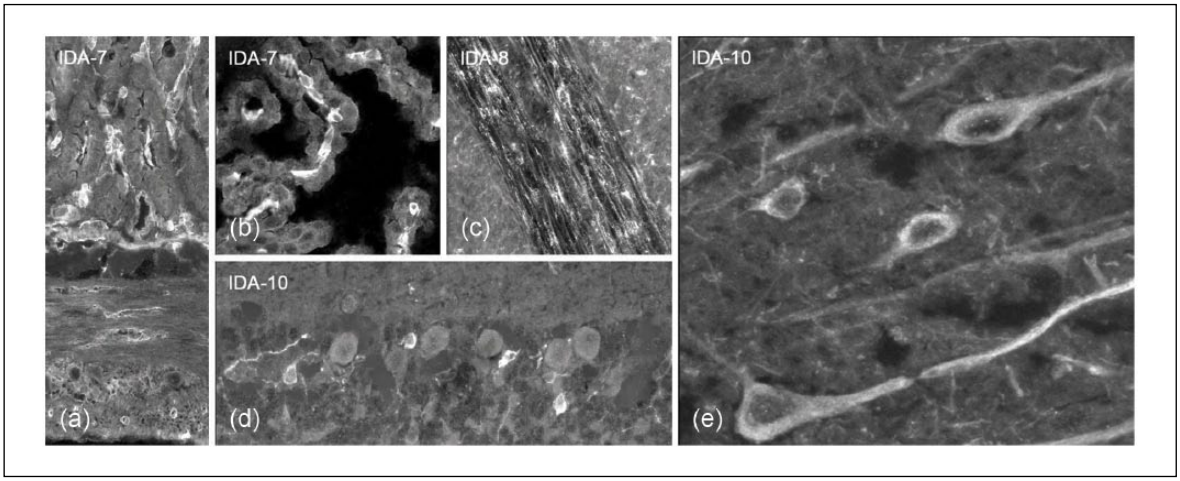


**Figure 4.** IDA-7,-8,-10. Blood vessels (a) in the small bowel and (b) in the choroid plexus stain positively in sections exposed to IDA-7 serum. (c) A white matter tract in the cerebrum immunostained with IDA-8 serum shows staining restricted to discrete points located along the length of a bundle of myelinated nerve fibers. (d and e) Sections exposed to IDA-10 serum show staining of a population of small cells located close to (d) Purkinje cells and large neurons (e) in the cerebral cortex. (a and b) ×250, (c) ×200, (d) ×300, (e) ×580.

kappa and lambda light chains, but this is rare and always difficult to demonstrate.

Second, the distribution of IgG and complement in and around actively demyelinating MS lesions is non–specific, that is, altered myelin sheaths and macrophages containing myelin remnants stain positively for complement and sometimes also IgG in lesional areas invaded by macrophages in ischemic infarction, progressive multifocal leukoencephalopathy, miliary tuberculosis, subacute sclerosing panencephalitis, and cytomegalovirus encephalitis. This can be accounted

