

MULTIPLE SCLEROSIS

Street-Experienced Peripheral B Cells Traffic to the Brain

Daniel R. Lu^{1,2} and William H. Robinson^{1,2,3*}Deep sequencing of immunoglobulin repertoires in multiple sclerosis patients reveals dysregulation of peripheral B cell immunity (Palanichamy *et al.* and Stern *et al.*, this issue).

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) that results from autoimmune-mediated destruction of the myelin sheath. Among the most prominent immunodiagnostic features of MS is the accumulation of B cell–secreted antibodies in the CNS and cerebrospinal fluid (CSF). Observations that the number of B cells in CSF correlates positively with MS progression and that the CNS microenvironment in MS patients promotes B cell persistence and expansion also support B cell involvement (1). Recent clinical findings demonstrate that B cell–targeted therapies profoundly mitigate disease activity in MS patients. Specifically, clinical studies show that CD20 B cell–depleting therapies significantly reduce the frequency of relapses and of new demyelinating lesions, and accumulating evidence suggests that immunotherapies that block immune-cell trafficking across the blood-brain barrier (BBB) reduce disease activity in part by impeding B cell function (2).

Although these observations indicate that B cells contribute to MS pathogenesis, little is known about the sites of B cell–affinity maturation that produce the putative pathogenic B cells or the mechanisms that regulate antigen-dependent selection and activation of the B cells that populate the CNS. In this issue of *Science Translational Medicine*, Palanichamy *et al.* (3) and Stern *et al.* (4) provide evidence of bidirectional B cell migration and suggest that antigen-experienced B cells mature in peripheral lymph nodes before transmigrating to the CNS. These results provide insight into the trafficking of the presumed pathogenic B cells in MS and a framework by which peripheral deletion or modulation of specific B cell subsets could provide therapeutic benefit.

BLOOD-TO-CSF CONNECTION

Deep sequencing of the variable region genes of immunoglobulin heavy chains (Ig-VH) in the CSF and peripheral blood of MS patients pinpointed a population of B cells in the peripheral blood that used the same Ig genes and complementarity-determining region 3 sequences as those of B cells in the CSF; these findings revealed the existence of bicompartamental lineages that connect peripheral and CNS B cells in MS (5). Palanichamy and colleagues (3) extend this line of investigation by delineating the functional subsets of peripheral B cells that share lineages with B cells in the CSF.

By comparing the Ig-VH repertoire of cell-sorted peripheral B cell subsets to that of B cells in patient-matched CSF, the authors found that peripheral class-switched memory B cells and plasma cells have the greatest degree of sequence overlap with CSF B cells, whereas peripheral unswitched memory B cells and naïve B cells have the least. Thus, it appears that antigen-experienced, and not naïve, B cells constitute the humoral immune axis that spans the peripheral blood and the CNS. Because B cells can differentiate into switched memory B cells and plasma cells only after a productive germinal-center reaction, these findings support the emerging concept that MS pathology derives from successive rounds of CNS tissue damage, by which early antigen encounters generate autoreactive memory B cells, and subsequent antigen encounters activate memory B cells, resulting in B cell–mediated tissue destruction in the CNS (Fig. 1).

WHERE B CELLS MATURE IN MS

Where do these autoreactive B cells initially undergo affinity maturation? In order for the humoral immune system to develop affinity-matured antibody responses, B cells must traffic to germinal centers, where activation signals trigger pro-survival signals, somatic hypermutation, and antibody class-switching. Stern and colleagues (4) compared the immunoglobulin repertoires in sectioned

MS brain tissues with those in patient-matched cervical lymph nodes that drain the brain so as to determine whether B cells associated with MS gain antigen experience in peripheral lymphoid tissues or within the CNS. They observe that B cell populations that contained sequences most closely resembling germline sequences—which they term “founder events”—are highly enriched in B cell lineages that span both tissues despite their low frequency. This implies that B cell lineages with members in both the CNS and CLN are prone to undergo additional rounds of affinity maturation.

The authors then examine lineage trees for the occurrence of founders within the CNS and cervical lymph nodes and found that ~90% of founders are present in the cervical lymph nodes. Although it is impossible to definitively conclude where B cells first encounter antigen on the basis of sequence data alone, these results support a role for peripheral immunity in shaping the repertoire of CNS B cells and suggest that the initial antigen-dependent maturation of intercompartmental B cells occurs predominantly in the periphery. This concept is supported by previous studies that identify neuronal-derived antigens in the cervical lymph nodes of MS patients (6).

CLINIC AND QUESTIONS

The studies by Palanichamy *et al.* (3) and Stern *et al.* (4) highlight distinct but complementary intricacies of bidirectional B cell exchange across the BBB in MS. Both studies conclude that bicompartamental Ig-VH sequences derive from post-germinal center B cells, as evidenced by class-switching to IgG and IgA and the accumulation of extensive somatic mutations. In some instances, the sequences of bicompartamental lineages that displayed a high number of mutations were present in the CNS tissues, and in other instances, they were present in the periphery. This distribution of highly mutated sequences suggests a bidirectional exchange of B cells across the BBB and argues that affinity maturation can occur in both the CNS tertiary lymphoid structures and the cervical lymph nodes (Fig. 1).

