B-Cell Modulation in Multiple Sclerosis

Riley M. Bove, MD

Partners Multiple Sclerosis Center, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts

Abstract Over the past decade, a role for B cells has emerged in the pathophysiology of multiple sclerosis (MS), which has traditionally been considered a T cell–mediated disease. At the 29th Congress of the European Committee for Treatment and Research in Multiple Sclerosis, Charcot Award recipient Stephen L. Hauser, MD, discussed the emerging role of B cells in MS and its treatment. This article reviews evidence from clinical trials of the therapeutic role of rituximab, a chimeric monoclonal antibody that targets CD20 receptors on the surface of B cells. When compared with placebo, rituximab decreases the number of gadolinium-enhancing lesions on magnetic resonance imaging and frequency of clinical relapse. The therapeutic potential of ocrelizumab, a humanized anti-CD20 monoclonal antibody currently being tested in patients with MS, is also discussed. Targeting inflammation resulting from B-cell activity may only be one component of a longer-term strategy for halting disease progression in MS.

T he Charcot Award, inaugurated in 1969 and given every 2 years by the Multiple Sclerosis International Federation (MSIF), recognizes lifetime achievement in outstanding research into our understanding or treatment of multiple sclerosis (MS). The winner is invited to give the Charcot Lecture at the annual congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) and at the biennial MSIF Council Meeting.

The award is given in commemoration of Jean-Martin Charcot (1825–1893), the founder of modern neurology. In 1882, he established the first neurology clinic in Europe at the Salpêtrière Hospital in Paris.

Among his many discoveries, Charcot described MS in 1868, calling it sclérose en plaques.

2013 CHARCOT AWARD WINNER

Stephen L. Hauser, MD, is Chair of the Department of Neurology at the University of California, San Francisco (UCSF). Dr. Hauser graduated from the Massachusetts Institute of Technology and Harvard Medical School and trained in neurology at the Massachusetts General Hospital. He moved to UCSF in 1992. Among his many academic leadership positions, Dr. Hauser is a past president of the American Neurological Association and editor-in-chief of Annals of Neurology. In addition to the Charcot Award, Dr. Hauser has received the Jacob Javits Neuroscience Investigator Award and the 2008 John Dystel Prize for Multiple Sclerosis Research. In April 2010, Dr. Hauser was appointed by President Barack Obama to the Presidential Commission for the Study of Bioethical Issues.

Dr. Hauser’s first major contribution to the field of MS was advancing our understanding of the genetic basis of MS. His efforts to uncover genes that confer increased susceptibility to MS—including the identification of specific genes in the major histocompatibility complex (MHC) human leukocyte antigen (HLA) system—culminated in the founding of the International Multiple Sclerosis Genetics Consortium (IMSGC) in 2002. The IMSGC led to the identification of the first two non-HLA genes involved in MS susceptibility: IL2RA (CD25) and IL7RA (CD127). Since then, the IMSGC has helped in identifying over 100 non-HLA risk alleles, most of which are associated with immune function.1

Dr. Hauser’s laboratory has published the complete genome sequences and epigenome of twins discordant for MS and has established the first national DNA repository for MS. His second major contribution to the field of MS, advancing our understanding of the role of B cells in MS pathogenesis and its therapeutic implications, was the basis of his 2013 Charcot Lecture.

FROM THE BENCH TO THE BEDSIDE AND BACK

Dr. Hauser began his address with words memorializing Dr. Christian Confavreux, a leading MS researcher in Lyon, France, and then acknowledged previous Charcot Award winners. He introduced his lecture as a story common to many translational scientists, “from the benchside and back,” highlighting moments of “incremental advance,” frequent “disappointment,” and “occasional transformational experiences” along the way.

Traditional View of MS Immunology

A simplified model of the immune pathophysiology of MS has emerged
from studies of immune responses in adult-onset MS, lessons from therapeutic trials targeting the immune response, and data generated from animal models. According to this model, the initial step in pathogenesis involves the peripheral activation of CD4+ T helper (Th) type 1 cells in response to a stimulating antigen. The assortment of molecules released, including costimulatory signals and cytokines, influences the response profile of the activated immune cells.

