Serum autoantibodies to cell surface determinants in multiple sclerosis: a flow cytometric study

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Summary

Multiple sclerosis is thought to be an autoimmune, inflammatory, cell-mediated disease. However, there is evidence suggesting that autoantibodies could play a role in the pathogenesis of multiple sclerosis. Many studies have looked for antibodies to candidate antigens such as myelin basic protein or myelin-oligodendrocyte glycoprotein, with inconclusive results. In order to determine whether antibodies to cell surface determinants on oligodendrocyte or neuronal cells were present in multiple sclerosis, we used flow cytometry to detect antibody binding to intact cultured human cell lines, comparing sera from multiple sclerosis patients with sera from patients with other inflammatory CNS diseases. Sera from healthy individuals were used to determine a normal range. Significant surface binding of IgG or IgM antibodies to oligodendrocyte precursor (OPC)-derived cell lines was seen in 50% of multiple sclerosis sera with no significant difference between secondary progressive (SPMS) and relapsing–remitting (RRMS) subgroups. In contrast, binding to a neuronal cell line, SK-N-SH, was seen with 70% of SPMS sera compared with 25% of RRMS sera (P < 0.001). No significant difference in antibody binding between multiple sclerosis sera and control sera was seen when OPCs were differentiated or when the cells were permeabilized to expose intracellular antigens. Results from all nine patients with ‘benign’ multiple sclerosis were indistinguishable from controls. This study represents a systematic approach to begin to define new antigenic targets in multiple sclerosis and demonstrates that antibodies to accessible antigens on the cell surface of both OPCs and neurons are present in some patients. The results lend support to the possibility that autoantibody-mediated processes are important in a subgroup of multiple sclerosis patients. Identification of the cell surface determinants to which the antibodies bind may shed light on new targets for therapeutic intervention.

Keywords: multiple sclerosis; autoantibodies; flow cytometry; neuroblastoma cells; oligodendrocyte precursor cells

Abbreviations: ADEM = acute disseminated encephalomyelitis; ECACC = European Collection of Cell Cultures; EDSS = Expanded Disability Status Score; FACS = fluorescence-activated cell sorter; OPC = oligodendrocyte precursor cell; PDGF = platelet-derived growth factor; RRMS = relapsing–remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis


Introduction

Multiple sclerosis is widely assumed to be an autoimmune disease. This assumption is made on the basis of indirect evidence from morphological studies on post-mortem brains, as well as epidemiological (Henderson et al., 2000), genetic (Willer and Ebers, 2000) and therapeutic studies (Coles et al., 1999). However, the presence of specific autoantibodies, almost invariably seen in other organ-specific autoimmune diseases, has always been controversial. Detection of antibodies by indirect immunofluorescence on brain sections (Abramsky et al., 1977; Traugott et al., 1979) or on cultured oligodendrocytes (Lubetzki et al., 1986) does not clearly distinguish multiple sclerosis patients from those with other inflammatory neurological disorders.

There is evidence that antibodies are relevant to the pathogenesis of multiple sclerosis. Oligoclonal immunoglobulin in the CSF is used as a diagnostic test (Johnson et al., 1999)...
1977). Recent mRNA studies of B cells in the CSF and brain lesions of multiple sclerosis patients suggest that there is a T-cell-mediated, antigen-driven clonal expansion of B cells (Owens et al., 1998), as may be expected in an autoimmune disease. Indeed, some other diseases demonstrating oligoclonal immunoglobulin in the CSF have now been shown to be autoantibody mediated, including neuromyotonia (Sinha et al., 1991) or some forms of limbic encephalitis (Buckley et al., 2001). Moreover, some treatments effective in autoantibody-mediated diseases such as intravenous immunoglobulin (Lisak, 1998) and plasma exchange (Weinshenker et al., 1999) may have benefits in some multiple sclerosis patients. Autoantibodies to myelin antigens such as myelin-oligodendrocyte protein (MOG) have been shown to contribute directly to demyelination in animal models (Raine et al., 1999). Remyelination is thought to be a result of proliferation and differentiation of oligodendrocyte progenitor cells (OPCs; Gensert and Goldman, 1997). These cells recently have been identified in adult human brain (Scolding et al., 1995) and are thought to make up 5–8% of the adult glial population and up to 70% of dividing cells (Horner et al., 2000). Specific markers for cells with OPC phenotype have allowed post-mortem study of these cells in multiple sclerosis lesions. These studies have shown wide heterogeneity in the number of OPCs in multiple sclerosis lesions and normal appearing white matter. In general, numbers of these cells appear to be greatly increased in acute lesions (Prineas et al., 1989; Maeda et al., 2001) but they are reduced in number or absent in more chronic lesions (Scolding et al., 1998; Wolswijk, 2002).