Therapeutic agents that target immune function by inhibiting B cell trafficking might abrogate cross-compartmental B cell movement and activation and thereby provide benefit in MS. Two therapeutics currently being used to treat MS are natalizumab, a humanized monoclonal antibody that targets a cell adhesion molecule necessary for

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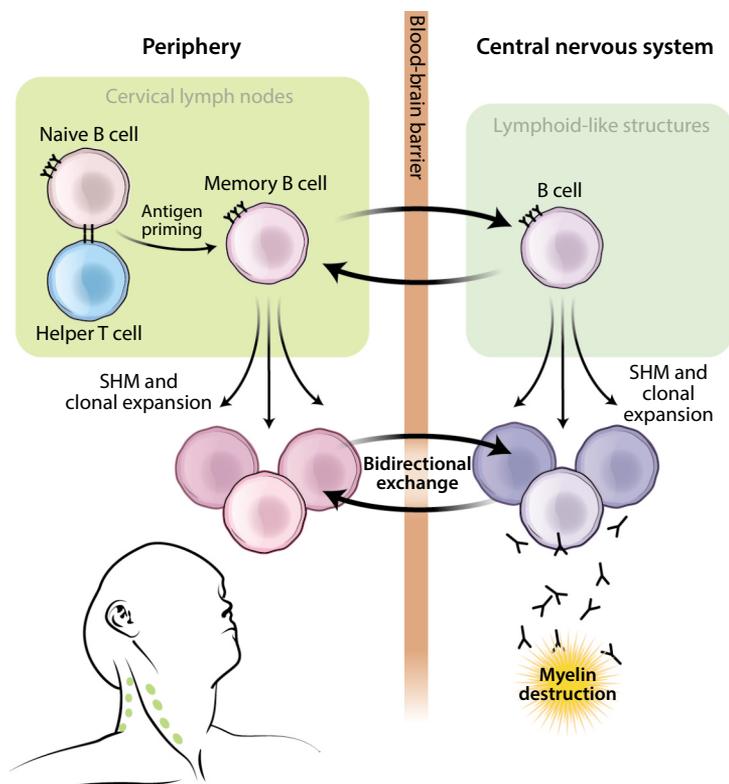


Fig. 1. Street-experienced B cell traffic. In MS, peripheral antigen exposure results in antigen priming of naïve B cells and the formation of immunological memory. These primed B cells undergo affinity maturation and clonal expansion in the cervical lymph nodes and then extravasate into the CNS. Formation of ectopic lymphoid-like structures in the CNS perpetuates the activation and affinity maturation of these B cells, which transmigrate between the CNS and periphery. Thus, peripheral antigen experience modulates CNS B cell responses in MS. B cells likely contribute to the pathogenesis of MS by producing autoantibodies, serving as professional antigen-presenting cells, and producing proinflammatory cytokines. SHM, somatic hypermutation.

immune cell movement across tissues, and FTY720, which binds to a G protein-coupled receptor thought to regulate immune cell movement into the circulation. But, additional research is needed to further define how these agents affect B cell trafficking and B cell-mediated inflammation. Bidirectional trafficking of B cells might also explain how therapies that deplete circulating B cells also reduce B cells in cerebral perivascular spaces (2, 7), which may be responsible for the therapeutic activity of such agents in MS.

The fact that it is affinity-matured memory B cells that contribute to the immune continuum between the periphery and CNS has implications for MS pathogenesis. Because formation of memory B cells requires prior encounters with antigen and the successful completion of the germinal center reaction, the new findings (3, 4) suggest that aberrant exposure of myelin antigens, neuronal antigens, or microbial antigenic mimics to

the immune system shapes the autoimmune response in MS patients (1). Treatments that reduce the magnitude and number of inflammatory events in at-risk individuals or hamper the formation of immunological memory may avert the development of clinical MS.

The exciting new findings (3, 4), while offering a snapshot of antibody repertoires at a single time point, raise a number of questions about the role of B cells in MS. At what stage do potentially autoreactive B cells first encounter the antigens that initiate their selection or activation, and can this be linked to clinically distinct inflammatory phenotypes? In addition, most of the patients sequenced had relapsing-remitting MS or progressive MS and thus had an advanced autoimmune response. Monitoring the antibody repertoires of individuals who are at risk of developing MS may provide greater insights into the role of B cells in MS pathol-

ogy by showing how the B cell repertoire evolves over the course of the disease.

B cells are thought to contribute to MS plaque formation through the secretion of proinflammatory cytokines, the presentation of antigen to other immune cells, or antibody-mediated mechanisms. Although Palanichamy *et al.* (3) and Stern *et al.* (4) assume that B cells that transmigrate across the BBB contribute to MS pathology, it is impossible to determine whether these B cells are activated and homing to the CNS in response to one or more antigens without knowing what antigens these B cells target. Various papers have identified autoantigens present in MS patients (8, 9), but it is not known whether these antigens are targeted in all or only a subset of these patients. Both papers report an overrepresentation of certain IGHV4 germline segments, a finding that suggests that selection of antibodies in MS is skewed toward particular antigen epitopes. Because MS is known to present with a diverse array of clinical features, improved classification of antibody-repertoire heterogeneity and specificity might enable current and future treatments to be more effectively targeted to relevant patient populations.

