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Mass cytometry provides unprecedented insight into the role of B cells during the pathogenesis of multiple sclerosis

Key take-home messages

- B cells can play a detrimental and protective role in the pathogenesis of multiple sclerosis
- Mass cytometry provides insight into the multitude of B cell subsets
- Interrogating B cell subsets will provide further insight into the pathogenesis of multiple sclerosis

Abstract

In recent years, it has become clear that B cells play a prominent role in the pathogenesis of multiple sclerosis (MS). This is most evident when considering the effectiveness of anti-CD20 monoclonal therapeutics including rituximab and ocrelizumab. In fact many successful therapeutics alter the level of switched memory B cells. It is however unlikely all switched memory B cells are detrimental in the context of MS. The ability to distinguish between various B cell subsets is hence important if we are to more specifically target detrimental from potentially beneficial B cells. Mass cytometry provides the ability to interrogate a larger number of markers in a single experiment, allowing unprecedented insight into B cell subsets and how they contribute to MS disease progression. This review highlights the importance of investigating B cells in the context of MS, and how mass cytometry provides the ability to interrogate a large number of subsets for an in-depth characterisation.

B cells can play a detrimental and protective role in the pathogenesis of multiple sclerosis

In recent years, it has become clear that B cells play a prominent role in the pathogenesis of multiple sclerosis (MS). This is most evident when considering the effectiveness of anti-CD20 monoclonal therapeutics including rituximab[1] and ocrelizumab.[2] The majority of successful disease-modifying therapeutics (DMTs) including monoclonal antibodies, are incapable of crossing the blood-brain barrier, cladribine[3,4] and fingolimod[5] being exceptions. The mechanism of action of successful therapeutics such as cladribine,[6] anti-CD19 (inebilizumab), anti-CD52 (alemtuzumab), S1P agonist (fingolimod), anti-VLA-4 (natalizumab) and dimethyl fumarate,[7] appears to involve modulating the level of circulating B cells within peripheral blood. More specifically, these studies have found $CD27^+$ memory B cells to be particularly affected, with efficacy correlating with large numbers of memory B cells being removed from circulation. It has therefore been proposed that memory B cells play a key role in MS pathogenesis.[8] As part of the adaptive immune response, memory B cells provide defence against previously encountered pathogens. In people with MS, the majority of B cells found within white matter lesions are $CD27^+$ memory B cells.[9] Although the exact role of memory B cells in the context of MS is yet to be fully understood, recent work by Jelcic et al.[10] found memory B cells were capable of activating brain-homing T cells that may contribute to disease pathogenesis. However, it is unlikely all circulating memory B cells contribute to disease pathogenesis, meaning that more work is needed to differentiate pathogenic from non-pathogenic subsets of memory B cells.

There is growing evidence not all B cells are detrimental in the context of MS, with some playing a protective role. These so called "regulatory B cells" or B_{Regs} are capable of suppressing an immune response and many studies have investigated B_{Regs} in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Mice deficient in IL-10-producing B cells are incapable of recovering from EAE.[11] While depleting B cells with anti-CD20 prior to EAE induction worsens disease, removal of B cells *after* signs of clinical disease reduces disease severity.[12] Thus, B cells are important for preventing the development of CNS-autoimmunity and limiting disease severity, but once disease has developed, different B cells subsets are pathogenic. Novel DMTs such as exposure of the skin to ultraviolet (UV) radiation which can protect mice from EAE[13] and delay the onset of MS[14], work in part by activating EAE-protecting B cells.[15]

In contrast to regulatory T cells, which are routinely defined by their high expression of CD25 and FoxP3 and low levels of CD127[16] there is no defined phenotype that enables the reliable identification of B_{Regs}. This has led to the hypothesis that any B cell has the potential to become regulatory, and that it depends on the environment in which it finds itself as to whether the B cell exerts immune regulation.[17] In fact, many subsets of B cells

have been found to produce immunoregulatory IL-10, including transitional B cells, naïve B cells, plasma cells, plasmablasts and even memory B cells.[7] Furthermore, B cells can suppress the immune response in IL-10-independent mechanisms, including through TGF-β or IL-35 production, or via expression of co-inhibitory molecules such as PD-L1 or GITRL.[7] Although IL-10 is most well-known for its suppressive capabilities, IL-10 can act as a B cell stimulant to promote immunoglobulin production.[18] The success and failure of some DMTs provide clinical clues as to which B cells in MS patients are likely to be pathogenic and which are potentially protective. The success of CD20-targeting monoclonal antibodies suggest that in the context of MS, pathogenic B cells may express high levels of CD20. Alternatively, MS-protective B cells may reside in the plasma cell lineage which express low or negligible amounts of CD20 on their surface. The results from a trial of atacicept (a fusion protein of immunoglobulin and TACI that blocks signals from BAFF and APRIL) showed that this DMT exacerbates disease in MS patients.[19] This failure suggests that either atacicept fails to deplete potentially pathogenic memory B cells,[8] or may starve regulatory B cells of the cytokines they need to survive.

