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B Cells and Autoantibodies in the Pathogenesis of Multiple Sclerosis and Related Inflammatory Demyelinating Diseases

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The mainstream view is that MS is caused by an autoimmune attack of the CNS myelin by myelin-specific CD4 T cells, and this perspective is supported by extensive work in the experimental autoimmune encephalomyelitis (EAE) model of MS as well as immunological and genetic studies in humans. However, it is important to keep in mind that other cell populations of the immune system are also essential in the complex series of events leading to MS, as exemplified by the profound clinical efficacy of B cell depletion with Rituximab. This review discusses the mechanisms by which B cells contribute to the pathogenesis of MS and dissects their role as antigen-presenting cells (APCs) to T cells with matching antigen specificity, the production of proinflammatory cytokines and chemokines, as well as the secretion of autoantibodies that target structures on the myelin sheath and the axon. Mechanistic dissection of the interplay between T cells and B cells in MS may permit the development of B cell based therapies that do not require depletion of this important cell population.

1. MS AND RELATED INFLAMMATORY DEMYELINATING CNS DISEASES

Multiple sclerosis (MS) is the most common neurological disease in young adults, affecting over 250,000 individuals in the United States and up to 1.2 million worldwide. It is believed to result from an autoimmune attack on protein components of myelin, the insulation which allows for rapid conductance of electrical signals along axons. MS is characterized by discrete regions of central nervous system (CNS) inflammation, lymphocyte infiltration, demyelination, axonal damage, and ultimately the death of myelin-producing oligodendrocytes. Depending on the localization of these plaques, MS patients suffer from a wide variety of symptoms, including weakness, sensory disturbances, ataxia, and visual impairment. Magnetic resonance imaging (MRI) allows visualization of active lesions in the absence of clinical symptoms, and has become a valuable tool for both diagnosis and monitoring of disease activity. A diagnosis of MS requires multiple episodes of demyelination separated in space and time (Poser *et al.*, 1983), which may be supported by clinical evidence of multiple demyelinating events or the presence of multiple lesions of different age on MRI (McDonald *et al.*, 2001). On the basis of the temporal pattern of demyelinating events and accumulation of permanent disability, MS is classified into

relapsing-remitting (RR), secondary progressive (SP), and primary progressive (PP) types (Hauser and Oksenberg, 2006; Noseworthy *et al.*, 2000). About 80–85% of MS patients initially experience a relapsing-remitting course (RRMS) and the intervals between and duration of relapses are highly variable, not necessarily correlating with the presence of lesions by MRI because most lesions are clinically silent (Goodin, 2006). Over time, many RRMS patients develop a progressive worsening of symptoms between relapses, known as SPMS. Cumulative axonal loss may be an important contributor to the progressive decline in neurological function (Hauser and Oksenberg, 2006; Sospedra and Martin, 2005; Trapp *et al.*, 1998). Within 15 years of diagnosis, 50–60% of RRMS patients cannot walk unassisted, and 70% are limited in performing activities central to daily life (Hauser and Oksenberg, 2006). The remaining 15–20% of patients experience a progressive pattern of disease (PPMS), characterized by a gradual accumulation of symptoms and concurrent decline in function. The progression of disability occurs despite a lower frequency of active (gadolinium-enhancing) lesions by MRI relative to RRMS (Thompson *et al.*, 1997).

MS is a complex disease, with contributions from multiple genetic and environmental factors. The frequency of MS varies between ethnic populations (Rosati, 2001), and descendents of Northern Europeans have an increased prevalence relative to African, Asian, and Native American populations. The relative risk of developing MS is significantly higher for individuals with an affected first- or second-degree relative (Chataway *et al.*, 1998; Hauser and Oksenberg, 2006). The MHC locus on human chromosome 6 shows the strongest linkage to disease susceptibility, in particular the MHC class II region and a specific MHC class II haplotype (DRB1*1501-DQB1*0602) (Haines *et al.*, 1996; Sawcer *et al.*, 1996). Recent genome-wide studies have also identified single nucleotide polymorphisms in the IL-2 receptor alpha and IL-7 receptor alpha chains (The International Multiple Sclerosis Genetics Consortium, 2007), again indicating that genetic variations of immune response genes contribute to MS susceptibility. Women are affected by MS twice as frequently as men, which may be due to genetic or hormonal factors (Czlonkowska *et al.*, 2005).

A large body of work in the experimental autoimmune encephalomyelitis (EAE) model of MS indicates that CD4 T cells specific for myelin antigens are essential for the development of inflammatory, demyelinating CNS lesions. EAE can be induced by immunization with myelin proteins or peptides in complete Freund's adjuvant (CFA) or by transfer of myelin-specific T cell clones (Stromnes and Goverman, 2006a,b). Linkage of disease susceptibility to the MHC class II locus in MS indicates that peptide presentation to CD4 T cells is also important in the human disease. Even though disease can be transferred with purified T cells with a single antigen specificity, it is important to keep in mind that other cell populations of the immune system also play an essential role, as exemplified by the clinical trials with Rituximab which depletes B cells. The function of B cells in the chronic inflammatory process in MS will be discussed in detail.

1.1. Related demyelinating CNS diseases

Neuromyelitis optica (NMO), acute disseminated encephalomyelitis (ADEM), and clinically isolated demyelinating syndromes (CIS) are part of the spectrum of inflammatory

demyelinating CNS diseases. CIS is an isolated demyelinating event, and 50–95% of CIS cases are diagnosed with MS following a second demyelinating episode (Miller, 2004). Initiation of therapy with interferon beta at the time of CIS has been shown to significantly prevent or delay progression to MS, especially in patients with multiple MRI lesions who may be at higher risk (Kappos *et al.*, 2007; Miller, 2004; Thrower, 2007).

