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INSIGHTS

The BAFFling persistence of memory B cells

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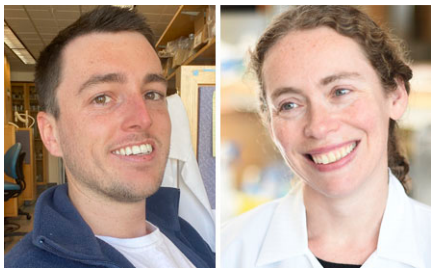
Although BAFF/BLyS and its receptor, BAFFR, play critical roles in naive B cell survival, the pathways involved in the persistence of memory B cells are largely unknown. In this issue of *JEM*, two groups, Müller-Winkler et al. (https://doi.org/10.1084/jem.20191393) and Lau et al. (https://doi.org/10.1084/jem.20191167), take complementary approaches to identify an essential role for BAFFR in the survival of memory B cells.

The cellular basis for long-lived humoral immunity (and for much of the protection conferred by vaccines) rests with two antigen-experienced B cell–derived populations, long-lived plasma cells (LLPCs) and memory B cells (MBCs). Emerging late in the course of the germinal center (GC) reaction, LLPC are the products of a Darwinian evolutionary process in which cells expressing the highest affinity B cell receptors (BCRs) are selected from among a randomly mutated repertoire. LLPCs migrate to and take up residence in a specialized bone marrow niche where they can persist for many years, constitutively secreting massive quantities of protective antibodies that (ideally) confer sterilizing immunity to the inciting pathogen. The other long-lived B cell population that is generated in the wake of a primary immune response, MBC, has been more challenging to study and is consequently less well understood.

MBCs generated during a primary immune response serve as a vital reservoir of broadly cross-reactive antibody specificities against pathogens (such as influenza) that undergo antigenic drift over time to escape recognition by preexisting LLPC-derived antibodies. MBC can mount rapid recall antibody responses, and they also have the capacity to reenter GC to undergo further affinity maturation and replenish both LLPC and MBC pools (Mesin et al., 2020; Turner et al., 2020). We now appreciate that there are multiple subpopulations of MBCs with specialized functions (see figure, panel A; Weisel and Shlomchik, 2017); in mice, PD-L2$^+$ MBCs (some of which are also CD80$^+$) harbor somatically mutated BCRs that are either class-switched or unswitched. These MBCs emerge from the peak GC reaction at time points earlier than LLPCs and are predisposed to rapidly differentiate into plasma cells to mount the classic "stronger, faster" (and higher affinity) recall response to antigen reencounter. By contrast, a subpopulation of largely unmutated and lower-affinity IgM$^+$ MBCs express neither PD-L2 nor CD80, and emerge in the first few days of a primary immune response before the development of GCs. It is proposed that this MBC subpopulation may be important to respond to more distantly related pathogens. However, the precise circumstances under which these lower-affinity MBCs are recruited into recall responses and how they contribute to host defense remain to be fully defined (Mesin et al., 2020; Turner et al., 2020).

Although substantial progress has been made in defining the cytokines and signaling pathways that are essential for LLPC survival, very little is understood about the basis for MBC persistence even though their long life is perhaps their most essential feature; the lifespan of MBC has been documented to exceed that of the mouse itself (Jones et al., 2015). Indeed, MBCs have been detected in survivors of the 1918 flu pandemic 90 yr later (Yu et al., 2008). This has proven hard to address, in part, because antigen-specific MBCs are rare and can be challenging to track in mice, and because experimental strategies to disentangle and isolate requirements for MBC survival from that of precursor B cells have been lacking.



Insights from Jeremy F. Brooks and Julie Zikherman.

In contrast to MBCs, naive follicular B cells in mice are abundant, and the molecular basis for their survival was established using a pioneering set of elegant conditional genetic approaches (Lam et al., 1997; Srinivasan et al., 2009). These studies revealed an essential role for tonic BCR signaling, in the absence of which naive B cells were lost in a matter of days (rather than the weeks to months of a typical mature B cell lifespan). Components of the proximal BCR signaling apparatus were subsequently shown to be required for MBC

