Multiple Sclerosis: From Bench to Bedside and Back Again

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Introduction

The goal of a career in biomedical research is to contribute meaningfully to medically useful discoveries: something that happens, if one is fortunate, perhaps a few times over the course of a career. For those of us involved in the B-cell story in multiple sclerosis (MS), this occurred in September 2006 with the unblinding of the phase II anti-CD20 rituximab (RTX) study (Hauser et al., 2008). First, we saw evidence of a potentially powerful new approach for treating relapsing MS (RMS). Second, despite this success, it was also clear that the rationale behind the clinical testing of RTX for MS was almost certainly incorrect. In many respects, this was the best possible result that one could wish for. A novel approach appeared to offer significant benefits for patients, and yet the data also sent us back to the bench in new and unexpected directions. The rubber meets the road when ideas born in the lab are formally tested at the bedside, and when data from real-life patients create new, testable ideas for research. Translational medicine is most effective when information flow is bidirectional, linking the laboratory with the clinic.

The Early Days

In the late 1970s, during my neurology residency, I was in a conference room at Massachusetts General Hospital in Boston with the chair of neurology, Raymond D. Adams. A postdoctoral fellow was presenting some work in experimental autoimmune encephalomyelitis (EAE) induced by myelin basic protein. Adams noted, with a touch of sarcasm, that the paralysis observed in the rodents likely resulted from peripheral nerve, and not CNS, disease. Indeed, he emphasized that the pathology of EAE and MS was quite different. T-cell-mediated acute EAE models in mice were dominated by an inflammatory panencephalitis with relatively sparse demyelination, unlike the primary macrophage-mediated demyelinating pathology typical of human MS. This experience motivated me to begin a long-term effort to model MS-like pathology in the laboratory.

Developing a Better Disease Model

In partnership with Norman Letvin, we began immunizing different species of nonhuman primates to search for pathologies that closely mimicked MS (Genain and Hauser, 1997). We were hopeful that a model could be generated in the New World marmoset *Callithrix jacchus*, a small primate approximately the size of a guinea pig but with a unique defining characteristic. *C. jacchus* pregnancies are typically multiple, involving gestation of several nonidentical embryos at a time. Each fetus shares a common blood supply, leading to the establishment of a permanent, stable, lifelong bone marrow chimerism among fraternal twins or triplets. We found that this chimeric state, as predicted, permitted the transfer of T-lymphocytes from one sibling to another without eliciting an alloresponse in the recipient. These data set the stage for adoptive transfer of encephalitogenic T-cells in a species phylogenetically close to humans, analogous to earlier experiments in inbred mice that were critical for defining the immunology of murine EAE.

After several years of starts and stops, a model of MS was successfully developed by Luca Massacesi, a postdoctoral fellow, in 1995 (Massacesi et al., 1995). The key step, which had eluded us before Luca's arrival, was the creative use of different immune adjutants (Genain and Hauser, 1996). Following immunization with a myelin extract in incomplete Freund's adjuvant, and later with myelin oligodendrocyte glycoprotein (MOG), animals developed a mild relapsing–remitting disease and an acute pathology characterized by large concentric areas of macrophage-mediated demyelination with relative axonal sparing and foci of remyelination; the myelin membrane was destroyed and reconstituted into vesicular fragments (Fig. 1), a pattern termed "vesicular demyelination" (Prineas and Connell, 1978). This was our first eureka moment—we had replicated the MS-like pathology that we had sought for a decade.

However, when we adoptively transferred MOG-reactive T-cell clones from an immunized *C. jacchus* animal into a chimeric sibling, we replicated the acute murine pathology of panencephalitis but not the distinctive MS-like pathology of vesicular demyelination (Massacesi et al., 1995). The explanation for this apparent conundrum was quickly solved by another postdoctoral fellow at the time, Claude Genain. Only by coadministering encephalitogenic T-cells plus pathogenic antibodies (Abs) could the MS-like demyelinating phenotype be reconstituted. This finding led us to focus on the concept that an MS-like, demyelinating lesion required both pathogenic T-cells plus autoantibodies; the autoantibodies alone were nonpathogenic, presumably because they required encephalitogenic T-cells to open the blood–brain barrier (BBB) and permit their passage into the CNS (Genain et al., 1995, 1996). Our confidence that these mechanisms were operational in MS was strengthened by older literature in guinea pig optic neuritis first described by Appel and Bornstein (1964), and much later by Linington, Olssen, and Wekerle in work with rat