NMO (also known as Devic's disease) primarily affects the optic nerve and spinal cord, leading to the defining symptoms of optic neuritis and transverse myelitis (Wingerchuk *et al.*, 2006). Longitudinally extensive spinal cord lesions are commonly found in NMO, although clinically silent brain lesions may also be seen on MRI. NMO is found in populations with a low incidence of MS, and affects women up to nine times more often than men. The majority of cases follow a relapsing course with incomplete recovery between episodes, but NMO has a worse long-term prognosis than does MS. More than 50% of patients are blind in at least one eye or cannot walk unassisted five years following diagnosis (Wingerchuk *et al.*, 2007). The recent discovery of a specific autoantibody in approximately 75% of NMO patients has aided in diagnosis and provided a therapeutic target, as discussed later in this chapter.

ADEM, as the name implies, is an acute and often rapidly progressing demyelinating condition and is more common in children than adults (Tenenbaum *et al.*, 2007). Patients with ADEM typically have a variety of neurological symptoms and diagnosis requires the presence of encephalopathy, defined as a change in behavior or consciousness (Krupp *et al.*, 2007; Tenenbaum *et al.*, 2007). ADEM often occurs within 4 weeks of infection or vaccination, although a causative relationship with infection can be difficult to establish. While the majority of ADEM patients experience a monophasic disease course with partial or complete recovery, some have a recurrence of the same symptoms and lesion location more than 3 months following the initial event (recurrent ADEM) or experience new lesions and symptoms (multiphasic ADEM) (Krupp *et al.*, 2007). A minority of individuals with ADEM can later develop MS, although the reported frequency of this occurrence is highly variable (0–28%) (Dale *et al.*, 2000; Leake *et al.*, 2004; Mikaeloff *et al.*, 2004, 2007; Tenenbaum *et al.*, 2002), requires many years of follow-up, and is dependent on the criteria used to define MS. For instance, Poser's criteria (Poser *et al.*, 1983) would consider multiphasic ADEM as MS, as both are a recurring demyelinating events separated in space and time. The overlapping symptoms and lack of specific biomarkers can cause difficulties in distinguishing between MS and ADEM, especially in pediatric populations (Belman *et al.*, 2007; Krupp *et al.*, 2007; Wingerchuk, 2003).

2. THERAPEUTIC DEPLETION OF B CELLS IN MS WITH RITUXIMAB

Recent clinical trials with Rituximab have shown that B cells play an important role in the pathogenesis of MS. Rituximab (marketed by Genentech as Rituxan®) is a monoclonal antibody directed against CD20, a transmembrane protein expressed on the surface of B cells, but absent from fully differentiated plasma cells (Sabahi and Anolik, 2006). Administration of this antibody rapidly depletes CD20-expressing cells from the circulation via complement-mediated lysis and cell-mediated cytotoxicity (Reff *et al.*, 1994), and B cell numbers remain low for 3–12 months following treatment. Rituximab has been in use as a

treatment for non-Hodgkin's B cell lymphomas since FDA approval in 1997 and has shown an excellent safety profile (Rastetter *et al.*, 2004). Most adverse events are related to the antibody infusion process, while recurrent infections occur at a similar frequency as in placebo control groups and opportunistic infections are rare. The preservation of antibody secretion by plasma cells may explain the low incidence of infection.

The efficacy and safety profile of Rituximab prompted its clinical testing in complex antibody-associated autoimmune diseases, initially rheumatoid arthritis (RA) (Edwards *et al.*, 2004). A phase III trial reported the combined use of methotrexate and Rituximab in patients with established RA who had previously failed tumor necrosis factor (TNF)-directed therapies (Cohen *et al.*, 2006). Treatment with Rituximab resulted in a significant reduction of disease severity compared to methotrexate alone, while patients in the control group experienced a worsening of RA symptoms. The response was not associated with a reduction in rheumatoid factor antibodies, and the mean serum levels of IgM, IgG, and IgA generally stayed within normal limits through the trial period. A follow-up study demonstrated the safety of subsequent infusions following B cell repopulation, with no increase in the infection rate (Keystone *et al.*, 2007). On the basis of these results, Rituximab was approved in 2006 for use in RA patients who do not respond to TNF-antagonists (Sabahi and Anolik, 2006). Rituximab has also shown benefit in several other autoimmune diseases, including autoantibody-associated neuropathies, immune thrombocytopenia and systemic lupus erythematosus (Edwards and Cambridge, 2006; Renaud *et al.*, 2003, 2006; Sabahi and Anolik, 2006; Tanaka *et al.*, 2007).