Mass cytometry provides insight into the multitude of B cell subsets

Recent advancements in flow cytometry, particularly mass cytometry, have enabled examination of more than 40 markers in a single panel. Mass cytometry is similar to conventional fluorescence flow cytometry in that cells are stained with antibodies, but rather than antibodies being conjugated to fluorochromes they are instead conjugated to Lanthanide heavy metal isotopes.[20] The use of heavy metal isotopes that are not naturally found in cells, rather than fluorochromes, avoids problems of spectral overlap enabling many more markers to be investigated in a single panel. Stained cells will then be run through a flow cytometer coupled with a mass spectrometer. Following incineration, only the heavy metal isotopes remain which are then subjected to time-of-flight mass spectrometry to differentiate between the metals based on their molecular mass. Computational extrapolation to the cellular flow event allows for the identification of specific markers on (and within) individual cells.

Mass cytometry has recently been used to identify 25 subsets of regulatory T cells within the bone marrow of multiple myeloma patients,[21] whilst Christophersen et al.[22] used a tetramer to identify and phenotype T cells that recognise the gluten antigen in coeliac disease patients. It is hence possible to not only identify a range of cell subsets, but also provide unparalleled insight into the potential function of these cells. In contrast to T cells, not nearly as much work has been done to identify B cell subsets to the same extent. In fact many immunophenotyping studies simply use CD19 to identify total B cells rather than individual subsets. Sundling et al.[23] utilised mass cytometry to identify 10 subsets of B cells within malaria patients. In our own studies, mass cytometry has identified 9 individual subsets of IgG3$^+$ B cells.[24] In this study, we found the proportion of circulating IgG3$^+$ B cells to increase as clinically-isolated syndrome patients convert to MS, whilst MS patients with active disease had a greater level compared to those with inactive MS. It is evident there are many more subsets of B cells in circulation than fairly represented in current studies, so it is important to differentiate between them at a phenotypic level to better understand their role in MS pathogenesis.

Concluding remarks

There is no longer any doubt that B cells contribute to disease pathogenesis in MS. Differentiating between detrimental and protective B cells is challenging but essential if we are to target these immune cells more specifically and effectively in the prevention and treatment of MS. Advancements in cytometry that allow for the evaluation of an increased number of parameters for a single cell provide greater power for interrogating B cell subsets. Mass cytometry allows more defined phenotyping of individual B cell subsets and revelation of their potential function. More work remains to be done, and mass cytometry will continue to provide important insight into the pathogenesis of MS, and how B cells may both contribute to and protect from such a disease.