The initial stimulating antigen is unknown, but the presence of an infectious antigen is likely; this antigen subsequently cross-reacts with a central nervous system (CNS) antigen, a process known as "molecular mimicry." Subsequently, activated T cells transmigrate across the blood-brain barrier by various steps involving adhesion, chemotraction, and active infiltration into the CNS. This leads to reactivation of infiltrating cells within the CNS, which contributes to perivascular inflammation and injury. Release of additional CNS antigens may result in the recruitment of T cells with specificity for additional CNS antigens, called "epitope spreading," which may further propagate a chronic immune response. Further modifications to this model include the appreciation of other factors, most importantly Th12,17.

Back to the Bench

In trying to understand why aspects of chronic relapsing, remitting MS (RRMS) were not adequately modeled in typical rodent models of MS or experimental autoimmune encephalomyelitis (EAE), Dr. Hauser acknowledged the advice of his mentor, Dr. Raymond D. Adams (1911–2008), Chief of Neurology at Massachusetts General Hospital, who encouraged his protégés to look to new models. In 1992, in collaboration with Drs. Norman Letvin and Luca Massacesi, macrophage-mediated vesicular demyelination was replicated in New World monkeys (Callithrix jaccus; marmosets),3 who developed chronic relapsing–remitting and sometimes progressive disease, with evidence of remyelination. Dr. Claude Genain, among others, demonstrated that both encephalitogenic T cells and pathogenic antibodies were needed to replicate the demyelinating phenotype.4 In collaboration with Dr. Cedric Raine, Dr. Hauser and coworkers found that autoantibodies that recognize the immunizing antigen were deposited within the vasculature myelin sheaths in this animal model of MS and that similar antibodies against diverse antigens appeared in human MS lesions.5 This discovery implied that humoral immunity might be a key factor in MS pathogenesis. Subsequently, Dr. Christian von Büdingen and colleagues recognized that CD20+ B cells were present in MS lesions.6 This finding would be instrumental down the road in the development of rituximab and the rationale for its use in patients with MS.

A ROLE FOR B CELLS

Over the past decade, several lines of evidence led Dr. Hauser and others to question the role of autoimmune B cells and humoral mechanisms in the immunology of MS. First, a puzzling aspect of the traditional model is that therapies based upon this theory, such as interferon beta-1a and glatiramer acetate, did not fully prevent relapse or the accumulation of disability. Second, an element included in the diagnostic criteria for MS was an elevation in immunoglobulin-G (IgG) synthesis in the cerebrospinal fluid (CSF) relative to peripheral blood IgG levels and the presence of oligoclonal bands. Third, most MS lesions demonstrate deposition of antibody and activation of complement, vesicular disintegration of the myelin membrane, and detectable autoantibodies targeting diverse antigens in the CSF.

Memory B cells, which cross the blood–brain barrier, are believed to undergo restimulation, antigen-driven affinity maturation, clonal expansion, and differentiation into antibody-secreting plasma cells within the CNS to trigger cellular-dependent and complement-dependent cytotoxic effects. B cells can influence the priming of effector T cells by functioning as antigen-presenting cells. Further, abnormalities in B-cell cytokine responses have been reported in MS patients, and production of cytokines and chemokines by B cells may be involved in the formation of ectopic lymphoid-like structures. Finally, B cells may be the reservoir for Epstein-Barr virus (EBV), and infection with EBV is a known risk factor for MS.7,8 Altogether, these observations suggest that B cells exert both antibody-dependent and antibody-independent effects in MS, and targeting B cells might disrupt critical processes in MS pathogenesis.