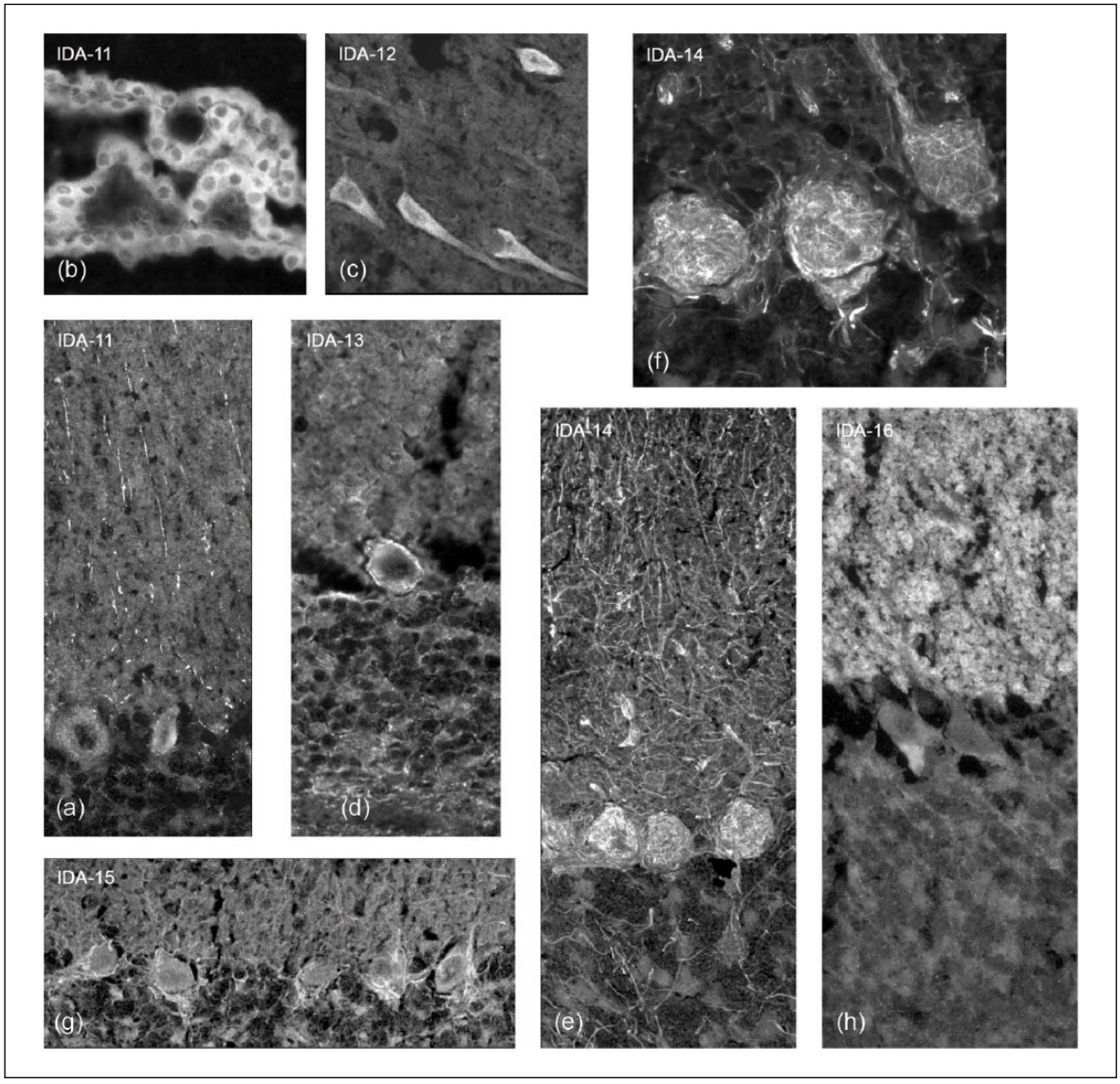


**Figure 5.** IDA-11, -12, -13, -14, -15, -16. (a) Positively stained structures in sections exposed to IDA-11 serum include fibers in the molecular layer of the cerebellum in parallel array. (b) Some IDA-11 sera also stained choroid plexus epithelial cell cytoplasm. (c) Positively stained cell bodies and dendrites of neurons in the cerebral cortex in sections exposed to IDA-12 serum. (d) Purkinje and granular cell cytoplasm together with molecular layer neuropil react positively in sections treated with IDA-13 serum. (e and f) Positively stained structures in sections treated with IDA-14 sera include cell processes throughout the (e) molecular layer of the cerebellar cortex and (f) cytoplasmic filaments in cell bodies of Purkinje cells. (g) An IDA-15 serum-treated section shows positively stained processes associated with the surface of Purkinje cells. (h) Neuropil of the molecular layer of the cerebellar cortex reacts positively with IDA-16 serum. (a) ×380, (b) ×180, (c) ×320, (d) ×380, (e) ×480, (f) ×920, (g) ×300, (h) ×480.

for by what is referred to as myelin stickiness. Where myelin sheaths are disrupted in vivo or in vitro in such a way as to expose the myelin intraperiod line—something that occurs in myelin isolates, vesiculated myelin, myelin engaged by macrophages, or myelin treated with complement—it acquires the property of activating complement and binding IgG.[11,32]

Third is the question of the distribution of IgG in the developing MS lesion. Usually, myelin sheaths engaged by macrophages in MS lesions stain negatively for IgG, or the staining is weak or equivocal compared to the intense staining seen in disrupted sheaths immunostained for C3d and membrane attack complex (MAC).[27,31] By contrast, intact normal appearing