REFERENCES

1. Cross AH, Stark JL, Lauber J, Ramsbottom MJ, Lyons JA. *Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients.* J Neuroimmunol. 2006;180:63-70.
2. Kappos L, Li D, Calabresi PA, O'Connor P, Bar-or A, Barkhof F, Yin M, Leppert D, Glanzman R, Tinbergen J, Hauser SL. 2011. *Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial.* Lancet. 2011;378:1779-87.
3. Saven A, Kawasaki H, Carrera CJ, Waltz T, Copeland B, Zyroff J, Kosty M, Carson DA, Beutler E, Piro LD. 1993. *2-Chlorodeoxyadenosine dose escalation in nonhematologic malignancies.* J Clin Oncol. 1993;11:671-8.
4. Kearns CM, Blakley RL, Santana VM, Crom WR. *Pharmacokinetics of cladribine (2-chlorodeoxyadenosine) in children with acute leukemia.* Cancer Res. 1994;54:1235-9.
5. Foster CA, Howard LM, Schweitzer A, Persohn E, Hiestand PC, Balatoni B, Reuschel R, Beerli C, Schwartz M, Billich A. 2007. *Brain penetration of the oral immunomodulatory drug FTY720 and its phosphorylation in the central nervous system during experimental autoimmune encephalomyelitis: consequences.*
6. Ceronie B, Jacobs BM, Baker D, Dubuisson N, Mao Z, Ammoscato F, Lock H, Longhurst HJ, Giovannoni G, Schmierer K. 2018. *Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells.* J Neurol. 2018.
7. Li R, Patterson KR, Bar-Or A. *Reassessing B cell contributions in multiple sclerosis.* Nat Immunol. 2018.
8. Baker D, Marta M, Pryce G, Giovannoni G, Schmierer K. *Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis.* EBioMedicine. 2017;16:41-50.
9. Serafini B, Severa M, Columba-Cabezas S, Rosicarelli B, Veroni C, Chiappetta G, Magliozzi R, Reynolds R, Coccia EM, Aloisi F. *Epstein-Barr virus latent infection and BAFF expression in B cells in the multiple sclerosis brain: implications for viral persistence and intrathecal B-cell activation.* J Neuropathol Exp Neurol. 2010;69:677-93.
10. Jelcic I, Al Nimer F, Wang J, Lentsch V, Planas R, Jelcic I, Madjovski A, Ruhrmann S, Faigle W, Frauenknecht K, Pinilla C, Santos R, Hammer C, Ortiz Y, Opitz L, Gronlund H, Rogler G, Boyman O, Reynolds R, Lutterotti A, Khademi M, Olsson T, Piehl F, Sospedra M, Martin R. 2018. *Memory B Cells Activate Brain-Homing, Autoreactive CD4(+) T Cells in Multiple Sclerosis.* Cell. 175:85-100 e23.
11. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. *B cells regulate autoimmunity by provision of IL-10.* Nat Immunol. 2002;3:944-50.
12. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. *Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression.* J Clin Invest. 2008;118:3420-30.
13. Hauser SL, Weiner HL, Che M, Shapiro ME, Gilles F, Letvin NL. 1984. *Prevention of experimental allergic encephalomyelitis (EAE) in the SJL/J mouse by whole body ultraviolet irradiation.* J Immunol. 1984;132:1276-81.
14. Hart PH, Jones AP, Trend S, Cha L, Fabis-Pedrini MJ, Cooper MN, D'Este C, Geldenhuys S, Carroll WM, Byrne S, Booth DR, Cole JM, Lucas RM, Kermode AG. *A randomised, controlled clinical trial of narrowband UVB phototherapy for clinically isolated syndrome: The PhoCIS study.* Multiple Sclerosis Journal - Experimental, Translational and Clinical. 4. 2018.
15. Kok LF, Marsh-Wakefield F, Marshall JE, Gillis C, Halliday GM, Byrne SN. 2016. *B cells are required for sunlight protection of mice from a CNS-targeted autoimmune attack.* J Autoimmun. 2016;73:10-23.
16. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, Solomon M, Selby W, Alexander SI, Nanan R, Kelleher A, Fazekas De St Groth B. *Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells.* J Exp Med. 2006;203:1693-700.
17. Rosser EC, Mauri C. *Regulatory B cells: origin, phenotype, and function. Immunity.* 2015;42:607-12.
18. Defrance T T, Vanbervliet B, Briere F, Durand I, Rousset F, Banchereau J. *Interleukin 10 and transforming growth factor beta cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A.* J Exp Med. 1992;175:671-82.
19. Kappos L, Hartung HP, Freedman MS, Boyko A, Radu EW, Mikol DD, Lamarine M, Hyvert Y, Freudensprung U, Plitz T, Van Beek J. *Atacicept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial.* The Lancet Neurology. 2014;13:353-363.
20. Bendall S, Simonds EF, Qiu P, Amir El AD, Krutzik PO, Finck R, Bruggner RV, Melamed R, Trejo A, Ornatsky OI, Balderas RS, Plevritis SK, Sachs K, Pe'er D, Tanner SD, Nolan GP. *Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum.* Scienc., 2011;332:687-96.
21. Marsh-Wakefield F, Kruzins A, McGuire HM, Yang S, Bryant C, Fazekas De St Groth B, Nassif N, Byrne SN, Gibson J, Brown C, Larsen S, McCulloch D, Boyle R, Clark G, Joshua D, Ho PJ, Vuckovic S. *Mass Cytometry Discovers Two Discrete Subsets of CD39(-)Treg Which Discriminate MGUS From Multiple Myeloma.* Front Immunol. 2019;10:1596.
22. Christopherson A, Lund EG, Snir O, Sola E, Kanduri C, Dahal-Koirala S, Zuhlke S, Molberg O, Utz PJ, Rohani-Pichavant M, Simard JF, Dekker CL, Lundin KEA, Sollid LM, Davis MM. *Distinct phenotype of CD4(+) T cells driving celiac disease identified in multiple autoimmune conditions.* Nat Med. 2019.
23. Sundling C, Ronnberg C, Yman V, Asghar M, Jahnmatz P, Lakshmikanth T, Chen Y, Mikes J, Forsell MN, Sonden K, Achour A, Brodin P, Persson KE, Farnert A. *B cell profiling in malaria reveals expansion and remodelling of CD11c+ B cell subsets.* JCI Insight, 5. 2019.
24. Marsh-Wakefield F, Ashhurst T, Trend S, McGuire HM, Juillard P, Zinger A, Jones AP, Kermode AG, Hawke S, Grau GE, Hart PH, Byrne SN. *IgG3+ B cells are associated with the development of multiple sclerosis.* Clin Transl Immunology. DOI 10.1002/cti2.1133. 2020.