A recent phase II randomized, placebo-controlled multicenter trial of Rituximab in RRMS showed substantial clinical benefit (Hauser *et al.*, 2008). Following the single infusion of antibody, depletion of B cells was rapid and persisted for greater than 24 weeks. The total number of lesions and number of new lesions were dramatically reduced in the treatment compared to control group, with 89.4% of patients in the treatment group experiencing one or fewer total lesions over the course of the trial. About 84.8% of patients receiving Rituximab had no new lesions, and although highly variable, the volume of lesions was decreased in the treatment group but increased in controls. The decrease in lesion activity was accompanied by a reduction in clinical relapses. The infection rate was similar in both groups, and no significant opportunistic infections were observed. These promising results are certain to be followed up with a large phase III trial to address safety and long-term efficacy issues. Smaller preceding trials support these findings. A report of one RRMS patient receiving two doses of Rituximab showed effective depletion of B cells for six months in both peripheral blood and CSF, and a complete absence of relapses and new lesions for nine months (Stuve *et al.*, 2005). A small trial in PPMS demonstrated rapid and effective peripheral B cell depletion, but an overall increase in CSF B cell frequencies after 2–18 months (Monson *et al.*, 2005). The majority of these cells were plasma cells, which are not targeted by Rituximab. In a larger study using Rituximab as an add-on therapy for RRMS patients with suboptimal responses to current treatment, B and T cell numbers were reduced in CSF following treatment, but no significant changes were observed in the number of oligoclonal bands, CSF IgG concentration or disability scores (Cross *et al.*, 2006). No serious complications or infections were observed in any of these trials.

Rituximab may also be useful for the treatment of other inflammatory demyelinating CNS diseases. Treatment with Rituximab led to significant neurological improvement in seven of eight NMO patients, including recovery of walking ability and regain of bladder and bowel control (Cree *et al.*, 2005), although the possibility of spontaneous recovery was not addressed with a control group in this open label trial.

3. WHICH B CELL FUNCTIONS ARE CRITICAL IN THE PATHOGENESIS OF MS?

It is obvious why Rituximab is an effective treatment for lymphoma – it eliminates the transformed cells. It is more difficult to dissect why treatment with Rituximab has such a profound effect on MS. The contribution of B cells may go beyond antibody production – B cells are potent APCs and the B cell – T cell interaction shapes the ensuing T cell response through expression of costimulatory molecules as well as production of cytokines and chemokines.

3.1. B cells as antigen presenting cells

Unlike other professional APCs, B cells have the unique capability to efficiently capture even minute amounts of antigen through the B cell receptor (BCR) (Fig. 4.1). B cells are thus the most efficient APCs for T cells with the same antigen specificity (Lanzavecchia, 1985). Antigen presentation by B cells is closely linked to activation. When crosslinked by a bound protein, the BCR signaling subunits (Ig- α and Ig- β) trigger receptor ubiquitination, internalization and targeting to an endocytic compartment optimized for peptide loading onto class II MHC (Drake *et al.*, 2006; West *et al.*, 1994). There, the BCR-associated protein antigen is degraded into peptides for presentation by MHC class II molecules. Intracellular trafficking of MHC class II molecules is also modulated by BCR ligation to promote maximal loading of antigen-derived peptides, even when present in low abundance (Vascotto *et al.*, 2007). Activation also induces recruitment of HLA-DM to this compartment (Lankar *et al.*, 2002) which catalyzes the exchange of low affinity MHC-associated peptides with higher affinity peptides; these complexes are subsequently transported to the cell surface for recognition by CD4 T cells. Costimulatory molecules including CD80, CD86, and ICOS ligand are also upregulated on the B cell surface following activation (Cambier *et al.*, 1994; Shilling *et al.*, 2006), and promote T cell activation upon peptide–MHC binding. Antigen-specific B and T cells form long-lasting immune synapses characterized by accumulation of peptide–MHC complexes at the site of cell-cell contact (Gordy *et al.*, 2004). The coordination of antigen uptake, class II loading, and costimulatory molecule expression allows antigen-specific B cells to activate cognate T cells far more efficiently than nonspecific B cells or monocytes (Lanzavecchia, 1985), and antigen-specific B cells are required to prime CD4 T cells with low doses of antigen (Rivera *et al.*, 2001). In addition to directly presenting antigens to autoreactive T cells, live B cells can also transfer antigen to macrophages by cell–cell contact, but the precise mechanism of this transfer is unknown (Harvey *et al.*, 2007; Townsend and Goodnow, 1998).

In the context of autoimmunity, which can be dominated by low-affinity T cells and limiting amounts of antigen, the potent APC function of B cells may be harmful. The

immunodominant antibody epitope of myelin basic protein (MBP) colocalizes with a major T cell epitope of the antigen (Wucherpfennig *et al.*, 1997), and binding to the BCR may thus protect the immunodominant epitope of MBP from degradation and promote its presentation to T cells. Similarly, the major epitope recognized by high-affinity insulin-specific autoantibodies is located in the same segment of the target antigen as the T cell epitope. The presence of autoantibodies with this specificity is highly predictive of later development of type 1 diabetes (Achenbach *et al.*, 2004; Kent *et al.*, 2005).

In an immune response to a pathogen, B cells are activated in a lymph node by antigen binding to the BCR, migrate to follicles, and continue to undergo antigen-driven maturation by forming germinal centers (MacLennan, 1994). Follicular dendritic cells display native proteins in the form of immune complexes to B cells, which take up the antigen through the BCR, process it and present antigen-derived peptides to CD4 T cells (McHeyzer-Williams *et al.*, 2006). In turn, T cells recognizing peptide-MHC complexes on the B cell become activated, upregulate expression of stimulatory molecules including CD40 ligand (CD154), and secrete cytokines, providing antigen-dependent survival and differentiation signals to the B cell. After acquiring T cell help, B cells rapidly undergo several rounds of proliferation in the germinal center, during which time the immunoglobulin variable sequences are mutated (Klein and Dalla-Favera, 2008; MacLennan, 1994). The resulting daughter cells once again sample antigens on follicular dendritic cells (FDCs), present peptides to T cells, and obtain survival signals.