### Table 1 Patient details

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Total number</th>
<th>M/F</th>
<th>Median age (years)</th>
<th>Median EDSS</th>
<th>Median disease duration (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>12</td>
<td>3/9</td>
<td>35</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ONDs</td>
<td>16</td>
<td>9/7</td>
<td>53</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RRMS</td>
<td>35</td>
<td>10/25</td>
<td>35</td>
<td>2.5</td>
<td>7.4</td>
</tr>
<tr>
<td>‘Benign’</td>
<td>9</td>
<td>2/7</td>
<td>40</td>
<td>2.5</td>
<td>14.4</td>
</tr>
<tr>
<td>In relapse at time of study</td>
<td>10</td>
<td>5/5</td>
<td>33</td>
<td>4.0</td>
<td>4.8</td>
</tr>
<tr>
<td>SPMS</td>
<td>23</td>
<td>7/16</td>
<td>42</td>
<td>6.0</td>
<td>13.3</td>
</tr>
<tr>
<td>Total multiple sclerosis</td>
<td>58</td>
<td>17/41</td>
<td>38</td>
<td>4.0</td>
<td>9.6</td>
</tr>
</tbody>
</table>

OND = other neurological diseases.

### Table 2 Control subjects with other CNS diseases (OND)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>F</td>
<td>Transverse myelitis (Zoster)</td>
</tr>
<tr>
<td>55</td>
<td>M</td>
<td>Total anterior circulation cerebral infarct—40 days</td>
</tr>
<tr>
<td>58</td>
<td>F</td>
<td>Pneumococcal meningitis</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>Herpes simplex encephalitis</td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>Transverse myelitis (viral)</td>
</tr>
<tr>
<td>34</td>
<td>M</td>
<td>Herpes simplex encephalitis</td>
</tr>
<tr>
<td>91</td>
<td>F</td>
<td>Total anterior circulation cerebral infarct—30 days</td>
</tr>
<tr>
<td>48</td>
<td>F</td>
<td>Total anterior circulation cerebral infarct—28 days</td>
</tr>
<tr>
<td>49</td>
<td>M</td>
<td>Viral encephalitis</td>
</tr>
<tr>
<td>76</td>
<td>M</td>
<td>Partial anterior circulation infarct—5 days</td>
</tr>
<tr>
<td>61</td>
<td>F</td>
<td>Total anterior circulation cerebral infarct—2 days</td>
</tr>
<tr>
<td>53</td>
<td>M</td>
<td>Subarachnoid haemorrhage</td>
</tr>
<tr>
<td>72</td>
<td>F</td>
<td>Posterior circulation cerebral infarct—4 days</td>
</tr>
<tr>
<td>44</td>
<td>M</td>
<td>Pneumococcal meningitis</td>
</tr>
<tr>
<td>37</td>
<td>M</td>
<td>Acute disseminated encephalomyelitis</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>Rasmussens encephalitis</td>
</tr>
</tbody>
</table>
previous studies looking for evidence of autoantibodies in multiple sclerosis have often concentrated on techniques to detect binding to specific myelin antigens (Reindl et al., 1999). Some of these antigens are intracellularly located, and are not likely to be directly involved in an antibody-mediated attack. Here, we have taken a wider approach and asked whether multiple sclerosis sera contain IgG or IgM antibodies to any cell surface determinants on human cells of neuronal or glial lineage. To ensure that our observations were without bias, and the results quantifiable, we used the fluorescence-activated cell sorter (FACS) machine to measure antibody binding to cultured human neuronal and oligodendrocyte cell lines at various stages of differentiation. A similar approach was used recently to identify antibodies of unknown specificity binding to a muscle cell line in patients with acetylcholine receptor antibody-negative myasthenia gravis (Blaes et al., 2000).