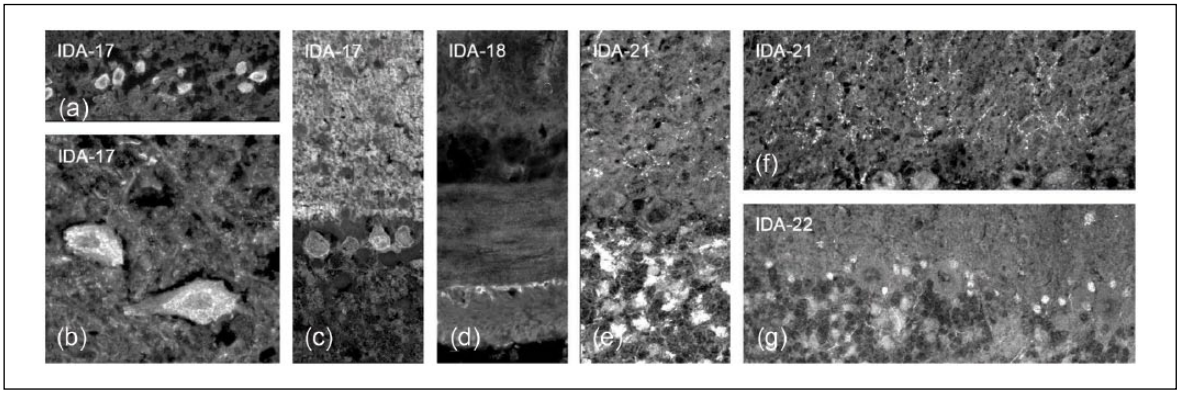


**Figure 6.** IDA-17, -18, -21, -22. Structures in sections of rat cerebral and cerebellar cortex that react with test sera. (a and c) IDA-17: Purkinje cell cytoplasm and (b) neurons elsewhere in the cerebrum. (c) Some IDA-17 sera also react with the molecular layer neuropil. (d) IDA-18: myenteric plexus. (e and f) IDA-21: lines of puncta in the molecular layer of the cerebellar cortex and clusters of mossy fiber puncta in the granular layer of the cerebellar cortex. (g) IDA-22: nuclei of Bergmann glia located among Purkinje cells. (a) ×150, (b, f, g) ×280, (c) ×240, (d) ×150, (e) ×240.