ENTER RITUXIMAB

The story of rituximab and one of its champions, Dr. Lee Marshall Nadler, stands as a legend of the modern era of targeted therapies. Rituximab is a genetically engineered, chimeric monoclonal antibody targeting the CD20 antigen expressed on B lymphocytes from the pre-B-cell stage through differentiation into mature B cells but excluding plasma cells.
In the 1980s, Dr. Nadler and colleagues employed the methods of monoclonal antibody research to focus on the discovery of molecules uniquely expressed on human B cells. Eventually, all known human B-cell–specific antigens (CD19, CD20, CD21, and CD22) were discovered in his laboratory. He used these monoclonal antibodies to classify human B-cell leukemia and lymphomas, and he was the first investigator to administer a monoclonal antibody to a human. Eventually, the anti-B1 monoclonal antibody he developed led to the discovery of the CD20 cell-surface antigen on B cells and the development of rituximab.

Rituximab depletes CD20+ B cells by activating both cell-mediated and complement-dependent cytotoxic processes and by promoting apoptosis. Rituximab currently is used in the treatment of a range of diseases, including non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, Wegener’s granulomatosis, and microscopic polyangiitis and off-label in systemic lupus erythematosus and idiopathic thrombocytopenic purpura.9

**HERMES Trial: Rituximab Promising in RRMS**

In considering the therapeutic potential of rituximab in MS, Dr. Hauser and colleagues hypothesized that B-cell depletion might decrease antibody production, reduce cytokine networks, and limit B-cell–mediated antigen presentation and activation of T cells and macrophages.

In 2008, Dr. Hauser and others reported their findings from the HERMES trial, a phase 2, double-blind, 48-week trial involving 104 patients diagnosed with RRMS who were randomized to receive rituximab or placebo. Participants were 18–55 years of age, had experienced at least one relapse, and had an Expanded Disability Status Scale (EDSS) score of 0–5. Treated patients received 1,000 mg of rituximab intravenously (IV) on days 1 and 15 with premedication (acetaminophen and diphenhydramine hydrochloride) to minimize infusion-related reactions.

The primary endpoint was the total number of gadolinium-enhancing lesions detected on cranial magnetic resonance imaging (MRI) scans at weeks 12, 16, 20, 24, and 24. Patients given IV rituximab had reduced counts of these lesions at 12, 16, 20, 24, and 48 weeks and fewer new gadolinium-enhancing lesions, lower T2 lesion volume, and fewer relapses at 24 and 48 weeks (Figure 1).7 The effects of rituximab treatment on outcomes became significantly different from those of placebo at weeks 8–12.

In terms of tolerability, within 24 hours of starting treatment, the group receiving IV rituximab experienced more adverse events than did the placebo group (78% vs 40%, respectively), but findings between the two groups were similar after the second event (20% vs 40%). The incidence of infection (eg, nasopharyngitis, upper respiratory tract infections, urinary tract infections, and sinusitis) was similar among the rituximab and placebo groups (70% vs 71%). More patients in the placebo group than in the rituximab-treated cohort discontinued therapy before week 48 (40% vs 16%); in most cases, however, the decision to stop treatment was based on either patient or physician choice rather than on relapse of disease or initiation of excluded therapy.

In his remarks at the ECTRIMS meeting, Dr. Hauser described the difficulties of getting approval from the US Food and Drug Administration for this trial, as the investigators were asked to cut their primary endpoint from 48 weeks to 24 weeks. He also highlighted the economic and political challenges, particularly interactions with the pharmaceutical industry, that his colleagues and he faced in designing and executing the trial.