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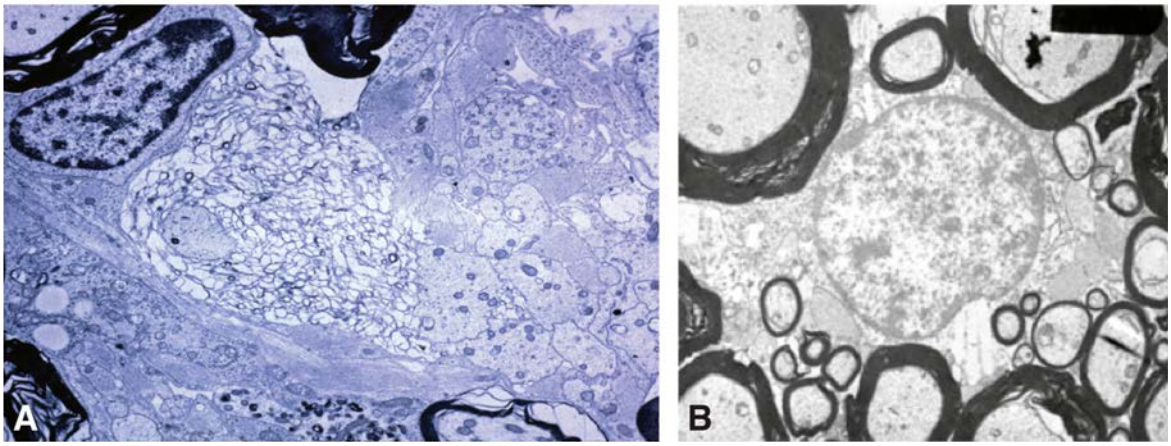

Figure 1. Ultrastructural features of *C. jacchus* EAE. In ***A***, primary demyelination with preservation of axons, macrophage infiltration (macrophage nucleus visible at the top right), and astrogliosis are present. In the center, morphological changes of myelin dissolution and fasciculation are visible. In ***B***, findings in chronic *C. jacchus* EAE are shown, illustrating areas of thin, compact myelin-encircling axons, indicative of remyelination. Reprinted with permission from Hauser (2015), Fig. 3. Copyright 2015, SAGE Publications.

EAE models (Linington et al., 1993; Lorentzen et al., 1995).

In 1999, we completed a deeper study of the lesion with Cedric Raine, revealing the presence of bound Abs in the demyelinated lesions of C. *jacchus* that recognized the immunizing antigen (Ag) MOG. However, when we then turned to human MS tissue, we found that deposited Abs were also bound to the myelin membrane but had specificities that were far more diverse than in EAE (Genain et al., 1999; Raine et al., 1999). This suggested that a highly focused immunotherapy is unlikely to be successful for MS.

Back to the Bedside

Given the heterogeneous nature of the Ab repertoire associated with myelin destruction in MS, it became clear that targeting any specific protein or epitope was a dubious therapeutic strategy. Thus, we turned to methods that could deplete or inactivate a broad range of Abs, plasma cells, or perhaps their progenitors, B-lymphocytes. The first two options were not feasible with available therapeutics, and we had previously found that indiscriminate Ab removal via plasmapheresis had little meaningful effect on chronic MS (Hauser et al., 1982, 1983); thus, our thoughts turned to B-cell-based therapy, and specifically to the anti-CD20 monoclonal Ab (MAb) RTX.

RTX was synthesized at Idec Pharmaceuticals in 1986. Idec entered into a codevelopment partnership with Genentech in 1995, and two years later, RTX (marketed as Rituxan) received U.S. Food and Drug Administration (FDA) approval for the treatment of B-cell lymphoma. In 2001, I began discussions with Genentech around RTX therapeutics for MS after our failed application to the National Institutes of Health (championed by Claude Genain with Michael Racke and Nancy Monson at University of Texas Southwestern Medical Center) had left us little hope that public resources could be found to support this trial. The referee comments from the application were dismissive, reflecting profound skepticism of the proposition that humoral immune mechanisms might be central to MS pathogenesis. Across much of academia at the time, MS research was dominated by concepts of T-cell mediation, analogy to murine EAE models, and a belief that CNS Igs, including oligoclonal bands (OCBs), represented meaningless "nonsense" Abs (Mattson et al., 1980). The field was not yet ready.

Industry proved to be a more flexible, and less risk-averse, partner. Discussions with Genentech progressed well, although the company estimated our chance of success at "less than 15%." Even if one accepted that autoantibodies were responsible for MS, a B-cell-based therapy would not immediately knock down Ab production by long-lived plasma cells. Their experience with RTX indicated that IgG Ab levels were largely unchanged following treatment, although lower-affinity IgM was modestly reduced by approximately 15%. At least in theory, one would need many years of treatment to reduce circulating levels of Ig. Our original plan was to begin with a placebo-controlled phase IIB clinical trial of

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two courses of RTX spaced six months apart, and a primary endpoint at 12 months, or six months after the final infusion. The FDA balked at this design, advising us that it was unethical to maintain MS patients on placebo therapy for one year. In response to these concerns, the trial was scaled back; fewer patients would be enrolled, only a single course of RTX would be administered, and the primary endpoint would be measured at six months. Our prospects for success seemed ever dimmer.

