# Humoral autoimmunity as a mediator of CNS repair

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Autoimmune responses directed against the central nervous system (CNS) have generally been considered pathogenic in nature. Although there are several well understood conditions in which this is the case, there is also a growing body of experimental evidence to show that both the cellular and humoral immune responses can promote tissue repair following CNS injury and disease. Our laboratory has used a mouse model of chronic demyelinating disease to characterize a class of polyreactive IgM autoantibodies that react with oligodendrocyte surface antigens and promote myelin repair. By screening a large number of human monoclonal antibodies, we have found that IgM antibodies that react with CNS tissue are relatively common. Autoreactive IgM antibodies might constitute an endogenous system for tissue repair, and therefore these antibodies could be of value as therapeutic reagents.

The central nervous system (CNS) is often considered a site of 'immune privilege', based, in part, on the physical separation of CNS tissue from peripheral immune function by the blood–brain barrier. However, the isolation of the CNS is often imperfect, and there are many examples in human disease and in animal models of disease in which both the cellular and humoral branches of the immune system interact with the CNS.

Evidence for CNS tissue repair mediated by the cellular branch of the immune system has recently been reviewed[1,2]. Here, we review recent work demonstrating that elements of humoral immunity might also play a role in tissue repair.

## Autoantibodies as pathogenic agents

The existence of pathogenic autoantibodies is well-established for several peripheral neurologic syndromes, including myasthenia gravis, Lambert–Eaton syndrome, Guillain–Barré syndrome and acquired neuromyotonia. Questions concerning antibody-mediated central nervous tissue injury are always complicated by issues of blood–brain barrier permeability, but antibody involvement is suspected in both Ramussen's and Bickerstaff's encephalitis[3].

The involvement of pathogenic autoantibodies in a particular disease has generally been defined based on several lines of experimental and clinical evidence. Antibodies to a defined target should be present in the majority of patients with the disease. The presence of these antibodies is often demonstrated by using purified antibodies as immunostaining reagents on tissues that express the target antigen. Immunization with the target antigen should induce the disease in experimental animals, and passive transfer of antibody to non-immunized animals, or transfer of antibody from patients with the disease, should also induce disease. Reduction of serum antibody levels, either by plasma exchange or by immunosuppression, usually leads to clinical improvement, and rising antibody levels following these treatments are mirrored by a return of clinical symptoms.

Criteria such as these can define a role for pathogenic antibodies in the development of a disease. We have applied many of the same criteria to define autoantibodies that

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promote tissue repair in an animal model of multiple sclerosis (MS), the most common human CNS demyelinating disease. Before we describe these observations, we will first consider data on the role of autoantibodies in human MS.

## Autoantibodies in MS

Elevated concentrations of immunoglobulin (oligoclonal bands) in the cerebral spinal fluid have long been used as a diagnostic marker for MS, and were originally interpreted as evidence for the involvement of the humoral immune response in the pathogenesis of MS. There have been many attempts to identify pathogenic antibodies in the serum of MS patients, and antibodies that are specific for a variety of myelin proteins and antigens have been detected, including antibodies against many of the major myelin proteins, such as myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) and myelin-associated glycoprotein (MAG). However, there is little direct evidence that these antibodies are actually involved in the pathogenesis of MS, and the presence of these antibodies is not highly specific for the disease; antibodies to many of these antigens are also found in the serum of healthy individuals or in patients with neurological diseases other than MS.

Although there is little direct evidence to support a role for humoral immunity in the pathogenesis of MS, the possibility is supported by observations in animals with experimental autoimmune encephalitis (EAE). Immunization with myelin components such as MBP or PLP, produces EAE, a T-cell-mediated inflammatory disease in which demyelination is often minimal. However, if antibodies to MOG are injected after induction of EAE, the severity of the disease is dramatically increased and large demyelinating lesions develop[4–6]. MOG autoantibodies have been detected in association with disintegrating myelin in both human MS patients and in a marmoset model of EAE (Ref. 7). These observations support the notion that autoreactive antibodies have the potential to induce or exacerbate demyelinating disease.