Dr. Hauser commented that the HERMES trial results led researchers “almost back to square one” (in his words) in terms of their understanding

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**FIGURE 1** Number of gadolinium-enhancing lesions in patients receiving rituximab or placebo, from baseline to week 48. (A) Mean total number of gadolinium-enhancing lesions by week. (B) Mean number of new gadolinium-enhancing lesions by week. Missing values were imputed by averaging the available data. Baseline magnetic resonance imaging information was obtained 4 weeks before baseline. Adapted, with permission, from Hauser et al.7
of the underlying immunopathology of MS, as the “immediate effect” implied a prominent role for B cells. In terms of the mechanism of action of rituximab in MS, Dr. Hauser and his coworkers postulated that rituximab treatment led to the lysis of memory B cells located in the peripheral blood and lymphoid tissues. Additionally, they hypothesized that rituximab interfered with antigen presentation by B cells and with activation of T cells or macrophages by pro-inflammatory B-cell cytokines. Because CD20 is not expressed on stem cells or plasma cells, median immunoglobulin levels were not affected during the trial. Dr. Hauser suggested that oligoclonal immunoglobulin might be a surrogate marker for B-cell clones.

OLYMPUS Trial: Rituximab Less Promising in Primary Progressive MS (PPMS)

The results of the OLYMPUS trial, in which patients with PPMS received IV rituximab or placebo on a 2:1 basis, revealed less potential for rituximab in patients with primary, progressive disease.5 In this double-blind, randomized clinical trial, patients received rituximab or placebo every 24 weeks over a period of 96 weeks (a total of four courses of treatment). No significant reduction in time to clinically definite progression (defined as an increase in EDSS score sustained over 12 weeks) was found between rituximab and placebo. Patients given rituximab had less of a decrease in T2 lesion volume, but total brain volume was similar in both patient groups.

Interestingly, in subgroup analysis, rituximab therapy was associated with delayed time to clinically definite progression in patients under 51 years of age, in those with gadolinium-enhancing lesions on MRI, and in patients who were both under 51 years old and had gadolinium-enhancing lesions. These analyses suggested that (1) some PPMS patients have evidence of inflammation early in the disease course, which influences the rate of progression; (2) early, aggressive treatment of inflammation in PPMS may be beneficial; and (3) age-related neurobiologic changes, such as immunosenes-
cence, occur in MS and carry implications for therapeutic decisions.

Ocrelizumab: A Better Benefit-to-Risk Ratio?

Given concerns about the immunogenic effect of repeated rituximab infusions, as well as political difficulties, Dr. Hauser and his colleagues hypothesized that ocrelizumab, a recombinant humanized monoclonal antibody that selectively targets CD20+ B lymphocytes, might offer similar therapeutic benefits to rituximab in MS with less risk of immunogenicity and infusion-site reactions.6 Ocrelizumab is biosimilar but not bioidentical to rituximab. In vitro, ocrelizumab demonstrates more antibody-dependent, cell-mediated cytotoxicity than rituximab and less complement-dependent cytotoxicity.11 Thus, by increasing antibody-dependent, cell-mediated, cytotoxic effects, ocrelizumab may modulate tissue-dependent mechanisms of pathogenic response more effectively than rituximab.

In 2011, Dr. Hauser joined Dr. Ludwig Kappos and coworkers6 in publishing the results of a phase 2, placebo-controlled trial involving 220 patients with RRMS who were randomly assigned to receive 600 or 2,000 mg of ocrelizumab, interferon beta-1a, or placebo. Patients with RRMS were included in the study if they were 18–55 years of age and had at least two relapses within the prior 3 years (including at least one in the prior year), an EDSS score of 1–6 at baseline, and evidence of inflammatory disease (at least six T2 lesions on MRI or two relapses in the prior year). Among exclusion criteria was an EDSS score ≤ 2 in patients who had had the disease for more than 15 years. Patients received four treatment cycles of 24 weeks followed by a 1-year treatment-free observation period.

In the primary analysis at 24 weeks, when compared with the placebo group, patients given 600 or 2,000 mg of ocrelizumab had 89% or 96% fewer T1 gadolinium-enhancing lesions, respectively (Figure 2).6 Both doses of ocrelizumab were superior to interferon beta-1a in reducing these lesions.

Two phase 3 pivotal trials in RRMS patients and the first phase 2 pivotal trial in PPMS patients (the ORCHESTRA trial) are ongoing. Twists and turns in this research program included the halting of an ocrelizumab program in rheumatoid arthritis patients in 2010 because of the appearance of opportunistic infections. Dr. Hauser noted that RA treatment involves polypharmacy in an older population.