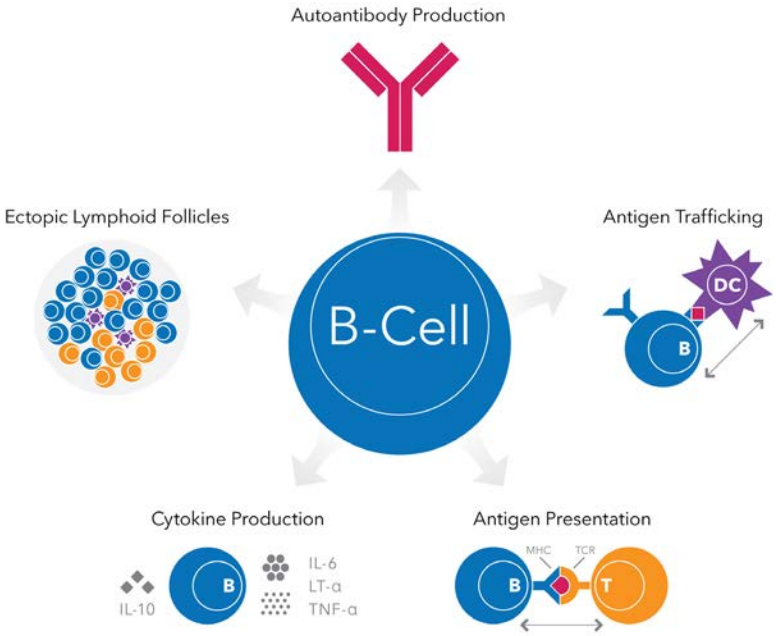

Figure 2. An overview of the diverse functional roles of B-cells. LT-α, lymphotoxin-alpha; TCR, T-cell receptor. Reprinted with permission from Hauser (2015), Fig. 4. Copyright 2015, SAGE Publications.

As noted earlier, when the data were unblinded in 2006, we observed a dramatic and almost immediate 91% reduction in gadolinium-enhancing magnetic resonance imaging (MRI) activity (the primary endpoint) plus a significant reduction in the rate of new relapses (Hauser et al., 2008). This was our second eureka moment. Perhaps the rapid onset of the benefit conferred by RTX was the most stunning aspect of the trial. Because the clinical effects happened so quickly, they were almost certainly not the result of any reduction in long-lived Abs but were more likely explained by some direct effect on B-cells themselves. In many respects, this was the best of all possible results for a clinical experiment. The data raised hope that an impactful new approach to MS therapy would result, but they were also perplexing, sending us back to the lab with information that the underlying hypothesis behind the clinical trial was almost certainly wrong. We now had a new focus on B-cell biology.

The Multifunctional B-Cell

B-cells are extremely diverse members of the universe of adaptive immunity. Although targeting autoantibodies provided the original conceptual framework for testing RTX in RMS, the resulting data made it likely that the robust efficacy was somehow related to a direct effect on B-cells themselves (von Büdingen et al., 2011). B-cells have numerous effector functions independent of their differentiation from Ab-secreting plasma cells (Fig. 2). B-cells are highly effective Ag-presenting cells (APCs), but unlike other conventional APCs that are promiscuous Ag presenters, B-cells are most efficient at presenting Ag that is initially recognized by the surface B-cell receptor (BCR), i.e., the clonally specific Ig molecule. Thus, B-cells can be viewed as extremely selective APCs. Ag initially bound to surface BCRs is internalized, complexed in endosomes with class II major histocompatibility complex (MHCII) molecules, and returned to the surface for Ag presentation to T-cells. B-cells are also highly motile, and in secondary lymphoid structures, they play a role in "Ag shuttling," a process in which Ag is grabbed from macrophages by B-cells via the BCR and transported to follicular dendritic cells (DCs), another class of APCs. Through secretion of cytokines, B-cells can also regulate, as bystanders, various effector immune functions mediated by both B-cells and T-cells. Some B-cells support pro-inflammatory function through secretion of tumor necrosis factor alpha (TNF-α) and lymphotoxin, whereas a different interleukin (IL)-10–producing B-cell population has a regulatory, anti-inflammatory role. Interestingly, MS B-cells may be inherently polarized toward a pro-inflammatory functional phenotype (Bar-Or et al., 2010). As noted earlier, the rapid response to B-cell depletion therapy for focal disease activity in RMS indicated that the mechanism of action was likely not, as initially hypothesized, inhibiting autoantibodies. Instead, it was more likely blocking B-cell APC function and subsequent T-cell activation, or perhaps acting via

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bystander effects on adaptive immunity. However, the potential inhibition of an as-yet unidentified autoantibody in MS could not be completely excluded (Khosroshahi et al., 2010).

B-cell development begins in the bone marrow and proceeds through stages of pro-B-cells and pre-B-cells before the cells exit into the circulation as naive, Ag-inexperienced B-cells. The vast majority of B-cells are located in follicles in secondary lymphoid tissues, including lymph nodes and spleen, and in mucosal sites. Binding of Ag through the BCR triggers activation, proliferation, and somatic hypermutation (SHM) of BCRs, resulting in maturation to memory (Ag-experienced) B-cells and differentiation to Ab-secreting plasma cells. B-cells are believed to reside in lymphoid follicles for only ~1 d before returning to the circulation, highlighting the dynamic nature of B-cell Ag capture, activation, and SHM of the BCR. It is thought that both memory B-cells and Ab-secreting plasmablasts and plasma cells can cross the BBB and enter the CNS in low numbers, and once there can reside in protective niches for long periods of time—a concept that has become increasingly relevant to research in progressive MS.