### Methods

#### Patient selection

Patients were recruited from out-patient clinics, neurology wards and multiple sclerosis treatment trials, after authorization from the local Research Ethics Committee. Informed consent was obtained according to the Declaration of Helsinki. All multiple sclerosis patients had clinically definite multiple sclerosis as defined by the Poser criteria (Poser et al., 1983). Multiple sclerosis patients were classified as either relapsing–remitting (RR) or secondary progressive (SP) by clinical history. All RRMS patients had active disease, defined as at least two relapses in the preceding 2 years. No multiple sclerosis patient had ever received disease-modifying treatment. Ten of the RRMS patients had suffered a relapse within 3 months prior to blood sampling. Appropriate clinical information was recorded including age, disease duration, numbers of relapses in the last 2 years and current disability measured by the Expanded Disability Status Score (EDSS; Kurtzke, 1984; Table 1). A subgroup of ‘benign’ patients was identified with disease duration of >10 years but minimal permanent disability (EDSS <2.5). Fourteen control patients were selected with CNS inflammation and necrosis from established non-autoimmune causes (other neurological diseases, Table 2) to control for autoantibody which may be produced as a non-specific response to tissue damage. One patient with acute disseminated encephalomyelitis (ADEM) following viral infection was also included, and one patient with Rasmussen’s encephalitis, which may be associated with serum antibodies to a neuronal surface antigen (Rogers et al., 1994). Both these patients had responded to plasmapheresis. Twelve age-matched healthy volunteers were recruited from staff at the Department of Clinical Neurology at the Radcliffe Infirmary, Oxford. No control patient had received steroids or any immunosuppressant drugs. After informed consent, 20 ml of blood was taken from each volunteer. Serum was stored at −70°C and centrifuged before use.

#### Preparation of cell cultures

OPCs, astrocyte, neuroblastoma, endothelial and muscle-like cell lines were used (Table 3). These were all grown as a monolayer in 175 cm² plastic culture flasks at 37°C in a 5% CO₂ humidified atmosphere. Glutamine (2 mM), penicillin and streptomycin were added to all culture media. Cells were passaged every third day. The identity, and appropriate characteristics, of the cell lines were confirmed by flow cytometry using monoclonal antibodies or specific antisera to different cell markers. The results are shown in Table 3.

Occasionally, culture conditions were altered to influence the expression of surface antigens. The SK-N-SH cells were differentiated using serum-free RPMI 1640 medium with 1 μM all-trans retinoic acid (ATRA; Lovat et al., 1993). Differentiation was