## Antibodies against myelin can promote CNS remyelination

A direct demonstration that autoreactive antibodies can enhance endogenous myelin repair came from studies in this laboratory using Theiler's murine encephalomyelitis virus (TMEV) to induce chronic demyelinating disease in mice[8]. After intracerebral infection with TMEV, the virus replicates in the gray matter of the brain, resulting in acute encephalitis that is resolved in 14–21 days, and which is followed by chronic viral persistence in spinal cord white matter. Persistent TMEV infection eventually leads to chronic demyelination and progressive loss of motor function, a clinical pattern that is very similar to that observed for progressive MS in humans. Myelin pathology in TMEV-infected mice is immune-mediated, with chronically infected animals demonstrating a wide range of disease phenotypes, depending on their specific genetic background. In the SJL strain, demyelination is evident within 30 days after infection, and by 1–3 months the animals begin to develop neurological deficits, such as spasticity and gait abnormalities, weakness of the lower extremities and bladder incontinence. Paralysis eventually occurs by 6–9 months. Spontaneous repair of damaged myelin is common in many mouse strains, but is relatively limited in the SJL strain; often less than 10% of the total demyelinated lesion area is repaired. The relative absence of spontaneous repair makes this an excellent model for the study of strategies to promote endogenous remyelination.

Our initial observation of a beneficial humoral immune response occurred when chronic TMEV-infected mice were immunized with spinal cord homogenate (SCH) in incomplete Freund's adjuvant. Histological examination of spinal cord lesions from immunized animals revealed substantial CNS remyelination compared with control animals that had been treated with adjuvant alone. Passive transfer of antiserum[9] or purified immunoglobulin[10] from uninfected animals that had been immunized with SCH also enhanced remyelination, demonstrating a beneficial role for the humoral immune response against SCH in promoting myelin repair.

To further explore the nature of this beneficial immune response, hybridomas were generated from SJL mice following SCH immunization, in an attempt to identify monoclonal antibodies (mAbs) that promote remyelination. Two mouse mAbs that enhance remyelination were subsequently identified and designated SCH94.03 and SCH79.08, respectively. Both antibodies are polyreactive IgM antibodies and both bind to antigens expressed on the surface of oligodendrocytes, suggesting that the remyelination-promoting activity of these antibodies might involve direct stimulation of myelin-producing cells[11]. Subsequently, four additional oligodendrocyte-specific mouse IgM antibodies were characterized and shown to promote CNS remyelination[12].

We have used oligodendrocyte binding as a screening assay for the identification of candidate human mAbs that might promote remyelination and therefore have potential as therapeutic reagents[13]. As a source of human mAbs, we used serum-derived human monoclonal IgMs (sHIgM) and serum-derived human monoclonal IgGs (sHIgG) isolated from patients with monoclonal gammopathy, a relatively common condition characterized by high concentrations of monoclonal serum antibody. We tested

52 sHIgMs and identified six that bound to the surface of morphologically mature rat oligodendrocytes in culture, whereas none of the 50 sHIgGs bound. The oligodendrocyte-binding sHIgMs were tested *in vivo* and two of these (sHIgM22 and sHIgM46) were found to have remyelination-promoting activity similar to that observed with the mouse monoclonals.

Figure 1a shows a well-remyelinated lesion from an animal treated with a 1 mg intraperitoneal injection of a serum-derived human monoclonal IgM, and an unremyelinated lesion from the saline treatment group (Fig. 1b). Histological examination of spinal-cord sections five weeks after treatment with either sHIgM22 or sHIgM46 antibodies revealed significantly greater repair of the lesioned area (17.06% and 27.12%, respectively), compared with animals treated with saline (6.74%) (Ref. 13). In general, increases in remyelination of approximately 3–5-fold are observed after treatment with remyelination-promoting antibodies. We estimate that this represents at least 30 000–50 000 remyelinated axons in an antibody-treated animal.

We have tested approximately 40 different mAbs, of varied isotype and with varying binding specificities, for their remyelination-promoting potential in TMEV-infected mice, and we have never observed a significant increase in the total demyelinated-lesion area following antibody treatment. This observation demonstrates that antibodies with pathogenic potential are relatively uncommon for this model system. We have tested antibodies against MOG and galactocerebroside (GalC) that have previously been reported as pathogenic in other experimental systems[4,6,14,15], but have not observed significant increases in the extent of demyelinated lesions following five weeks of antibody treatment. One antibody against GalC, mAb O1, actually promotes repair in this system[12]. The reasons for these differences are unclear, but there could be some species- and model specificity to the observed effects of these antibodies. It is worth noting, however, that the patients from whom sHIgM22 and sHIgM46 were isolated have very high levels of oligodendrocyte-reactive antibodies in their serum, but have shown no signs of neurological disease.