CD20 is an ideal target for B-cell immunotherapy. The CD20 molecule is expressed on pre-B-cells and throughout the life cycle of naive and memory B-cells; CD20 is not expressed on stem cells or pro-B-cells at the earliest stages of the B-cell differentiation program, nor is it expressed on plasmablasts or terminally differentiated plasma cells (Fig. 3, top). Following removal of CD20 B-cells with RTX, there is consistent repletion from early B-cell progenitors residing in the bone marrow, generally beginning four to six months after treatment (Fig. 3, bottom). Because long-lived plasma cells are unaltered, Ab responses to infectious agents or to vaccinations are largely preserved during periods of B-cell depletion. This feature may also explain the favorable safety record (after ~3 million doses) of RTX. MAbs against CD20 do not effectively remove B-cells residing in protective niches within secondary lymphoid structures. Circulating B-cells, representing only ~2% of the total B-cell pool in humans, are the B-cell compartment most efficiently depleted by these agents.

If B-cells residing in pathogenic niches (e.g., the CNS in MS, or synovium in rheumatoid arthritis [RA]) are relatively protected from anti-CD20 therapy, then how does the treatment work? The most likely explanation is that sustained depletion of circulating B-cells, which in autoimmune disease likely includes recirculating, restimulated memory B-cells destined to return to the target tissue, prevents their reentry into white matter regions in MS or joint tissue in RA (Silverman and Boyle, 2008).

Technology Moves Faster Than Clinical Research

It took 18 months for the RTX data to find their way into final print (Hauser et al., 2008), but by this time, the prospects for advancing to phase III clinical trials of RTX were dead. The reasons for this were multiple but included complex governance of the RTX franchise between the two participating pharmaceutical companies, Biogen Idec and Genentech (Biogen and Idec Pharmaceutical agreed to merge in 2003) and RTX's expiring patent life; an FDA requirement that we carry out a dose-finding study of RTX before moving forward with phase III; the development of a new humanized anti-CD20 MAb, ocrelizumab (OCR), by Genentech; and lastly, Roche's acquisition of Genentech in 2009. A plan was put forward to no longer pursue RTX but instead to develop OCR for MS.

Different MAbs are not necessarily biologically identical even if they target the same molecule; this is certainly the case for Abs that target CD20. RTX and OCR target different epitopes of CD20 and kill B-cells through different cytolytic pathways. RTX has stronger complement-dependent cytotoxicity (CDC) and less Ab-dependent cell-mediated cytotoxicity (ADCC), whereas the converse is true for OCR. Greater ADCC activity by OCR results from a higher affinity of Fc binding to the Fc-gamma receptor IIIa (FcγRIIIa) on host natural killer cells. The dose of Ab used, and the frequency of administration, may also influence ADCC activity. These differences between RTX and OCR, as well as differences in dose plus the use of polytherapy, may help to explain a complication observed in a trial of OCR as add-on therapy for RA in which several serious opportunistic infections developed in older Asian RA patients treated with high doses of OCR (Rigby et al., 2012). This complication in the OCR trial was quite unexpected, as no safety signal of this type had been noted in the nearly 200,000 RA patients treated with RTX as add-on therapy (Rubbert-Roth et al., 2010). Although the RA trial of OCR was halted, the MS phase II trial of low-dose OCR as monotherapy proceeded, and when the results were unblinded, a robust treatment response identical to that found for RTX was observed with acceptable safety (Kappos et al., 2011). Also important, our hope that OCR, a humanized MAb working primarily through ADCC, would produce a lower incidence of infusion reactions compared