Because the germinal center reaction is highly competitive, B cells containing rearranged receptors with lower affinity for antigen do not receive the required signals from T cells and undergo death by apoptosis. Cells with higher affinity for antigen proliferate and leave the follicle as memory B cells or plasmablasts, the precursors of long-lived plasma cells. Although capable of establishing residence in the bone marrow, newly generated plasma cells can also traffic to sites of inflammation by chemokine and integrin dependent homing (Kunkel and Butcher, 2003). The process of immunoglobulin diversification in germinal centers can give rise to self-reactive B cells, which should undergo apoptosis in the absence of cognate T cell interactions. However, the mechanisms of central and peripheral T cell tolerance are not perfect, and cells which escape can promote the survival and maturation of autoreactive B cells. The control of self-reactive B and T cell activation are closely linked in the germinal center reaction, making it an attractive target for treatment of autoimmune diseases.

There is substantial evidence for such antigen-driven B cell responses in MS. Structures similar to lymph node B cell follicles, termed tertiary lymphoid organs, develop in the target organs of inflammatory autoimmune diseases and are induced by a positive feedback loop of tissue chemokine expression, lymphocyte recruitment and activation, and cytokine production (Aloisi and Pujol-Borrell, 2006). Follicles containing B cells and FDCs can be found in the meninges of patients with secondary-progressive MS, and their presence is associated with a younger age of onset and more severe pathology (Magliozzi *et al.*, 2007; Serafini *et al.*, 2004). Similar structures have also been found in mice with progressive relapsing EAE (Magliozzi *et al.*, 2004). The formation of these ectopic follicles indicates that B cells migrate to the brain, are activated locally and present antigens, and differentiate

into memory B cells or plasmablasts within the CNS, rather than being activated in the periphery and homing to the CNS in a fully mature state. The establishment of germinal centers in the brain may reflect differences in the immune response in RRMS and SPMS (Lyons *et al.*, 1999; Magliozzi *et al.*, 2007).

An important role for antigen presentation by B cells has also been demonstrated in mouse models of autoimmunity. Mice with a mutated immunoglobulin heavy chain (mIgM mice) express membrane-bound BCR but cannot secrete antibodies. When bred onto autoimmune-prone backgrounds such as NOD or MRL/lpr, mIgM mice developed type 1 diabetes and lupus nephritis, respectively (Chan *et al.*, 1999a; Wong *et al.*, 2004), while B cell deficient mice are resistant to the development of these autoimmune diseases (Chan and Shlomchik, 1998; Chan *et al.*, 1999b; Serreze *et al.*, 1996).

A strong B cell dependence has also been found in particular EAE models in which myelin oligodendrocyte glycoprotein (MOG) is used as the target antigen. These studies showed that B cells are required for the development of severe EAE following immunization with whole MOG protein, but are dispensable following MOG peptide immunization (Hjelmstrom *et al.*, 1998; Svensson *et al.*, 2002). The models involving immunization with whole proteins may be more relevant to the human disease because APCs in MS lesions phagocytose large fragments of myelin containing intact proteins. The differences between protein and peptide immunization may at least in part be due to the dose of the immunogen: smaller molar quantities are typically administered when intact proteins are utilized and the efficient antigen presentation function of B cells may then be critical. Furthermore, T cell–B cell collaboration can result in the production of conformation-sensitive antibodies which can induce demyelination. In contrast, peptide immunization only induces antibodies against this linear epitope and such antibodies do not bind to native MOG (von Budingen *et al.*, 2004).

The synergistic action of myelin-specific T cells and B cells has also been highlighted by recently developed spontaneous models of CNS autoimmunity. Mice that only express a transgenic MOG-specific TCR develop isolated optic neuritis in the absence of classical EAE symptoms (Bettelli *et al.*, 2003). Breeding of these TCR transgenic mice with an anti-MOG heavy chain knock-in results in a high frequency of MOG-specific T cells and B cells and an aggressive autoimmune disease in which lesions are largely confined to the optic nerves and spinal cord, as seen in NMO (Bettelli *et al.*, 2006; Krishnamoorthy *et al.*, 2006).

3.2. B cells as a source of cytokines

The costimulatory molecules and cytokines present during antigen recognition by a naive T cell determine whether that cell becomes activated and what effector phenotype it acquires. As shown in Figure 4.2, activated B cells can be induced to produce a variety of immunomodulatory cytokines and growth factors (Pistoia, 1997). B cells can differentiate into effector subsets with distinct cytokine profiles, both *in vitro* and *in vivo*. Production of type I cytokines, including interferon-gamma (IFN- γ) and IL-12, induces T cells to adopt a Th1 phenotype characterized by expression of IFN- γ . This type of response is optimized for the clearance of intracellular pathogens and leads to activation of cytotoxic CD8 T cells, NK cells, and production of complement-fixing antibody isotypes, which can all contribute to

tissue damage in autoimmune diseases. TNF α production by B cells can also amplify Th1 differentiation and IFN γ production by T cells (Menard *et al.*, 2007). B cell derived IL-6, in combination with TGF β , promotes T cell differentiation into highly pathogenic Th17 cells, which secrete high levels of IL-17 and other proinflammatory cytokines (Bettelli *et al.*, 2007). Secretion of type 2 cytokines by B cells is associated with development of Th2 cells, which modulate inflammation by production of cytokines that promote the clearance of extracellular pathogens (Harris *et al.*, 2000). Although characteristic of allergies, Th2 responses are protective in MS and animal models, and are promoted by approved MS therapies, including interferon β and glatiramer acetate (Sospedra and Martin, 2005).