Recovery Period After B-Cell Depletion

The prolonged benefits of B-cell depletion after exposure to rituximab

![FIGURE 2](http://example.com/figure2.png)
suggest that protection may extend beyond the period of B-cell depletion. Dr. Hauser cited the contributions of many of his colleagues to investigation of the immunologic changes occurring in the peripheral blood and CSF during this “recovery” period. In the peripheral circulation, immunologic changes include dominance of naïve and immature B cells, an increase in the numbers of interleukin-10-secreting B regulatory cells and CD25+FoxP3+ T regulatory cells, and a decline in Th1 and Th17 proinflammatory responses. In the CSF, the number of T and B cells decreases, and resting CD19+ bright B cells predominate. Nonetheless, the effect of rituximab treatment on oligoclonal bands is incompletely understood. Early evidence suggests no decrease after just one course of B-cell depletion. Repeated treatment courses may be important, as they have proven to be in the treatment of rheumatoid arthritis.

**EMERGING INVESTIGATIONS INTO B-CELL FUNCTION IN MS**

Myelin oligodendrocyte glycoprotein (MOG) is a glycoprotein that appears to be involved in either completion or maintenance of the myelin sheath. It has emerged as a potential antigen involved in the pathogenesis of MS.

At the University of California, San Francisco, researchers in Dr. Scott Zamvil’s laboratory immunized both healthy mice and MHC II-deficient mice with EAE. B-cell class II-deficient mice repopulated with normal B cells developed normal EAE to extracellular mouse MOG but were protected against extracellular human MOG. This observation suggested that a simple substitution may have induced a conformational change and led the human MOG antigen to be completely B-cell dependent; this protection could not be restored by injecting MOG antibody. Thus, there may be a repertoire of B-cell–dependent antigens that might help to elucidate underlying triggers in MS.

Dr. Hauser also described recent genetic studies that “solved how heritable our antibody repertoire is.” Twin studies have revealed identical expression of heavy-chain variable and D segments on antibodies, but the hypervariable complementarity-determining regions that bind antigens are “absolutely environmental,” Dr. Hauser said.

Next, he reviewed the IgG sequences identified by Dr. Christian von Büdingen, among others, to identify “clonotypes” of CSF and peripheral-blood B cells and oligoclonal bands. These studies revealed oligoclonal B cells in CSF that are fingerprints for MS. In lineage analyses, multiple different oligoclonal bands belong to the same clone and may be responding to a smaller number of antigenic determinants than the bands would suggest. Members of these oligoclonal bands could be identified both in the CSF and in peripheral blood mononuclear cells. In addition, some B cells found only in the peripheral blood were associated with oligoclonal bands found only in the CSF, suggesting some exchange of B cells across the blood-brain barrier (Figure 3). B-cell therapy may disrupt this circuitry.

Finally, Dr. Hauser mentioned genetic studies that revealed many single nucleotide polymorphisms involved in B-cell activation and B-cell receptor signaling pathways that may influence susceptibility to a “hyperpolarized, proinflammatory B-cell state,” concluding that the genetics “needs to link to function to understand how inheritance predisposes to this B-cell problem.”

**UNFINISHED BUSINESS**

In his closing remarks, Dr. Hauser related the story of a “leading citizen of California” with long-standing RRMS who had experienced ongoing inflammatory activity despite adherence to many disease-modifying therapies. He was treated with rituximab off label since 2003. To date, he has demonstrated an excellent response to rituximab in terms of inflammatory activity. Still, his disease continues to progress clinically. Over the past 10 years, he has gone from an EDSS score of 3 to requiring bilateral crutches. MRI tractography has revealed ongoing atrophy of the motor cortex. Dr. Hauser commented, “I think that the best question here is, are there B cells in lymphoid compartments.
follicles that are contributing to percolating [of disease activity]? This anecdote highlights our incomplete understanding of MS.

REFERENCES