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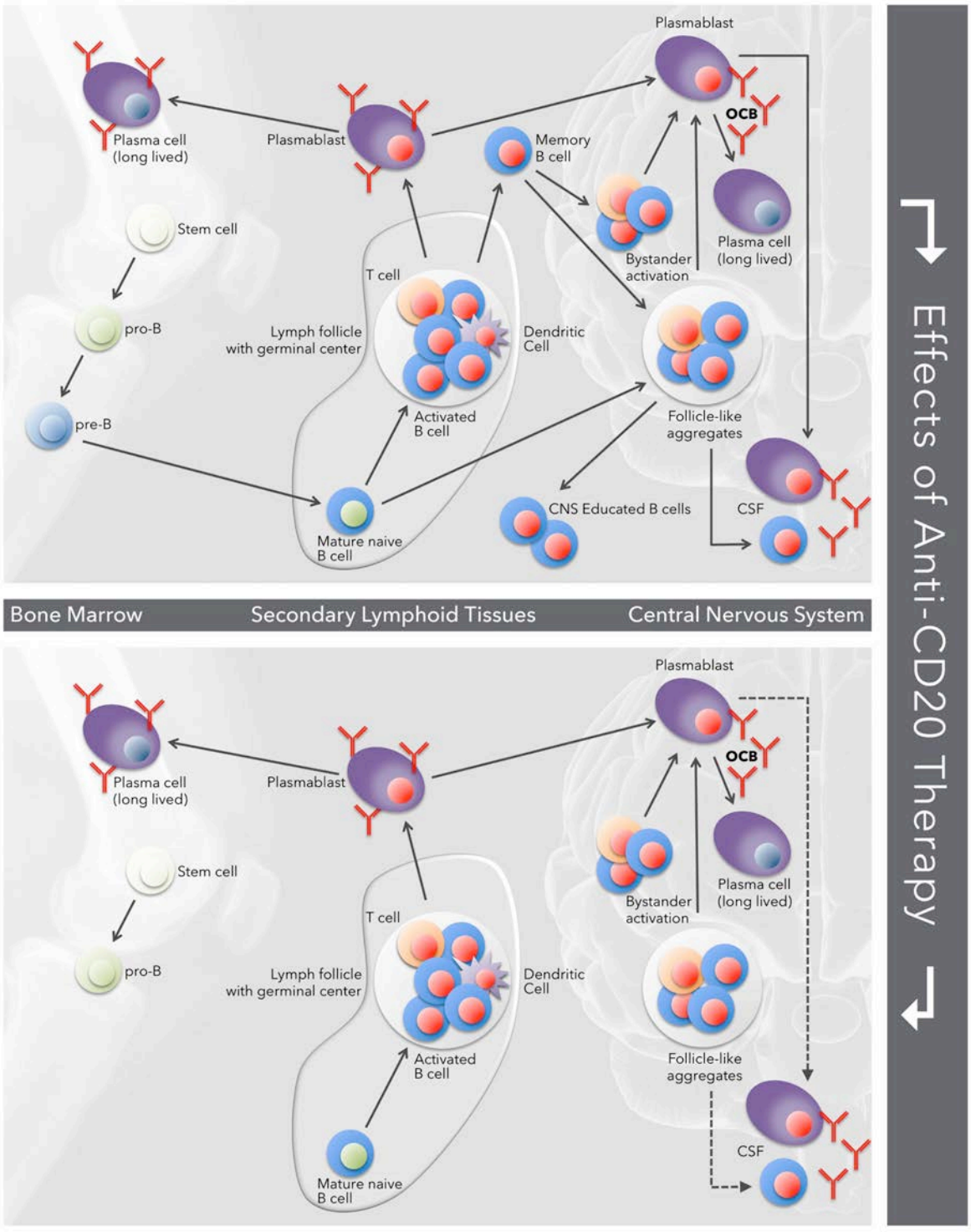

Figure 3. The effects of anti-CD20 therapy on recirculating B-cells. Top panel, summary of the life cycle of B-cells destined for the CNS. Bottom panel, highlights of the effects of depletion of circulating B-cells with anti-CD20 therapy; B-cells residing in lymphoid tissues and the CNS are likely to be resistant to depletion with anti-CD20 therapy. Reprinted with permission from Hauser (2015), Fig. 5. Copyright 2015, SAGE Publications.

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with the chimeric RTX mediating lysis via CDC, was confirmed, making OCR a far more attractive agent for chronic use. All involved breathed a deep collective sigh of relief as we advanced to the pivotal phase III clinical trials.

Although OCR's success was anticipated in RMS, the results of the two pivotal OCR trials, published earlier this year, exceeded expectations (Hauser et al., 2017; Montalban et al., 2017). The trials revealed dramatic effects on all key clinical and MRI outcomes in RMS and demonstrated clear benefits for the previously untreatable form of the disease, primary progressive MS (PPMS). In the RMS trials, OCR produced stunning reductions in the MRI endpoint of gadolinium enhancement and new lesion formation: almost 99% compared with baseline levels, indicating nearly complete elimination of new lesion formation in brain white matter. In a single pivotal study in PPMS, confirmed progression of disability (the primary endpoint) favored OCR. However, a modest risk reduction of 24% and multiple secondary clinical and MRI endpoints, including timed walk, white matter lesion volume, and brain atrophy, also showed benefits favoring treatment. OCR (marketed as Ocrevus) was recently approved by the FDA for RMS and PPMS, and decisions by other regulatory agencies are expected to be forthcoming.