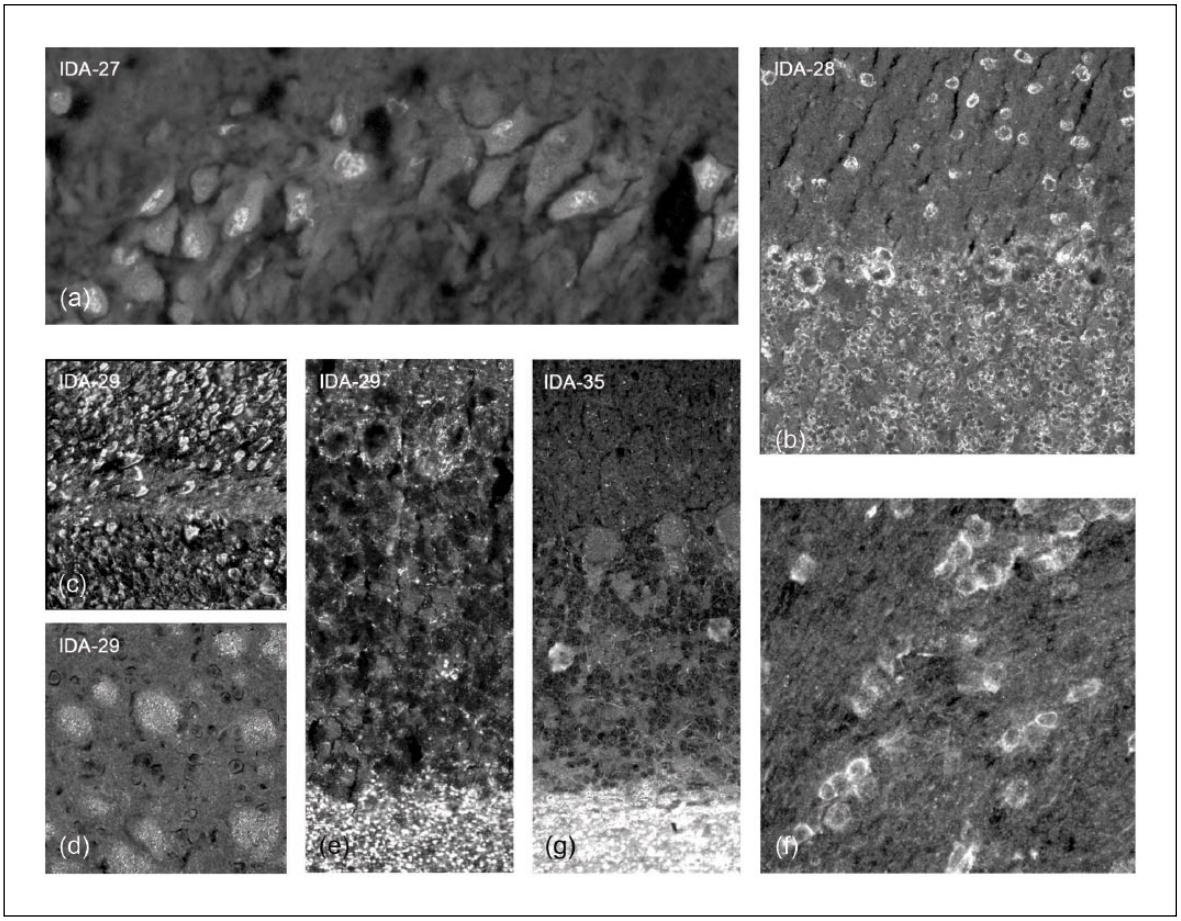


**Figure 7.** IDA-27, -28, -29, -35. Structures in sections of rat brain that reacted with test sera. (a) Purkinje cell nuclei (IDA-27). (b) Cytoplasm of neurons and glia in the cerebellar cortex (IDA-28). (c) Myelinated nerve fibers with positively stained myelin sheaths (IDA-29). (d) Bundles of myelinated fibers in the cerebrum ("pencil fibers") (IDA-29). (e) White matter core of a cerebellar cortical villous together with flecks of immunopositive myelin close to Purkinje cells (IDA-29). (f) Oligodendrocytes located in a white matter tract. (g) The myelinated core of a cerebellar cortical villous together with labeled Golgi neurons (IDA-35). (a) ×420, (b) ×240, (c) ×240, (d) ×120, (e) ×340, (f) × 320, (g) ×260.

myelin sheaths bordering actively demyelinating and recently active lesions, even in tissue steeped in IgG and where axons and the cell bodies of neurons, astrocytes, and oligodendrocytes stain positively for IgG, myelin sheaths show no immunoreactivity for IgG.[31,33]

*What initiates myelin destruction in MS if not an anti-myelin antibody?* There are alternative explanations regarding myelin destruction in MS that do not invoke anti-myelin antibodies—the most plausible, phagocytosis of myelin by activated microglia and monocyte-derived macrophages via scavenger and complement receptors of myelin opsonized by complement secondary to activated caspase 3 oligodendrocyte apoptosis.[34,35]

In summary, anti-CNS autoantibodies of diverse specificities are detectable by IIF in sera from approximately one in four patients with MS. These antibodies appear not to differ from serum antibodies detectable in some patients with unrelated diseases of the CNS and in some healthy controls. The present catalogue of such antibodies may be useful in studies aimed at their further characterization.

## Acknowledgements

Technical assistance was provided by Hung Jiew Lee.

## Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: J.D.E.P. received funding for travel and speaking honoraria from Biogen, Genzyme/Sanofi, Merck Serono, Novartis and Teva.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The study was supported by Multiple Sclerosis Research Australia; the Nerve Research Foundation, University of Sydney; the National Multiple Sclerosis Society (United States RG2731-A-8); and the NSW Ministry for Science and Medical Research.

## References

1. Ryberg B. Multiple specificities of antibrain antibodies in multiple sclerosis and chronic myelopathy. *J Neurol Sci* 1978; 38: 357–382.
2. Lisak RP, Zweiman B, Burns JB, et al. Immune responses to myelin antigens in multiple sclerosis. *Ann NY Acad Sc* 1984; 436: 221–230.
3. Garren H, Steinman L and Lock C. The specificity of the antibody response in multiple sclerosis. *Ann Neurol* 1998; 43: 4–6.
4. Archelos JJ, Storch MK and Hartung HP. The role of B cells and autoantibodies in multiple sclerosis. *Ann Neurol* 2000; 47: 694–706.
5. Raine CS, Canella B, Hauser SL, et al. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: A case for antigen-specific antibody mediation. *Ann Neurol* 1999; 46: 144–160.
6. Lucchinetti C, Bruck W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. *Ann Neurol* 2000; 47: 707–717.
7. Lucchinetti CF, Bruck W and Lassmann H. Evidence for pathogenic heterogeneity in multiple sclerosis. *Ann Neurol* 2004; 56: 308.
8. Srivastava R, Aslam M, Kalluri SR, et al. Potassium channel KIR4.1 as an immune target in multiple sclerosis. *N Engl J Med* 2012; 367: 115–123.
9. Schluesener HJ, Sobel RA, Linington C, et al. A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. *J Immunol* 1987; 139: 4016–4021.
10. McFarland HF. The path to damage in multiple sclerosis. *Ann Neurol* 1999; 46: 141–142.
11. Aarli JA, Aparicio SR, Lumsden CE, et al. Binding of normal human IgG to myelin sheaths, glia and neurons. *Immunology* 1975; 28: 171–1851.
12. Seil FJ. Tissue culture studies of demyelinating disease: A critical review. *Ann Neurol* 1977; 2: 345–355.
13. Kennedy PG and Lisak RP. A search for antibodies against glial cells in the serum and cerebrospinal fluid of patients with multiple sclerosis and Guillain-Barre syndrome. *J Neurol Sci* 1979; 44: 125–133.
14. Nairn RC. *Fluorescent protein tracing*. 4th ed. London: Churchill Livingstone, 1976, pp. 131–147.
15. Traugott U, Snyder S and Raine CS. Oligodendrocyte staining by multiple sclerosis serum is nonspecific. *Ann Neurol* 1979; 6: 13–20.
16. Traugott U and Raine CS. Antioligodendrocyte antibodies in cerebrospinal fluid of multiple sclerosis and other neurologic diseases. *Neurology* 1981; 31: 695–700.
17. McKeon A, Lennon VA, Jacob A, et al. Coexistence of myasthenia gravis and serological markers of neurological autoimmunity in neuromyelitis optica. *Muscle Nerve* 2009; 39: 87–90.

18. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: Distinction from multiple sclerosis. *Lancet* 2004; 364: 2106–2112.

19. Mayer MC, Breithaupt C, Reindl M, et al. Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases. *J Immunol* 2013; 191: 3594–3604.

20. Hohlfeld R, Dornmair K, Meinl E, et al. The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. *Lancet Neurol* 2016; 15: 317–331.

21. Lassmann H, Vass K, Brunner CH, et al. Characterization of inflammatory infiltrates in experimental allergic encephalomyelitis. In: Zimmerman HM (ed.) *Progress in neuropathology* (vol. 6). New York: Raven Press, 1986, pp. 33–62.

22. Yu Z, Kryzer TJ, Griesmann GE, et al. CRMP-5 neuronal autoantibody: Marker of lung cancer and thymoma-related autoimmunity. *Ann Neurol* 2001; 49: 146–154.

23. Lisak RP, Zweiman B and Norman M. Antimyelin antibodies in neurologic diseases. Immunofluorescent demonstration. *Arch Neurol* 1975; 32: 163–167.

24. Pittock SJ, McClelland RL, Achenbach SJ, et al. Clinical course, pathological correlations, and outcome of biopsy proved inflammatory demyelinating disease. *J Neurol Neurosurg Psychiatry* 2005; 76: 1693–1697.

25. Bruck W, Popescu B, Lucchinetti CF, et al. Neuromyelitis optica lesions may inform multiple sclerosis heterogeneity debate. *Ann Neurol* 2012; 72: 385–394.

26. Gay FW, Drye TJ, Dick GW, et al. The application of multifactorial cluster analysis in the staging of plaques in early multiple sclerosis. Identification and characterization of the primary demyelinating lesion. *Brain* 1997; 120(Pt 8): 1461–1483.

27. Barnett MH and Prineas JW. Relapsing and remitting multiple sclerosis: Pathology of the newly forming lesion. *Ann Neurol* 2004; 55: 458–468.

28. Breij EC, Brink BP, Veerhuis R, et al. Homogeneity of active demyelinating lesions in established multiple sclerosis. *Ann Neurol* 2008; 63: 16–25.

29. Henderson AP, Barnett MH, Parratt JD, et al. Multiple sclerosis: Distribution of inflammatory cells in newly forming lesions. *Ann Neurol* 2009; 66: 739–753.

30. Ingram G, Loveless S, Howell OW, et al. Complement activation in multiple sclerosis plaques: An immunohistochemical analysis. *Acta Neuropathol Commun* 2014; 2: 53.

31. Prineas JW and Graham JS. Multiple sclerosis: Capping of surface immunoglobulin G on macrophages engaged in myelin breakdown. *Ann Neurol* 1981; 10: 149–158.

32. DeJong BA and Smith ME. A role for complement in phagocytosis of myelin. *Neurochem Res* 1997; 22: 491–498.

33. Prineas JW, Kwon EE, Goldenberg PZ, et al. Interaction of astrocytes and newly formed oligodendrocytes in resolving multiple sclerosis lesions. *Lab Invest* 1990; 63: 624–636.

34. Rodriguez M, Scheithauer BW, Forbes G, et al. Oligodendrocyte injury is an early event in lesions of multiple sclerosis. *Mayo Clin Proc* 1993; 68: 627–636.

35. Prineas JW and Parratt JD. Oligodendrocytes and the early multiple sclerosis lesion. *Ann Neurol* 2012; 72: 18–31.