B cell-derived cytokines are also critical for the establishment of germinal centers in lymph nodes and inflamed tissues. In mice, expression of lymphotoxin (LT) and TNF by B cells is required for the maturation of follicular dendritic cells and organization of germinal centers in the spleen (Endres *et al.*, 1999; Fu *et al.*, 1998; Gonzalez *et al.*, 1998). The induction of lymphoid follicles in the gut and at sites of inflammation is also dependent on provision of LT by B cells (Gommerman and Browning, 2003; Lorenz *et al.*, 2003). Ectopic germinal centers are formed in the CNS of MS patients, and both TNF and LT have been detected in lesions (Selmaj *et al.*, 1991). Increased levels of serum TNF and LT have also been detected in MS patients, although other studies have not found a difference between MS and controls (Ledeen and Chakraborty, 1998). Monoclonal antibody inhibitors of TNF are approved therapies for RA, but MS patients receiving treatment had an increased attack rate relative to controls, and a clinical trial was discontinued prematurely due to this negative outcome (The Lenercept Multiple Sclerosis Study Group, 1999).

The context of activation plays an important role in determining the cytokine profile of a B cell: activation through the BCR with T cell help (CD40 ligation) leads to production of proinflammatory cytokines, while ligation of CD40 in the absence of specific BCR activation promotes secretion of the immunosuppressive cytokine IL-10 (Duddy *et al.*, 2004). IL-10 has potent antiinflammatory properties and can inhibit antigen presentation to CD4 T cells, indirectly downregulating their activation, cytokine production, and proliferation (Roncarolo *et al.*, 2006). Production of IL-10 by B cells is essential for recovery from MOG peptide-induced EAE (Fillatreau *et al.*, 2002), and overexpression of IL-10 protects mice from EAE (Cua *et al.*, 1999). B cells from MS patients produce less IL-10 than B cells from healthy individuals, but high levels of IL-10 and TGF- β are produced by newly generated B cells following Rituximab treatment (Duddy *et al.*, 2007).

The antigen-driven B cell maturation process in the MS brain is made possible by local changes in the immune environment. Expression of the lymphocyte adhesion molecule LFA-1 is increased in active MS plaques relative to noninflammatory neurological diseases (Cannella and Raine, 1995), signifying the active migration of cells into the plaque. Under normal conditions, astrocytes maintain an immunosuppressive cytokine environment in the CNS, with detectable levels of IL-10 and TGF β but no TNF (Hickey, 2001). In MS, the balance of cytokines is tipped towards inflammation rather than tolerance, with increased levels of IL-1, IL-2, TNF, and IFN γ (Cannella and Raine, 1995). Normally secreted at low levels by astrocytes, the potent B cell activating factor BAFF is upregulated in MS, to levels comparable to secondary lymphoid tissues (Krumbholz *et al.*, 2005). Autoreactive B cells

compete poorly with non-autoreactive cells for BAFF-induced survival signals, and mice engineered to overexpress BAFF develop B cell-associated autoimmunity (Lesley *et al.*, 2004). Regulation of local BAFF concentration can be an effective method of preventing autoimmunity, and loss of this control contributes to autoreactive B cell proliferation and survival in the brains of MS patients.

3.3. Autoantibody production by B cells

B cells are unique in their ability to produce antibodies, which circulate throughout the body and target specific antigen-bearing targets for clearance. Antibodies to self proteins are directly responsible for the pathology in both systemic and organ-specific autoimmune diseases, and have multiple mechanisms of causing tissue damage. In some cases, autoantibodies are directed to a cell-surface receptor, such as the acetylcholine receptor in myasthenia gravis or the thyroid stimulating hormone receptor in Grave's disease (Kohn and Harii, 2003; Vincent, 2002). Antibodies can also form immune complexes with circulating autoantigens, which accumulate in the kidneys and joint synovium in patients with SLE and RA (Davidson and Aranow, 2006; Dorner *et al.*, 2004; Rahman and Isenberg, 2008; Singh, 2005). Other autoantibodies bind to circulating cells such as platelets or erythrocytes, triggering complement-mediated lysis or phagocytosis by Fc-receptor bearing cells (Elson and Barker, 2000). Much effort has been placed on determining the specificities of antibodies found in the lesions, serum, and CSF of patients with MS and related demyelinating diseases, and a wide variety of antibody targets on myelin, axons, and astrocytes have been studied (Fig. 4.3).

A common finding in MS patients is the intrathecal synthesis of IgG: oligoclonal IgG bands are detectable in more than 95% of MS patients and increased CSF concentrations of IgG relative to albumin are found in up to 70% of cases (Link and Huang, 2006). The oligoclonal nature of these antibodies suggests an antigen-driven process, and determining the specificities of such antibodies could thus provide insights into the causes of MS. Several candidate autoantigens have been proposed as targets, as well as antigens from infectious agents, but there is currently no agreement on the specificities or significance of oligoclonal bands in MS, although they are the only immunological feature used to support the diagnosis (Cepok *et al.*, 2005; Correale and de los Milagros Bassani Molinas, 2002; Franciotta *et al.*, 2005).