Additional Insights from the Trials

In the original phase II RTX study in MS, focal disease activity remained reduced even after B-cells had returned to the peripheral blood (PBL). This point was driven home in a preliminary open-label, open-extension phase of the OCR phase II study. After four courses of treatment with OCR, MRI and clinical disease activity remained quiescent 18 months after the last dose. Equally important, in the phase II studies, no evidence of rebound was present at any time point. These data suggest that anti-CD20 treatment might reset the immune system in some way and confer protection against the development of new focal MS lesions beyond the period of B-cell depletion. Studies of PBL in RTX-treated individuals indicated that, following repletion, there are persistent changes in both B-cell and T-cell subpopulations that could, at least in theory, promote immune homeostasis and reduce pro-inflammatory responses. Repleting B-cells express predominantly naive and immature (CD5, CD38hi) phenotypes (Duddy et al., 2007); pro-inflammatory T-cells are decreased (Bar-Or et al., 2010); and regulatory T-cells are increased (Vallerskog et al., 2007). Reductions in pro-inflammatory immune cells are also present in CSF, with reduced numbers of T-cells and B-cells (Cross et al., 2006; Piccio et al., 2010) and a predominance of resting B-cells (Monson et al., 2005).

Another Surprise: CD20-Positive T-Cells

Work led by Christian von Büdingen confirmed earlier suggestions that CD20 T-cells exist in the healthy human circulation (Palanichamy et al., 2014b). This heterogeneous population, representing ~7% of total mature circulating T-cells, is composed of numerous T-cell subsets, including both CD4 helper and CD8 cytotoxic T-cells as well as naive and various memory T-cell populations. $CD20^{+}$ T-cells have a lower surface density of CD20 compared with B-cells (hence the designation $CD3^{+}$ $CD20^{dim}$), but nonetheless, the vast majority of these cells are depleted from the peripheral circulation with anti-CD20 therapy. It remains possible—and would certainly be ironic if true—that the effects of anti-CD20 therapy on MS result from elimination of pathogenic $CD20^{+}$ T-cells.

Back to the Bench

Scott Zamvil developed bone marrow (BM) chimeric mice containing B-cells that were selectively deficient in expression of MHCII molecules; other APCs, including DCs and monocytes, expressed MHCII normally. Following immunization with the extracellular region of mouse MOG, or with an immunodominant p35–55 MOG peptide, mice lacking MHCII on B-cells developed EAE normally. However, following immunization with recombinant human MOG, these mice became resistant to EAE induction, and susceptibility could not be restored by administering MOG Ab (Molnarfi et al., 2013). How can one interpret the finding that B-cells competent to serve as APCs were absolutely required for EAE against human MOG (hMOG) but not against murine MOG? This B-cell dependence can probably be attributed to a single amino acid change in the immunodominant region of MOG (e.g., a substitution of proline in place of serine at position 42 in human MOG). Moreover, transgenic mice that expressed surface MOG-reactive Ig on their B-cells could not secrete Ab. When crossed with a transgenic MOG-reactive T-cell line, progeny developed spontaneous EAE (associated with Th17 polarization, B-cell activation, and formation of ectopic germinal centers in the meninges), all in the absence of secreted Ab. Thus, B-cell APC function, in the absence of autoantibodies, is sufficient to promote T-cell activation and an MS-like disorder.

In EAE, B-cells tend to be involved as APCs when the immunization regimen employs whole myelin

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proteins such as hMOG, but not when myelin peptides (e.g., p35–55 MOG) are used. Peptide immunization models are B-cell independent because the BCR that binds mostly conformational rather than short linear epitopes is not involved in Ag capture (Lyons et al., 1999; Fillatreau et al., 2002). Interestingly, in EAE induced by whole MOG protein, B-cell depletion is protective, but in EAE induced by MOG peptide, B-cell depletion worsens disease severity, probably by depleting IL-10-secreting regulatory B-cells (Weber et al., 2010). In humans, a clinical trial of atacicept—a decoy receptor for the B-cell growth factors B-cell activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL)—paradoxically worsened MS, possibly by altering regulatory B-cell tone (Kappos et al., 2014). These cautionary data emphasize that B-cell depletion can be deleterious in some situations, and they highlight the potential clinical relevance of information gleaned from EAE even when the models are imperfect representations of human MS.

Thus, B-cells can be pro-inflammatory or regulatory, and the predominance of one or another function is one determinant of the outcome of an ongoing immune response. Clearly what is needed is to better understand how B-cell polarization might be aberrant in MS. Gene variants that are expressed by B-cells make up an important component of the more than 200 variants thus far known to be associated with inherited risk for MS (International Multiple Sclerosis Genetics Consortium et al., 2013; Farh et al., 2015). Similarly, a number of functional changes in B-cells have been described in MS patients, including changes in cytokine profiles indicating a pro-inflammatory bias, and a defect in inducing B-cell tolerance in PBL (Kinnunen et al., 2013).

Identifying and Tracking Culprit B-Cells

BCRs are heterodimeric proteins with the Ag-binding portion formed by the variable regions of heavy and light chains. With respect to Ag recognition, the heavy-chain variable region (VH) is generally believed to play the primary role; VH results from the splicing of three gene segments into a mature transcript: one copy of a variable (*V*), diversity (*D*), and joining (*J*) gene segment. *V*, *D*, and *J* genes exist as multiple copies in each genome, contributing significantly to the diversity of Ab transcripts. Most Ab diversity is generated by variation in how gene segments splice together, and especially by somatic mutations in the complementarity-determining regions of *V* genes that shape the Ab response. Following Ag contact in secondary, and possibly in ectopic, lymphoid tissues, BCRs undergo somatic diversification as their phenotype advances from naive to memory B-cells, and then to Ab-secreting plasmablasts and plasma cells. The propensity of B-cells to select some members of *V* gene families over others is highly heritable, but the clonal repertoire of BCRs expressed by any individual is stochastic and not influenced by differences in the architecture of germline genes (Baranzini et al., 2010; Glanville et al., 2011).