### Table 3 Human cell lines used for screening multiple sclerosis sera

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Source</th>
<th>Medium</th>
<th>Phenotypic markers</th>
<th>Morphology</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOG</td>
<td>Glyn Dawson, Chicago</td>
<td>DMEM + 10% FCS</td>
<td>Galactocerebroside, NG-2, PDGFα receptor</td>
<td>Oligodendrocyte precursors</td>
<td>Post and Dawson (1992)</td>
</tr>
<tr>
<td>HOG differentiated</td>
<td>Tridiodothyronine 6 days</td>
<td>DMEM + 10% FCS</td>
<td>Galactocerebroside</td>
<td>Oligodendrocyte</td>
<td>de Arriba Zerpa et al. (2000)</td>
</tr>
<tr>
<td>MO3.13 differentiated</td>
<td>Tridiodothyronine 6 days</td>
<td>DMEM + 10% FCS</td>
<td>Galactocerebroside</td>
<td>Immature oligodendrocyte</td>
<td>Craighead et al. (1999)</td>
</tr>
<tr>
<td>CCF-STTG1</td>
<td>ECACC</td>
<td>RPMI + 10% FCS</td>
<td>GFAP, PDGFα receptor</td>
<td>Astrocyte</td>
<td>Krul and Tang (1992)</td>
</tr>
<tr>
<td>SK-N-SH</td>
<td>ECACC</td>
<td>RPMI + 10% FCS</td>
<td>NCAM, Hu, Neurofilament 200</td>
<td>Neuronal</td>
<td>Lovat et al. (1993)</td>
</tr>
<tr>
<td>SK-N-SH differentiated</td>
<td>Retinoic acid 6 days</td>
<td>RPMI + 10% FCS</td>
<td>NCAM, Hu, Neurofilament 200</td>
<td>Neuronal</td>
<td>Lovat et al. (1993)</td>
</tr>
<tr>
<td>IMR-32</td>
<td>ECACC</td>
<td>EMEM + 10% FCS</td>
<td>NCAM, Hu, Neurofilament 200</td>
<td>Neuronal</td>
<td>Verstraeten et al. (2002)</td>
</tr>
<tr>
<td>TE-671</td>
<td>ECACC</td>
<td>DMEM + 5% FCS</td>
<td>Acetylcholine receptor</td>
<td>Striated muscle</td>
<td>Blaes et al. (2000)</td>
</tr>
<tr>
<td>SK-HEP-1</td>
<td>ECACC</td>
<td>EMEM + 10% FCS</td>
<td>VCAM (not retested by our lab)</td>
<td>Endothelium</td>
<td>Watchorn et al. (2002)</td>
</tr>
</tbody>
</table>

All cell lines were passaged every 3 days using 0.25% trypsin/EDTA according to standard procedures.

GFAP = glial fibrillary acidic protein; NCAM = neural cell adhesion molecule; VCAM = vascular cell adhesion molecule.
confirmed by morphological changes observed by light microscopy. The immature oligodendrocyte lines were differentiated using serum-free medium with triiodothyronine (T3; Baas et al., 1997). This was Dulbecco’s modified Eagle medium, as above, with 10 μg/ml insulin, 5.5 μg/ml transferrin, 5 ng/ml selenium and 2 ng/ml T3. In this case, differentiation was confirmed by observing changes to cell surface antigens by flow cytometry (see below). In both cases, the cells were cultured in ‘differentiation medium’ for 6 days prior to flow cytometric analysis.

Pre-absorption of sera
Prior to some experiments, sera were pre-absorbed with different cell types. A total of 10^7 intact cells were incubated for 1 h at room temperature with 100 μl of serum diluted 1:5 in culture medium with gentle agitation. After microcentrifugation at 4000 r.p.m. for 5 min to remove the cells, supernatants were used for subsequent experiments.

Incubation with sera
The cells were removed from the flask using 0.1% trypsin. They were washed in phosphate-buffered saline (PBS), and 10^5 cells in 100 μl of medium were added to each well of a round-bottomed 96-well plate. Patient serum was diluted 1:5 with PBS and 100 μl added to each well to make a final dilution of 1:10. Antisera were used at the manufacturer’s recommended concentrations. For some experiments, cells were fixed for 10 min with 1% formaldehyde (in PBS) and permeabilized by addition of 0.1% Triton X-100 with the primary antibody. The plates were incubated for 1 h on ice. Subsequently the plates were centrifuged for 5 min and the liquid aspirated. The pellets were resuspended in PBS and centrifuged again to wash the cells. This process was repeated three times to remove non-specific and low-affinity binding.

To detect the bound antibody, 50 μl of an appropriate fluorescein isothiocyanate (FITC)-conjugated secondary antibody to IgG or to IgM was incubated in each well for a further 30 min on ice. In the preliminary experiments, the IgG and IgM secondary antibodies were mixed in equal proportions. In later experiments, they were added to separate wells. The FITC conjugates were protected from the light at all times by wrapping in silver foil. Following this, the cells were washed again as above. Finally, the cells were suspended in 1% formalin in PBS and analysed by flow cytometry within 48 h.