Clonally expanded B cells have been detected by single-cell PCR in the cerebrospinal fluid and lesions of patients with MS and optic neuritis, and one study found expansion of MBP-specific B cells in the CSF (Lambracht-Washington *et al.*, 2007). The immunoglobulin gene rearrangements found in CSF B cells are rarely observed in the periphery (Colombo *et al.*, 2000), and memory B cells and plasma cells with similar rearrangements have been detected in the same patient (Haubold *et al.*, 2004). These cells carry mutations typical of germinal center-derived memory B cells (Harp *et al.*, 2007), with overrepresentation of specific immunoglobulin variable regions, especially V_H4 (Baranzini *et al.*, 1999; Owens *et al.*, 1998). The variable region sequences of MS lesion-resident B cells are mutated to a similar extent as those in B cells undergoing an antigen-driven response to CNS measles virus

infection (Smith-Jensen *et al.*, 2000). Together, these findings provide support for a local antigen-dependent B cell maturation process in the CNS of MS patients.

Histological analysis of MS tissue has demonstrated deposition of antibodies and complement on the myelin sheath of a substantial fraction of lesions, but also indicated significant heterogeneity in the composition of demyelinating lesions. Lucchinetti and colleagues have classified MS lesion biopsies into four categories based on the observed inflammatory and neurodegenerative characteristics (Lassmann *et al.*, 2001; Lucchinetti *et al.*, 2000). The first two patterns are characterized by demarcated regions of infiltrating T cells and activated macrophages, centered around small veins. Areas of demyelination, with a simultaneous loss of multiple myelin proteins including MBP, MOG, proteolipid protein (PLP), and myelin-associated glycoprotein (MAG), and remyelination were observed in both patterns. The presence (pattern II) or absence (pattern I) of antibody and complement deposition distinguishes these two types of lesions, suggestive of different levels of B cell involvement. Infiltrating T cells and macrophages were also found in pattern III and IV lesions, but other features pointed to an oligodendrocyte defect with a preferential loss of MAG (pattern III) or nearly complete loss of oligodendrocytes (pattern IV) and complete lack of remyelination. Although different between patients, the patterns observed in multiple lesions from the same patient were always identical. Pattern II lesions were the most common, observed in 115 areas from 16/27 patients and including all clinical subtypes of MS. Pattern III lesions were primarily found in acute disease, with a duration of less than 2 months before biopsy, and although rare, pattern IV was only found in primary-progressive MS. The IgG found in pattern II lesions may directly contribute to disease progression, as patients with pattern II lesions, but not pattern I or III, respond to therapeutic plasma exchange (Keegan *et al.*, 2005). Development of novel methods for determining the composition of lesions may enable classification of MS based on the mechanism of pathogenesis rather than clinical presentation and facilitate individualized treatments.

Antibodies specific for myelin (Genain *et al.*, 1999; O'Connor *et al.*, 2005; Warren and Catz, 1993) and axonal proteins (Mathey *et al.*, 2007; Zhang *et al.*, 2005) have been eluted from MS lesions. The terminal complement complex C9neo is also found in active lesions containing IgG (Prineas and Graham, 1981; Storch *et al.*, 1998). Histological analysis of lesions has also shown active phagocytosis of antibody-decorated myelin by macrophages and opsonized myelin fragments have been detected within endocytic vesicles (Prineas and Graham, 1981; Storch *et al.*, 1998). Although IgG-containing lesions can be found in all types of MS, different antigens may be targeted in different subtypes.

MOG has been extensively studied as a target for autoantibodies in MS because it is selectively expressed by oligodendrocytes in the CNS. Unlike intracellular antigens such as MBP and PLP, MOG is localized to the surface of oligodendrocytes and the myelin sheath and therefore available for antibody binding in the absence of prior tissue damage (Brunner *et al.*, 1989). MOG was originally discovered by affinity purification with the well-characterized 8–18C5 antibody (Linington *et al.*, 1984), which recognizes a conformational epitope and can exacerbate demyelination when administered to animals with mild EAE (Breithaupt *et al.*, 2003; Linington *et al.*, 1988). Administration of antibodies to MOG to a healthy mouse cannot cause disease, as the blood–brain barrier must be compromised to

allow access to the target antigen. The conformation-specific antibodies produced following immunization with refolded MOG protein can directly contribute to demyelination in both rodents and nonhuman primates and also exacerbate EAE by passive transfer (Genain *et al.*, 1995; Lyons *et al.*, 2002; von Budingen *et al.*, 2002). However, antibodies to linear epitopes or peptides cannot bind MOG on the cell surface and are not pathogenic (Brehm *et al.*, 1999; von Budingen *et al.*, 2004).