Although the sequencing approaches used by Palanichamy *et al.* (3) and Stern *et al.* (4) do not provide pairing of heavy- and light-chain immunoglobulin genes expressed by individual B cells, high-throughput antibody repertoire sequencing approaches that enable such pairing are now available (10). Such methodologies will enable more comprehensive characterization of antibody repertoires in MS and other autoimmune diseases. Further, these next-generation technologies enable recombinant production of representative antibodies and thus identification of the antibodies' autoantigen targets, as well as assessment of their pathogenicity. Such characterization of representative antibodies will allow the "functional annotation" of antibody repertoires and thereby link the repertoires to autoimmune specificity and disease pathogenesis.

REFERENCES AND NOTES

1. M. Krumbholz, T. Derfuss, R. Hohlfeld, E. Meinl, B cells and antibodies in multiple sclerosis pathogenesis and therapy. *Nat. Rev. Neurol.* **8**, 613–623 (2012).
2. K. Lehmann-Horn, H. C. Kronsbein, M. S. Weber, Targeting B cells in the treatment of multiple sclerosis: Recent advances and remaining challenges. *Ther. Adv. Neurol. Disord.* **6**, 161–173 (2013).
3. A. Palanichamy, L. Apeltsin, T. C. Kuo, M. Sirota, S. Wang, S. J. Pitts, P. D. Sundar, D. Telman, L. Z. Zhao, M. Derstine, A. Abounasr, S. L. Hauser, H.-C. von Büdingen, Immunoglobulin class-switched B cells form an active immune

- axis between CNS and periphery in multiple sclerosis. *Sci. Transl. Med.* **6**, 248ra106 (2014).
4. J. N. H. Stern, G. Yaari, J. A. Vander Heiden, G. Church, W. F. Donahue, R. Q. Hintzen, A. J. Huttner, J. D. Laman, R. M. Nagra, A. Nylander, D. Pitt, S. Ramanan, B. A. Siddiqui, F. Vigneault, S. H. Kleinstein, D. A. Hafler, K. C. O'Connor, B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. *Sci. Transl. Med.* **6**, 248ra107 (2014).
 5. H. C. von Büdingen, T. C. Kuo, M. Sirota, C. J. van Belle, L. Apeltsin, J. Glanville, B. A. Cree, P. A. Gourraud, A. Schwartzburg, G. Huerta, D. Telman, P. D. Sundar, T. Casey, D. R. Cox, S. L. Hauser, B cell exchange across the blood-brain barrier in multiple sclerosis. *J. Clin. Invest.* **122**, 4533–4543 (2012).
 6. B. O. Fabrick, J. N. Zwemmer, C. E. Teunissen, C. D. Dijkstra, C. H. Polman, J. D. Laman, J. A. Castelijns, In vivo detection of myelin proteins in cervical lymph nodes of MS patients using ultrasound-guided fine-needle aspiration cytology. *J. Neuroimmunol.* **161**, 190–194 (2005).
 7. M. del Pilar Martin, P. D. Cravens, R. Winger, B. C. Kieseier, S. Cepok, T. N. Eagar, S. S. Zamvil, M. S. Weber, E. M. Frohman, B. K. Kleinschmidt-Demasters, T. J. Montine, B. Hemmer, C. M. Marra, O. Stüve, Depletion of B lymphocytes from cerebral perivascular spaces by rituximab. *Arch. Neurol.* **66**, 1016–1020 (2009).
 8. R. Srivastava, M. Aslam, S. R. Kalluri, L. Schirmer, D. Buck, B. Tackenberg, V. Rothhammer, A. Chan, R. Gold, A. Berthele, J. L. Bennett, T. Korn, B. Hemmer, Potassium channel KIR4.1 as an immune target in multiple sclerosis. *N. Engl. J. Med.* **367**, 115–123 (2012).
 9. F. J. Quintana, M. F. Farez, V. Vigiuetta, A. H. Iglesias, Y. Merbl, G. Izquierdo, M. Lucas, A. S. Basso, S. J. Khoury, C. F. Lucchinetti, I. R. Cohen, H. L. Weiner, Antigen microarrays identify unique serum autoantibody signatures in clinical and pathologic subtypes of multiple sclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 18889–18894 (2008).
 10. Y. C. Tan, S. Kongpachith, L. K. Blum, C. H. Ju, L. J. Lahey, D. R. Lu, X. Cai, C. A. Wagner, T. M. Lindstrom, J. Sokolove, W. H. Robinson, Barcode-enabled sequencing of plasma-blast antibody repertoires in rheumatoid arthritis. *Arthritis Rheumatol.* (2014).

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