None of the antibodies that induce remyelination react with Theiler's virus, and treatment with these antibodies does not decrease virus levels in infected animals. Therefore, virus neutralization does not explain the tissue repair observed following antibody treatment.

## Characteristic properties of remyelination-promoting antibodies

As outlined above, there are several properties that have been used to define certain autoantibodies as pathogenic agents. Similar criteria can be applied for the definition of autoreactive antibodies that are involved in tissue repair.

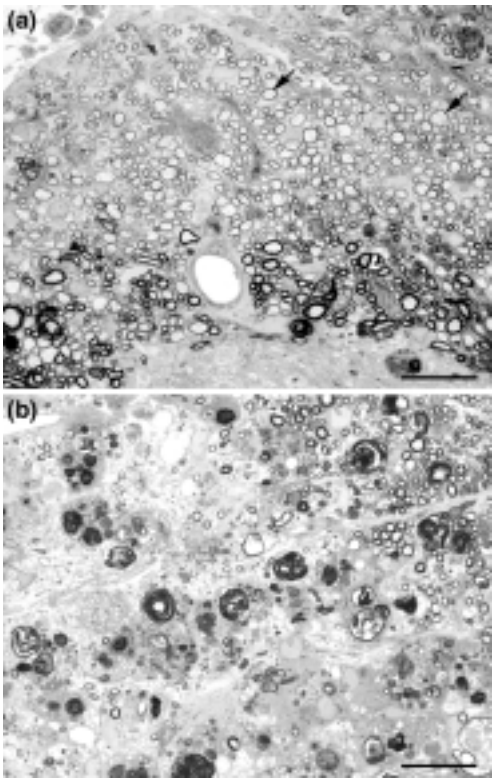


**Figure 1. Remyelination of Theiler's murine encephalomyelitis virus (TMEV)-induced lesions**

SJL mice with TMEV-induced demyelinating lesions were treated with either (a) the human monoclonal antibody sHIgM22 or (b) saline. Spinal cord cross-sections were stained for myelin with p-phenylenediamine. Remyelination of lesions is significant after five weeks of treatment with a remyelination-promoting antibody (a). Remyelinated axons (arrows) are identified by their relatively thin myelin sheaths compared with normal myelin. Thicker and darker-staining normal myelin sheaths are visible at the bottom of panel (a) and in the upper-right corner of panel (b). Animals treated with saline have many demyelinated lesions in the spinal cord; disintegrating myelin sheaths and myelin debris are clearly apparent (b). Scale bars, 25 μm.

Recognition of appropriate tissues or molecules is an important defining characteristic. All of the antibodies that promote remyelination bind to antigens on the surfaces of oligodendrocytes, suggesting that these antibodies might function through direct stimulation of the myelin-producing cells. However, these antibodies are highly polyreactive and recognize a variety of chemical haptens and proteins when binding is assayed by enzyme-linked immunosorbent assay. They also recognize intracellular proteins when used to stain a variety of permeabilized cells, but bind to a much more limited array of antigens on the surfaces of living oligodendrocytes. The oligodendrocyte surface antigens that are bound by several of these antibodies have been characterized, and are generally lipid or carbohydrate in nature rather than cell surface proteins[12,16]. Surface staining of a cultured oligodendrocyte with a human serum IgM is shown in Fig. 2(a).

Remyelination-promoting antibodies appear to be naturally occurring autoantibodies. Antibodies of this type are present in the serum of normal individuals and are often polyreactive IgM autoantibodies that are capable of binding to a variety of structurally unrelated, self- and non-self antigens[17]. It has been proposed that these antibodies represent a primordial form of the