Flow cytometry
Cell preparations were analysed using an analytical flow cytometer (Becton-Dickinson FACScan). The FACS machine was first used to
gate a population of $10^4$ live whole cells, excluding cell fragments or dead cells (Fig. 1a). Binding of antibody or isotype-matched control to these live cells was then identified with a fluorochrome-labelled second antibody. Binding of anti-NG-2 serum to the HOG cell line is shown in Fig. 1b. Figure 1c shows the mean fluorescence intensity of binding of antibodies to platelet-derived growth factor α (PDGFα) receptor, NG-2 and galactocerebroside on undifferentiated oligodendrocyte cell lines, or to the cells after differentiation by T3. The oligodendrocyte precursor surface markers NG-2 and PDGFα receptor disappeared after differentiation, while the galactocerebroside remained.

The cell lines were then tested for binding of IgG and IgM antibodies from multiple sclerosis and control sera. The antibody binding of each serum was expressed as a percentage of that of a positive control, after subtraction of the mean value for the healthy controls. An example is shown in Fig. 1d. If the high and low control values were similar, indicating that the cells were not expressing the antigen effectively, as happened in two out of 20 experiments, the plate was not analysed further.

**Statistical analysis**

Populations were compared using two-tailed Mann–Whitney U test with $P < 0.05$ considered significant. Correlations were assessed using Spearman’s $\rho$. As a guide, antibody binding above the mean of the healthy subjects plus 2 SDs was considered significant.

**Results**

**Binding of multiple sclerosis serum antibodies to cell lines with oligodendrocyte precursor phenotypes**

Two human oligodendrocyte-derived cell lines were used. The HOG cell line is a subclone of a well-differentiated oligodendroglioma (Post and Dawson, 1992). The MO3.13 line was produced by fusion of a primary human oligodendrocyte culture and a rhabdomyosarcoma line (McLaurin et al., 1995). Both cell lines have been shown previously to possess many of the characteristics of OPCs (Craighead et al., 1999; de Arriba Zerpa et al., 2000). Cells were tested without differentiation, or after differentiation with T3. Antibody binding to the surface of HOG oligodendroglial cells was significantly higher in multiple sclerosis sera than in control sera ($P=0.01$, Mann–Whitney U; Fig. 2a). Two other cell lines (MO3.13 and CCF-STTG1), which express OPC surface...
markers, also showed significantly greater binding of multiple sclerosis sera than of control sera ($P < 0.0003$ and 0.016, respectively, Fig. 2b and c), even though the CCF-STTG1 cells have morphology characteristic of astrocytes rather than oligodendrocytes (Krul and Tang, 1992).

Culturing the HOG cells in differentiating medium, which caused a reduction in the expression of PDGF receptor and NG-2 markers (Fig. 1c), resulted in loss of binding of multiple sclerosis sera, and the results were not significantly different between multiple sclerosis and controls (Fig. 2d). However, the differentiated MO3.13 cells did retain some reactivity with the multiple sclerosis sera (Fig. 2e).

**Fig. 3** Flow cytometric measurement of antibody binding to cultured cells incubated with sera from controls ($n = 28$), and patients with ‘benign’ multiple sclerosis ($n = 9$), RRMS ($n = 35$) and SPMS ($n = 23$). The results are shown with HOG oligodendroglial cells (a), MO3.13 oligodendroglial cells (b), CCF-STTG1 astrocytoma cells (c) and SK-N-SH neuroblastoma cells (d). The left hand shows all results while the right hand shows means and standard errors.