The ability of conformation-sensitive antibodies to exacerbate demyelination in animal models has made MOG an attractive candidate antigen for autoantibody-mediated injury in MS. A provocative report that antibodies to MOG are present in patients with CIS and correlate with an increased risk of progression to MS (Berger *et al.*, 2003) has not been reproduced by several groups, though antibodies may be associated with a higher MRI lesion load (Kuhle *et al.*, 2007; Lim *et al.*, 2005; Pelayo *et al.*, 2007). Increased frequencies of antibodies to MOG have been reported in the serum and CSF of MS patients relative to controls (Kennel De March *et al.*, 2003), but other studies have found similar levels of anti-MOG in MS and other neurological diseases or controls (Brokstad *et al.*, 1994; Lampasona *et al.*, 2004; Mantegazza *et al.*, 2004; Markovic *et al.*, 2003; O'Connor *et al.*, 2007; Reindl *et al.*, 1999). Although detectable in some cases, the reported frequency of anti-MOG in serum and CSF from MS patients varies considerably between studies. This variability is primarily attributable to differences in the assay methodologies and antigens utilized. Antibodies that bind to native MOG are most relevant to the disease, but are not detectable with all assays. Many of these studies used Western blots, which only detect antibodies to denatured antigen, or ELISAs, which cannot distinguish between responses to folded and unfolded proteins (Mathey *et al.*, 2004). Furthermore, the results obtained with Western blots and ELISAs do not necessarily correlate, even with the same sera (Pittock *et al.*, 2007). New methods have therefore been developed that enable specific detection of antibodies to properly folded and glycosylated MOG protein. The first method involves labeling of MOG transfectants with serum or CSF antibodies (Brehm *et al.*, 1999; Lalive *et al.*, 2006; O'Connor *et al.*, 2007; Zhou *et al.*, 2006), but the frequency of anti-MOG in MS still varies considerably between these studies, suggesting heterogeneity in the studied patient populations. The second method is a sensitive radioimmunoassay with a tetrameric version of folded and glycosylated MOG protein. The tetrameric nature of the antigen enables bivalent binding by IgG antibodies and thus increases the avidity of the interaction. With this assay, MOG antibodies were detected in nearly 20% of ADEM patients but only infrequently in adult-onset MS patients (O'Connor *et al.*, 2007). These results suggest that antibodies to MOG are more prevalent in ADEM than in adult-onset MS.

Antibodies to other myelin components including MBP, PLP, MAG, and cyclic nucleotide phosphodiesterase (CNP) have each been detected in some studies of MS serum or CSF (Hafler *et al.*, 2005). The frequency of these antibodies in MS and controls, like anti-MOG, varies considerably between studies, and none are useful as a diagnostic or prognostic marker. Furthermore, the contribution of antibodies to intracellular proteins such as MBP to the initiation of myelin damage is likely to be minimal, as they are only exposed once the demyelinating process has begun, but MBP-specific B cells may nevertheless be relevant as APC. The myelin sheath contains a variety of lipids, some of which may also be recognized

by autoreactive B cells. Using arrays of 50 unique brain and microbial lipids, Kanter *et al.* have defined an antibody response to sulfatide in MS CSF, and addition of sulfatide to a myelin peptide immunization worsens the course of EAE in mice (Kanter *et al.*, 2006).

Axonal degeneration appears to be an important factor in the cumulative neurological disability that develops over time in MS patients. A recent study demonstrated that an axonal protein is targeted by autoantibodies in a subset of MS patients, suggesting that the B cell response may not only contribute to demyelination but also to axonal damage. Approximately one-third of MS sera contained antibodies to neurofascin, an adhesion molecule expressed by oligodendrocytes and neurons which localizes to the myelin–axon interface at the nodes of Ranvier (Mathey *et al.*, 2007). Antibodies to neurofascin caused rapid worsening of EAE following systemic administration due to complement-mediated damage of the axon segments exposed at the nodes. High-throughput proteomic methods are likely to identify yet other autoantibody targets in MS.

The recent discovery of an autoantibody associated with NMO demonstrates that different inflammatory demyelinating diseases can be distinguished using antibodies as biomarkers. Serum IgG reactivity against the blood–brain barrier of mouse CNS tissue was found in the majority of patients with NMO (73%) or an optic-spinal form of MS (58%), but rarely in MS or controls, with 91–100% specificity for optic-spinal conditions (Lennon *et al.*, 2004). This “NMO-IgG” was also detectable in individuals with longitudinally extensive myelitis or recurrent optic neuritis, who are at a high risk of developing NMO. A single autoantigen, aquaporin-4 (Aqp4), was later found to be targeted by the autoantibody in NMO (Lennon *et al.*, 2005). Aquaporins are a family of water channels responsible for maintaining fluid balance in the renal medulla and gastric mucosa, and are also expressed by astrocytes in the CNS, localizing to the foot processes at the blood–brain barrier (Fig. 4.3). Serum antibodies from NMO patients showed identical reactivity to a monoclonal anti-Aqp4, and no reactivity was observed in tissue sections from Aqp4-null mice (Lennon *et al.*, 2005; Paul *et al.*, 2007; Takahashi *et al.*, 2007). The high specificity and sensitivity of anti-Aqp4 assays were confirmed in larger studies of patients with NMO and other neurological or autoimmune diseases including pediatric and adult-onset MS (Banwell *et al.*, 2008; Paul *et al.*, 2007; Takahashi *et al.*, 2007).

Antibody titer in NMO patients was found to correlate with disease severity (Takahashi *et al.*, 2007), and anti-Aqp4 status has since been incorporated into diagnostic criteria for NMO (Wingerchuk *et al.*, 2006). Antibodies to aquaporin play an active role in the progression of NMO, as Aqp4 is expressed at high levels in the spinal cord and optic nerve (Nielsen *et al.*, 1997) and demyelinated brain lesions are found more often in regions of high aquaporin expression (Pittock *et al.*, 2006). Aqp4 protein is lost in both active and inactive NMO lesions (Roemer *et al.*, 2007). When incubated with serum or purified IgG from NMO patients, cells transfected with Aqp4 rapidly internalize the antigen and can also undergo complement-mediated lysis (Hinson *et al.*, 2007). Patients with NMO respond positively to plasmapheresis (Weinshenker *et al.*, 1999), although the effect may be attributable to a change in the balance of cytokines rather than merely depletion of autoantibodies. Because autoantibody levels typically do not change following treatment, the benefit of Rituximab in

NMO and MS is likely due to disruption of antibody-independent B cell functions, such as antigen presentation and cytokine production within the CNS.