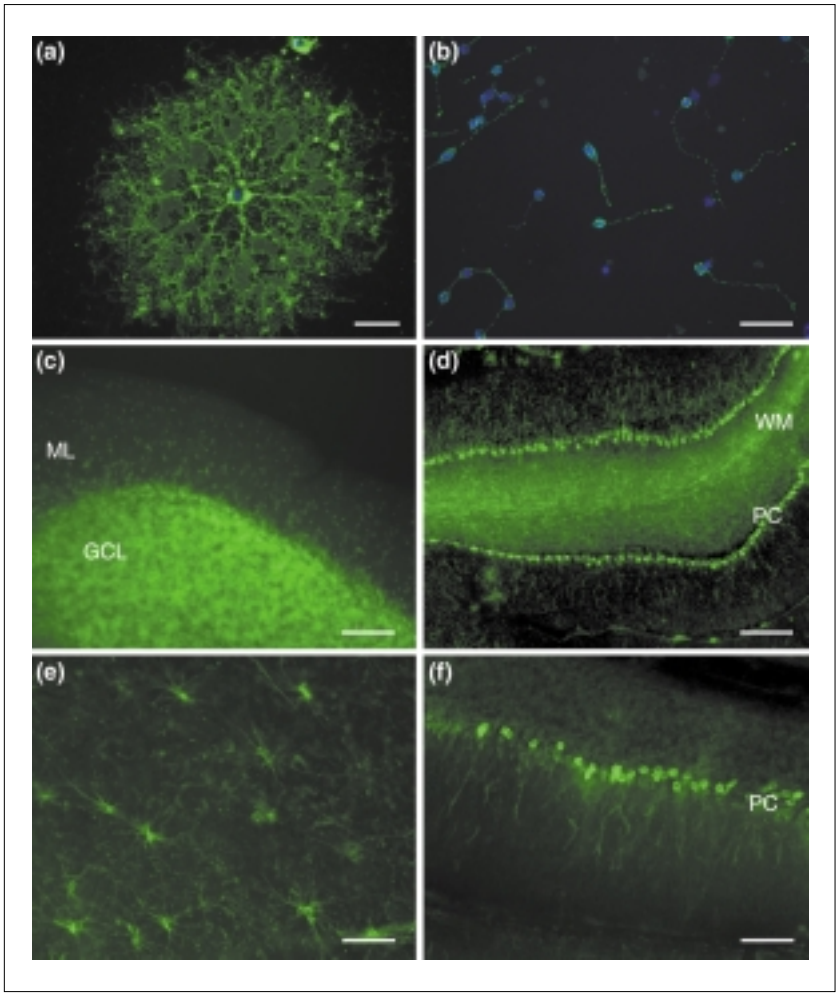


**Figure 2. Natural human serum IgMs are polyreactive with a variety of cell types within the central nervous system**

Slices of central nervous system tissue and cells were labeled with human antibodies before fixation or freezing to avoid artifacts. **(a)** sHIgM22 binds to the surface of oligodendrocytes obtained from the cortex of adult rats. **(b)** sHIgM42 binds to the surface of rat cerebellar granule cells after one day in culture. **(c)** Within a slice of adult SJL mouse cerebellum, AKJR4 binds strongly to the granule cell layer (GCL) and a population of basket cells within the molecular layer (ML). **(d)** sHIgM14 binds strongly to Purkinje cells (PC) and their arborizations as well as the central white matter (WM). **(e)** sHIgM32 labels the cytoskeleton of astrocytes at the surface of a slice of human cortical white matter. **(f)** In a slice of mouse cerebellum, MSI16 binds strongly to PC soma, less so to their dendritic arborizations, and does not bind to the central white matter. Scale bars are 20 μm in (a), 50 μm in (b), (e) and (f), 100 μm in (c), and 250 μm in (d).

immune system that might have performed largely physiological functions[18,19]. One of the functions of this system of antibodies could be to promote tissue repair. Whether or not this is the case, systemic administration of these antibodies to animals with myelin damage appears to have therapeutic value for enhancing repair.

The notion of an endogenous repair system is consistent with our initial observation of enhanced remyelination following immunization of experimental animals with spinal cord homogenate or individual myelin components[9]. Immunization might mimic the immune system exposure to CNS antigens that occurs after injury, resulting in an up-regulation of natural autoantibodies with CNS reactivity. We cannot say with certainty whether the specificity of the response evoked by immunization is the same as the endogenous response. However, the up-regulation of antibodies with reparative potential following immunization is clearly indicated by the fact that passive transfer of the immunoglobulin fraction from immunized animals promotes repair in the recipient[10]. In patients with MS, increases in myelin-reactive antibodies are frequently observed and might result as a reaction to exposure of CNS antigens following tissue damage.

The idea of a naturally occurring IgM-based system for tissue repair is supported by our recent observations that treatment with normal polyclonal human IgM effectively promotes remyelination, whereas the human IgG fraction is far less effective[13]. Our characterization of human mAbs from patients with monoclonal gammopathy revealed a high frequency of myelin-reactive IgM antibodies, further demonstrating that such antibodies are common in the serum of individuals with no history of neurological damage.

The binding of remyelination-promoting antibodies to the myelin-forming cells of the CNS, the presence of antibodies of this type in normal individuals, and their potential up-regulation in response to immunization with myelin or in patients with myelin damage, are all consistent with the presence of an endogenous antibody-based system of tissue repair.