Sequencing IgG-VH repertoires in MS patients revealed that CSF B-cells represent a clonally restricted population that had undergone highly selective activation and affinity maturation within the CNS compartment. By performing parallel sequencing of many thousands of IgG-VH transcripts per sample, it was possible to construct lineage trees representing clonally related CSF B-cells defined by their BCRs and to identify clonally related BCR sequences from PBL in the same individual. Results revealed a deep connection of these highly selected, clonally related B-cells between the CSF and PBL compartments (Fig. 4) (von Büdingen et al., 2012; Palanichamy et al., 2014b). Further, when CSF IgG-VH sequences were matched with mass-spectrometric proteomic analyses of isoelectric-focused CSF IgG, remarkably, the proteomic data and IgG-VH transcripts matched (Obermeier et al., 2008). Most peptides sequenced from OCBs could be shown to map to CSF-derived IgG-VH sequences, and in a given individual, different bands composing the OCBs were shown to be clonally related—that is, they belonged to the same BCR lineage tree (Obermeier et al., 2008; von Büdingen et al., 2012; Bankoti et al., 2014). Thus, there is evidence that ongoing stimulation and maturation to clonally restricted Ab-expressing B-cells occur primarily inside the CNS compartment. In some individuals, B-cells participating in OCB production can also be identified in PBL; these cells appear to migrate across the BBB and may undergo further Ag stimulation in the periphery (Bankoti et al., 2014; Palanichamy et al., 2014a). Thus, OCBs are not merely the terminal result of a focused immune response in MS but represent a component of active B-cell immunity that is dynamically supported on both sides of the BBB. Although it is unclear where in the periphery activation and/or SHM of B-cells responding to brain Ags might occur, recent data suggest that draining cervical lymph nodes are one potential site (Stern et al., 2014).

Our work in CSF (von Büdingen et al., 2012; Bankoti et al., 2014; Palanichamy et al., 2014a) and

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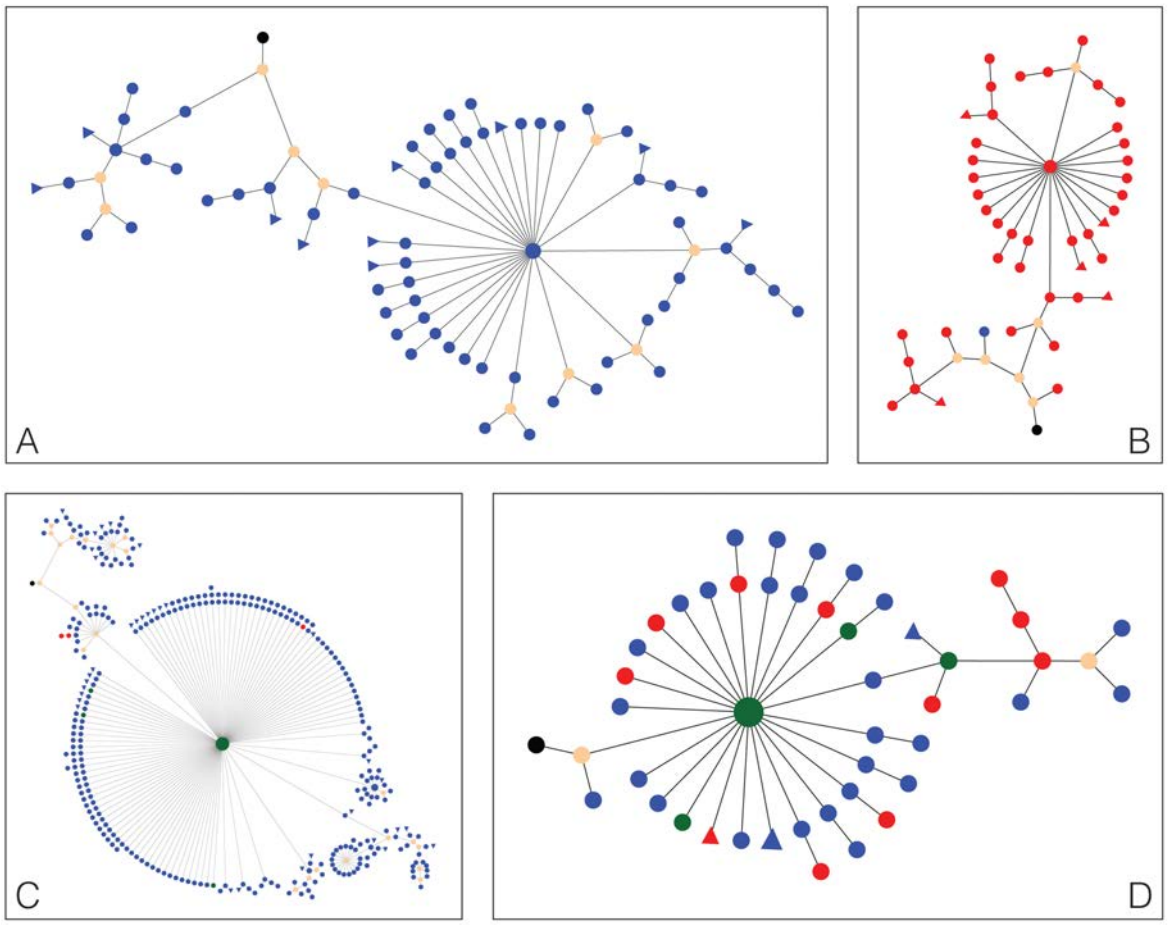