4. CONCLUSIONS AND FUTURE DIRECTIONS

The significant clinical and pathogenic heterogeneity of MS and related diseases make it challenging to define the mechanisms of demyelination in an individual, and biomarkers that reflect pathogenetic mechanisms may thus be valuable for individualized treatment. Autoantibodies circulating in the blood and CSF are attractive candidates for the development of biomarkers, and have already proven useful in differentiating NMO and MS. Autoantibodies to multiple islet antigens are highly predictive of progression to type I diabetes (Verge *et al.*, 1996; Ziegler *et al.*, 1999), and measurement of autoantibodies may also become clinically useful in MS and related demyelinating diseases once more antigens are identified and characterized. The MS community has traditionally focused on well-defined candidate autoantigens such as MBP, PLP, and MOG, but recent systematic approaches for the identification of novel antigens have yielded several new protein and lipid targets. Definition of the repertoire of autoantibodies may allow classification of demyelinating diseases based on pathogenetic mechanisms rather than clinical presentation, facilitating individualized treatment or prediction of disease progression. For example, the presence of Aqp4 antibodies in NMO patients predicts a better response to plasma exchange or intravenous immunoglobulin. Antibodies may be a key component of the primary immune response responsible for initiation of tissue damage in MS, or may be merely reflect tissue damage. *In vivo* studies with purified or recombinant antibodies will be necessary to distinguish between these possibilities.

Now that clinical trials with Rituximab have proven that B cells play an important role in the pathogenesis of MS, more selective approaches can be tested that target either defined B cell populations or functions. Because plasma cells are preserved and autoantibody titers do not decrease in all patients following B cell depletion, the beneficial effects of Rituximab in MS suggest that other B cell functions are critical, such as antigen presentation to T cells with a matching antigen specificity and/or cytokine and chemokine production. Rather than depleting B cells systemically, it may be preferable to decrease levels of pro-survival factors for which autoreactive cells must compete or block the cytokines required for perpetuation of an autoimmune response. A soluble version of the BAFF receptor has successfully been used to treat and prevent MOG-induced EAE (Huntington *et al.*, 2006). Recovery was associated with decreased anti-MOG titer and alterations in T cell cytokine production with increased levels of TGF β and a reduction of IFN γ . Small interfering RNAs (siRNAs) have been targeted to specific cells in the periphery and CNS as complexes with soluble antibodies or antibody-decorated liposomes (Kumar *et al.*, 2007; Song *et al.*, 2005). siRNAs could thus be used to target antigen presenting molecules or cytokines in either the entire B cell pool, or cells with a defined activation/differentiation state.

B cells are now recognized to play a central role in MS and many other chronic inflammatory diseases. Although antibodies to myelin and neurons are likely to contribute to demyelination, the capacity of B cells to activate antigen-specific T cells or promote a proinflammatory cytokine environment is critical for disease progression. The complex

interplay between B cells, T cells and other cells of the immune system may thus offer a number of other approaches to therapy that preserve the B cell repertoire.

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FIGURE 4.1.

Antigen presentation by B cells to T cells with matching specificity. Crosslinking of the BCR by antigen induces signaling events leading to B cell activation and receptor endocytosis (1). MHC class II molecules assemble in the ER with the invariant chain (2). The internalized antigen is targeted to an intracellular compartment enriched in MHC (3), where proteases break down the antigen into peptides. In this compartment, the invariant is proteolytically cleaved such that only the CLIP peptide remains bound. DM facilitates the exchange of the MHC-associated CLIP for other peptides, including those derived from the endocytosed antigen. MHC molecules bearing high affinity peptides leave the loading compartment and are exported to the cell surface with co-stimulatory molecules (4). Antigen-derived peptide-MHC complexes can then activate specific CD4 T cells. Initial T cell activation leads to expression of CD40 ligand, which provides signals required for B cell survival and differentiation into memory cells through CD40 on the B cell surface.

FIGURE 4.2.

B cell-derived cytokines direct the ensuing immune response. Antigen-specific immune responses are perpetuated by collaboration of T and B cells in germinal centers, the formation of which is dependent on production of lymphotoxin α and TNF by activated B cells. The cytokines present during antigen presentation to T cells determine their effector phenotype. In the presence of IL-4, T cells follow the Th2 maturation pathway, which is involved in allergic responses and protective in MS. Expression of IL-12 or TGF β and IL-6 by APCs lead to differentiation of proinflammatory Th1 and Th17 cells, respectively, which are pathogenic in animal models of MS. These proinflammatory cells can be inhibited by IL-10, produced by APCs and a subset of regulatory T cells.

FIGURE 4.3.

Locations of autoantibody targets in the CNS. Oligodendrocytes (blue) ensheath axons with the spiral myelin membrane, a specialized extension of the plasma membrane. Each oligodendrocyte produces a segmental myelin structure for several surrounding axons. MBP and PLP are the primary protein components of myelin, but are located within the many layers and therefore unavailable to antibody binding in noninjured myelin. Although less abundant, MOG is specifically expressed on the outer surface of myelin, making it a target for demyelinating antibodies. Antibodies to neurofascin, found at the myelin-axon junction

and exposed on axons at the nodes of Ranvier, can contribute to both demyelination and axonal damage. Specific lipids and glycolipids can be released from the membranes of damaged oligodendrocytes, and are also recognized by antibodies in MS. The foot processes of astrocytes (green) form the blood-brain barrier which restricts access of molecules from the circulatory system to the CNS. Aquaporin-4, a water channel, is enriched in these structures, and is the target of autoantibodies specifically found in NMO.