## Mechanisms of autoantibody-mediated remyelination

All of the remyelination-promoting antibodies bind to oligodendrocytes or myelin, and it seems reasonable to suggest that this has a direct effect on the cells being recognized. Work in other laboratories has demonstrated that oligodendrocyte-specific antibodies can cause biochemical and morphological changes in these cells. Dyer and colleagues have shown that antibodies against oligodendrocyte surface epitopes, including antibodies to GalC, sulfatide and myelin/oligodendrocyte-specific protein (MOSP), can induce changes in the organization of oligodendrocyte membrane and cytoskeletal structure[20–22]. These changes in cellular structure were preceded by antibody-induced calcium influx[23–25]. The influx of calcium might therefore play an important role in the regulation of oligodendrocyte structure and function, and could conceivably play a role in antibody-induced remyelination. Recently, our laboratory has observed similar calcium fluxes in oligodendrocytes after treatment of mixed primary glial cultures with remyelination-promoting antibodies. There appears to be a high degree of correlation between the ability of an antibody to promote

remyelination and its ability to stimulate calcium influx, suggesting a connection between these two phenomena (Allan Bieber *et al.*, unpublished observations).

Myelin-reactive autoantibodies might also work to enhance myelin repair through more indirect mechanisms. Antibody binding to damaged oligodendrocytes and myelin might stimulate repair by enhancing the opsonization and clearance of myelin debris by macrophages. The remyelination-promoting antibodies are all of the IgM isotype, and one of the properties of IgM antibodies is their efficient activation of complement. Complement is an important factor for the efficient phagocytosis of myelin by cultured macrophages, and antibody-mediated activation of complement might function to fragment myelin debris, making removal more efficient[26]. Large numbers of macrophages are often observed in demyelinated lesions, and phagocytosis of myelin debris might be an important prerequisite to efficient remyelination.

## Prospects and implications

Demyelinated axons have reduced conduction velocities and are highly vulnerable to conduction block and transection. Remyelination is therefore an important therapeutic goal for the treatment of demyelinating disease. Human antibodies that promote remyelination in animal models are obvious candidates for development as reagents for the treatment of diseases such as MS.

Huang and colleagues[27] recently reported that mice pre-immunized with homologous spinal cord homogenate demonstrated enhanced axonal regeneration and functional recovery after dorsal hemisection of the spinal cord. Immunization resulted in increased levels of myelin-reactive antibodies that stimulated the outgrowth of neurites on myelin substrates. This observation was interpreted as the result of antibody-mediated blocking of myelin-associated inhibitors of axon outgrowth, an effect that might also explain the enhanced axonal regeneration and functional recovery seen *in vivo*. These experiments are reminiscent of our earlier studies in the TMEV model, in which immunization with SCH or the passive transfer of immune serum was followed by repair of damaged myelin. It is not clear whether antibodies that might block myelin-associated inhibition of axon outgrowth and those that promote remyelination have similar or completely unrelated specificities. Whichever is the case, myelin-reactive antibodies might be useful, not only to promote myelin repair following demyelinating disease, but also for the treatment of axonal damage following spinal cord injury. Such antibodies could be administered exogenously or generated *in vivo* by appropriate immunization strategies. Recent work with the IN-1 antibody, also an oligodendrocyte-reactive IgM, leads to very similar conclusions[28–30].

Many of the human mAbs that were screened for oligodendrocyte reactivity bound not only to oligodendrocytes but also to other CNS cells such as neurons. Figure 2 demonstrates the diversity of reactivity that was observed among monoclonal human IgM antibodies. Many antibodies react strongly with oligodendrocytes and CNS white matter, but many also reacted with various populations of neurons, and fewer reacted with astrocytes. The high frequency of IgMs that bind to neurons raises the possibility that these antibodies might play a role in neuronal survival and regeneration following CNS injury. Many of the neuron-reactive antibodies can serve as permissive substrates for neurite outgrowth in culture, demonstrating the potential for this type of function *in vivo* and for their use in the treatment of axonal damage (Allan Bieber *et al.*, unpublished observations).

Currently, there are few effective therapies to promote tissue repair or to prevent or reverse neurological deficits following CNS injury or disease. The characterization of endogenous immune-mediated repair mechanisms is therefore of obvious importance. An understanding of these mechanisms should open up significant new areas for the development of antibody-based therapeutics and perhaps also for small-molecule-based therapeutics and vaccines for induction of the reparative response.

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