Figure 4. Intimate connections between CNS and peripheral B-cells in MS. Representative lineages of clonally related IgG-VH found in CSF (***A***), or in CSF and PBL (***B–D***) of MS patients as calculated by IgTree software and visualized in Cytoscape version 3.1 (organic layout) (Cytoscape Consortium, San Diego, CA). Each round node represents at least one unique IgG-VH sequence ranging from at least the 5' end of H-CDR1 to the 3' end of H-CDR3; larger nodes represent up to hundreds of identical sequences. Blue nodes, CSF-derived IgG-VH sequences; red nodes, PBL-derived sequences; green nodes, identical sequences found in both compartments. Black nodes, putative germline sequences represent the lineage root; beige nodes, hypothetical intermediates calculated by IgTree. Triangular nodes contain two or more singleton sequences in leaves. ***A***, intrathecal affinity maturation; ***B***, IgG-VH lineage with predominantly PBL-derived IgG-VH suggestive of B-cell migration from the CNS to the PBL or seeding from the PBL into the CNS; ***C*** suggests B-cell migration from the PBL into the CNS, with traces of the clusters remaining in the PBL and with extensive intrathecal B-cell SHM; ***D*** suggests ongoing B-cell exchange across the BBB, or affinity maturation occurring in both compartments in parallel. H-CDR1, heavy-chain complementarity-determining region 1. Reprinted with permission from Hauser (2015), Fig. 7. Copyright 2015, SAGE Publications.

that of others studying CSF (Owens et al., 2007) and brain tissue (Owens et al., 1998) clearly show that the activated B-cell clones in the CNS of MS patients display a bias in terms of increased usage of members of the IgG VH4 family. These data raise the possibility that even more-selective therapies based on targeting restricted populations of B-cells defined by their surface Ab receptors could be effective.

B-Cells and Progressive MS

As discussed earlier, the anti-CD20 therapies eliminate mostly circulating B-cells, leaving B-cells in secondary lymphoid organs and other sites partially unaffected. This feature could account for their favorable safety profile; however, at least in theory, it could also pose a challenge to effectively treating progressive MS (Hauser et al., 2013). If established B-cell nests residing in lymphoid follicle-like structures in the meninges are drivers of a chronic neurodegenerative process that ultimately results in progressive MS (Magliozzi et al., 2007), then anti-CD20 therapy would likely fail to deplete B-cells from these sites. This resistance of B-cells in protective niches could explain the relatively meager

response of PPMS (Hawker et al., 2009; Montalban et al., 2017) and the observation that RMS can evolve to secondary progressive MS despite ongoing RTX treatment. It is also possible that long-lived, CD20-negative plasma cells and their Ab products play some role in progressive MS. OCBs can persist in the CSF even after chronic treatment with RTX, indicating that aberrant humoral immune responses have not been eliminated from the CNS. Eliminating these CNS-restricted humoral immune responses might require the development of MAbs that disrupt protective niches (Radford et al., 2013), penetrate the BBB more effectively, and/or directly lyse Ig-secreting plasma cells. Another area of promise is to develop small molecules that inhibit critical B-cell signaling pathways (Puri et al., 2013; Byrd et al., 2014; Furman et al., 2014).

Conclusions

Looking back to that distant seminar room in Boston, it would have been impossible to imagine that almost 40 years later, B-cells would rest, arguably, at the epicenter of MS immunology. The B-cell saga in MS has provided a cornucopia of surprises, thrilling insights, several disappointments, numerous unsolved conundrums, and a few generic lessons. Foremost among the latter is the importance of road-testing ideas developed in the laboratory in real-life clinical situations and vice versa. Finally, the long process from conception to an initial clinical suggestion of efficacy, to completing the definitive clinical trials, amply demonstrates the bumpy and uncertain road that accompanies forays between academia and industry. This bidirectional process must be made more efficient if we are to effectively translate new discoveries into treatments and cures for our patients.

Acknowledgments

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