**Binding of multiple sclerosis serum antibodies to lines with neuronal, muscle and endothelial phenotypes**

Two neuroblastoma cell lines were tested as models for neuronal cells. Surprisingly, antibody binding to the human neuroblastoma cell line SK-N-SH was also significantly higher in multiple sclerosis than non-multiple sclerosis sera ($P < 0.0001$, Fig. 2f). Binding to IMR-32 neuroblastoma cells was not significantly different between multiple sclerosis and non-multiple sclerosis sera (Fig. 2g). Since the result with the SK-N-SH cells was unexpected, we tested the sera on two non-neuronal cell lines, TE671 cells, which are derived from a human rhabdomyosarcoma, and SK-HEP, which express endothelial markers. There was no significant binding in either case (Fig. 2h and i), providing no evidence that the multiple sclerosis sera were binding non-specifically to the SK-N-SH cells. Differentiation of SK-N-SH cells with ATRA made no difference to the results (data not shown).

**Presence of antibodies in different subgroups of multiple sclerosis patients**

The sera from the multiple sclerosis patients could be divided into three groups according to the nature or stage of the disease that they had reached (Table 1). These were RRMS ($n = 26$), SPMS ($n = 23$) and ‘benign’ multiple sclerosis ($n = 9$). The ‘benign’ multiple sclerosis group is a subset of the RRMS group, but is also shown separately on Fig. 3. The
age ranges were not significantly different, but the nine patients with benign multiple sclerosis had median disease duration of 12 years (range 10–28 year), whereas the patients with RRMS had shorter disease duration (median 5.5 years, range 1–12 years), and those with SPMS predictably had longer duration (median 14 years, range 2–28 years). Corresponding with the disease subgroup, the EDSS scores were higher in the patients with SPMS (median 2.5 for RRMS, 6 for SPMS). Ten RRMS patients had undergone relapses within 3 months of the serum sample being taken. The median EDSS for these patients was significantly greater than the other RRMS patients (median EDSS 5.5 for relapse group, 2 for other RRMS patients).

The antibody data were divided into these three subgroups and the results for the cell lines that bound multiple sclerosis sera significantly are shown in Fig. 3, with individual values on the left and mean values on the right. With each of the OPC lines (HOG and MO3.13), and the astrocyte-like cell line (CCF-STTG1), binding of RRMS serum antibodies tended to be higher than that of SPMS (Fig. 3A–C) without being significantly different. There was no significant difference in binding to any cell line when comparing sera from patients following a recent relapse with sera from the other RRMS patients (median EDSS 5.5 for relapse group, 2 for other RRMS patients).

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Strikingly, binding of serum antibodies from SPMS patients was significantly higher than RRMS or benign multiple sclerosis when tested on the SK-N-SH cell line (P < 0.001). The results shown in Fig. 3d are the mean values of three consecutive experiments testing the control and multiple sclerosis sera against SK-N-SH patients, with intra-assay variation of 6.4% and inter-assay variation of 18.7%.

Binding to the second neuroblastoma line, IMR-32, was also significantly higher from SPMS sera than RRMS sera (data not shown, P = 0.004). A number of the inflammatory controls also bound significantly to this cell line, however, explaining why there was no significant difference in binding between multiple sclerosis and non-multiple sclerosis sera overall (Fig. 1g).

Using the mean plus 2 SDs of the healthy control values as shown in Fig. 3 (left) to determine a cut-off value between negative and positive results (excluding the patient with ADEM), the sera from 16 out of 35 RRMS patients bound significantly to one or more OPC lines, and the sera from 16 out of 23 SPMS patients bound to the SKN cells. The results are summarized in Table 4. There was significant correlation of antibody binding to each of the three OPC lines, but no significant correlation between binding of sera to HOG and SKN-SH cells (data not shown).

To confirm that the antibodies were binding specifically to the different cell lines, we absorbed the sera against undifferentiated HOG and SK-N-SH cell lines before applying the absorbed sera to HOG or SK-N-SH cells (Fig. 4a). Although technically difficult, the results confirm that binding to HOG cells was not absorbed by pre-incubation with SK-N-SH cells, and vice versa.

We also tested the cells after permeabilization to allow the antibodies access to the cytoplasm. There was considerably more binding of IgG than with unpermeabilized cells, but no differences in the median binding to HOG or SK-N-SH cells when comparing multiple sclerosis sera with controls (Fig. 4b).

CSF samples were not available from the patients whose serum was tested, but eight CSF samples were made available subsequently from individuals with RRMS undergoing lumbar puncture for diagnosis of multiple sclerosis. All were positive for oligoclonal IgG. Of these, five out of eight were positive for binding to HOG cells, while none were positive for binding to SK-N-SH cells (Fig. 4c). Nine control CSF specimens from patients with viral meningitis did not show significant antibody binding to any cell line.
Class of immunoglobulin binding to OPC or SK-N-SH cells

All sera were first tested using a mixture of fluorescein-labelled antibodies to IgG and IgM, and positive results were then analysed separately for the class of immunoglobulin bound. Interestingly, the antibodies binding to the OPC cell lines were both IgG and IgM (Fig. 5a and b), whereas the antibodies binding to the SK-N-SH cell line were predominantly IgM (Fig. 5c). The antibodies from the ADEM serum that bound to HOG cells were also mostly IgM.

Discussion

Many studies have tried to demonstrate the presence of antibodies to CNS antigens in multiple sclerosis, but in the majority of cases the results have been disappointing, largely because a proportion of sera from healthy controls were also positive. Here we have used a new approach to systematically characterize the binding of multiple sclerosis sera, from different subgroups of patients, to both oligodendrocyte and neuronal cell lines. The results indicate that IgG antibodies to OPCs are present in a proportion of patients, largely those with relapsing–remitting disease, and do not bind to differentiated oligodendrocytes. In addition, some multiple sclerosis patients, mostly those in the secondary progressive phase of the disease, have IgM antibodies that bind to a neuroblastoma cell line that is widely agreed to express neuronal markers. These results show the presence of autoantibodies that bind to the surface of human brain cells, which could play a pathogenic role in multiple sclerosis.

Previous reports of specific autoantibodies in multiple sclerosis have often been dismissed as epiphenomena (Ruutiainen et al., 1981). One reason for this is the inflammatory nature of multiple sclerosis, which could increase levels of non-specific ‘natural’ antibodies, as is the case in other inflammatory autoimmune conditions (Jasani et al., 1999). However, there is little evidence for a general increase in natural autoantibodies in the serum of multiple sclerosis patients (Matsiota et al., 1988). In our experiments, levels of autoantibodies measured by flow cytometry were not found to be appreciably raised in a group of patients with other inflammatory CNS diseases, except for one patient with ADEM. Additionally, there was no relationship between antibody levels and inflammatory activity in the multiple sclerosis patients (e.g. during relapse). Antibodies binding to specific cells could be absorbed by pre-incubation with the same cells but not by irrelevant cell lines, suggesting high specificity for certain cell types.
Another widely held view is that necrotic cell death from any cause can lead to the exposure of intracellular autoantigens and the production of autoantibodies as a purely secondary process. The pathogenic activity of these secondary autoantibodies has not been established. In all our binding experiments, significant differences between multiple sclerosis patients and controls were only seen using cells with an intact cell membrane, suggesting that the autoantigens are on the cell surface. These antigens are therefore continually accessible to the immune system, and autoantibodies directed against them are likely to be pathogenic. In addition, since no autoantibodies were seen in control patients such as stroke victims, who suffer massive neuronal damage, we feel these autoantibodies cannot easily be dismissed as secondary phenomena.

Our results seem to support reports of heterogeneity in the pathogenesis of multiple sclerosis. In the paper by Lucchinetti et al. (2000), 60% of patients had evidence of lesion antibody deposition and/or complement activation, while the other cases appeared to demonstrate oligodendrocyte apoptosis. These patients were autopsy or biopsy cases and unlikely to be a representative sample of multiple sclerosis patients. In our experiments, 62% (36 out of 58) of multiple sclerosis sera demonstrated significant antibody binding to one or more human CNS cell lines. Our relatively non-specific method for antibody detection is likely to give some false-negative results. However, it is interesting that all the patients with ‘benign’ multiple sclerosis were antibody negative, and the absence of autoantibodies may indicate a relatively good prognosis.

The antigenic targets of serum autoantibodies from multiple sclerosis patients were surprising. Our experiments confirmed previous reports that there was little or no antibody directed against mature differentiated oligodendrocytes. However, 50% of multiple sclerosis patients tested showed significant antibody binding to at least one OPC line. The experiment with CCF-STTG1 cells demonstrated that the expression of OPC surface antigens was more important than oligodendrocyte morphology to elicit binding of multiple sclerosis serum antibodies. These cells were originally intended as ‘non-oligodendrocyte’ control glial cells, and we were surprised when they were found to express PDGFr receptor. This is a highly specific marker for OPCs (Nishiyama et al., 1996) and was not expressed by the neuronal, endothelial or muscle-like lines which we used. In fact, expression of OPC antigens, especially PDGFr receptor, is extremely common in all tumours of glial cell origin, and it has been proposed that all glial tumours, whatever their final morphology, may arise from dividing OPCs (Shoshan et al., 1999). This highlights the pitfalls of using tumour-derived cell lines as correlates of normal tissues, and we made sure we could re-confirm the expected antigenic profile of every other cell line used.

Antibody directed against OPCs has been demonstrated previously in the CSF of multiple sclerosis patients (Niehaus et al., 2000), and the idea that OPCs may be destroyed or depleted as a result of multiple sclerosis has been predicted for several years (Elias, 1987). This has been put forward as the explanation for the lower levels of remyelination seen in multiple sclerosis compared with animal models of demyelination. The presence of specific autoantibodies against OPCs could indicate that they are directly disabled or destroyed by an autoimmune process. The lack of demonstrable autoimmunity towards mature oligodendrocytes could imply that autoimmune destruction of OPCs may be the primary cause of the inflammatory multiple sclerosis lesions in some patients, with mature oligodendrocytes damaged as a non-specific result of the inflammatory cascade. This would explain the distribution of the inflammation, which mirrors the patchy distribution of adult OPCs (Ono et al., 1997). Further, it is a commonly held belief that OPCs are limited in numbers and could be depleted by chronic demyelination. If they are the focus of autoimmune attack, then this would

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Fig. 5 Flow cytometric measurement of antibody binding to cultured cells after incubation with serum samples from multiple sclerosis patients and controls, showing the contribution of IgG and IgM class antibodies. Results are from individual experiments using HOG oligodendroglial cells (a), CCF-STTG1 astrocytoma cells (b) and SK-N-SH neuroblastoma cells (c).
explain the unusual natural history of multiple sclerosis, where the frequency of relapses and inflammatory activity usually decreases with time.

Antibodies to various gangliosides (Sadatipour et al., 1998), neurofilament (Silber et al., 2002) and other neuronal antigens (Rawes et al., 1997) have been described previously in multiple sclerosis patients. Anti-neuronal antibodies are directly implicated in the pathogenesis of HTLV-1-associated myelopathy, which bears many clinical similarities to progressive multiple sclerosis (Levin et al., 2002). However, our study is the first to describe antibody from multiple sclerosis serum binding to the surface of intact viable neuronal cells in culture. The importance of these auto-antibodies is their association with a progressive course and the positive correlation with permanent disability. It is unlikely that these antibodies cause multiple sclerosis, but their presence in the serum could indicate the spreading of the autoimmune response to involve neuronal antigens. This ‘determinant spreading’ could lead to subsequent autoimmune neuronal damage and the changing of the disease to a progressive course. It could explain why some ‘benign’ patients, who never demonstrate ‘determinant spreading’, do not suffer significant permanent disability. Although this is merely conjecture, determinant spreading between myelin antigens is seen in animal models of demyelination (Vanderlugt et al., 2000) and may be important in the progression of experimental allergic encephalomyelitis.

Clearly more work needs to be done to elucidate further the exact antigens targeted by the immune system in multiple sclerosis. Knowing the cellular targets should help us to find the molecular targets, with the promise of developing diagnostic and prognostic immunological tests, and perhaps future treatments based on immunotherapy.

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