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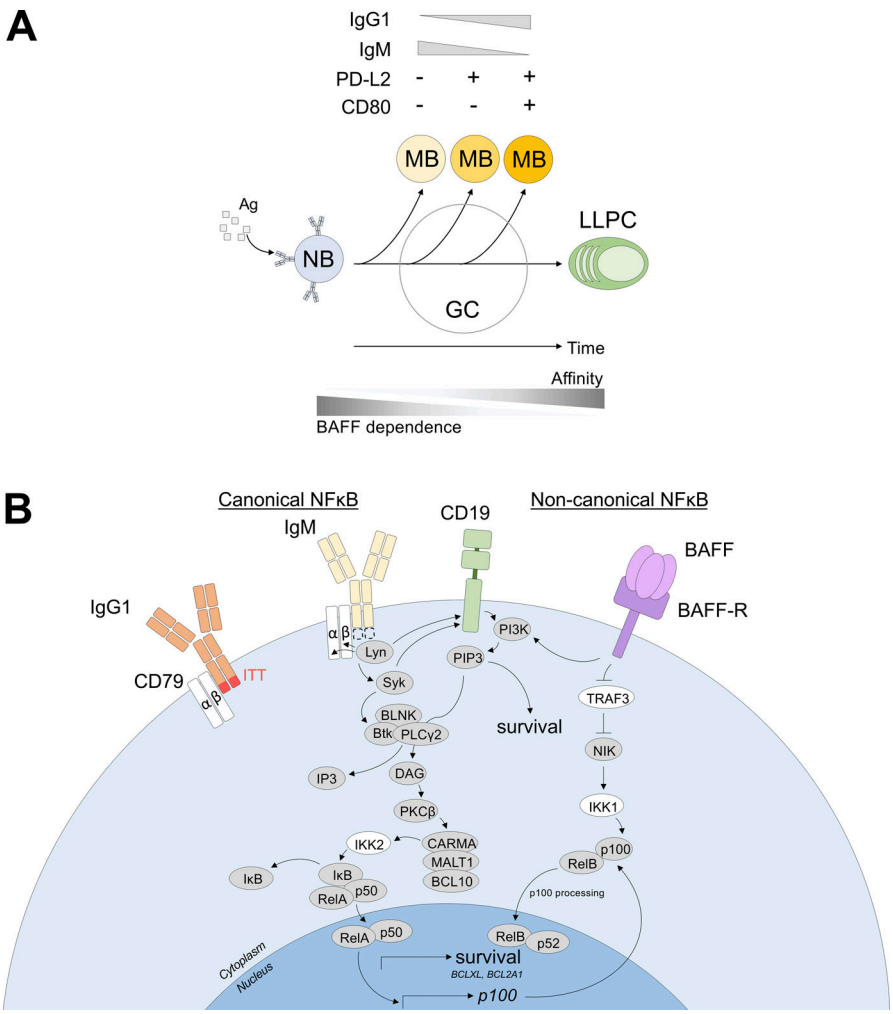

Cellular and molecular dynamics of BAFF dependence in B cells. (A) Activation of naive B cells (NB) generates LLPC and a heterogeneous pool of MBC (MB) by GC-independent and GC-dependent pathways. GC-independent, predominantly PD-L2$^-$CD80$^-$ unswitched, low-affinity MBCs are generated early in the response. By contrast, after progressing through the GC, some MBCs acquire affinity-matured, class-switched BCRs and can be distinguished primarily by the expression of PD-L2 and CD80 (Weisel and Shlomchik, 2017). Based on studies in which BAFF has been depleted or BAFFR expression has been genetically deleted, B cell dependence on BAFF can be positioned along a spectrum, ordered by NB > unswitched PD-L2$^-$ MBC > switched PD-L2 MBC. (B) MBC survival is transduced via NF-κB. BCR signaling triggers the canonical NF-κB pathway (via IKK1/IKK2/NEMO) to activate RelA/p50 (NF-κB1), while BAFF:BAFFR triggers the complementary noncanonical pathway (via IKK1) to activate RelB/p52 (NF-κB2) via processing of p100. However, cross-talk between two pathways is important; BCR signaling is required to replenish p100 substrate for processing downstream of BAFFR activation (Cancro, 2009). Additionally, BAFFR and BCR cross-talk is mediated by CD19 and PI3K. Genetic perturbations assessed in the two current papers to TRAF3, IKK1, IKK2, CD79 (all highlighted in white), BCR, and BAFFR reveal that MBC survival is dependent on both NF-κB pathways. Unlike IgM BCR, an immunoglobulin tail tyrosine (ITT) motif in the IgG1 BCR can facilitate signal transduction and may function to reduce dependence on CD79α in MBC.

survival, while cognate antigen was dispensable, suggesting an analogous dependence upon tonic BCR signaling (Weisel and Shlomchik, 2017).

A more complete understanding of naive B cell homeostasis fell into place with the discovery of the TNF receptor superfamily member BAFFR (also known as BR3 or TNFRSF13C) and its ligand BAFF/BLyS. This ligand–receptor pair functions as a "thermostat" to control both follicular and marginal zone B cell number and explains how a consistent population of mature B cells is maintained over time and why self-reactive B cells are competitively eliminated in the presence of nonself-reactive B cells (Cancro, 2009; Jackson and Davidson, 2019). Importantly, this set of discoveries led ultimately to the development of an anti-BAFF antibody, belimumab, as the first Food and Drug Administration–approved therapy for systemic lupus erythematosus (SLE) since prednisone itself was approved decades earlier. Naturally, investigators rushed to understand in mice and in humans how BAFF blockade would impact B cell subsets, including MBCs. Two oft-cited studies reported that BAFF was dispensable for MBC maintenance and for humoral recall responses in mice, although one of these studies reported a partial dependence of splenic IgM$^+$ MBC on BAFF (Benson et al., 2008; Scholz et al., 2008). In this issue, two independent groups take elegant and complementary approaches to revisit the role of BAFF and BAFFR in MBC survival and converge on a new conclusion—contrary to prior studies, MBC do depend critically for their persistence on BAFFR signaling.

Müller-Winkler et al. (2020) use a precise, conditional genetic approach to inducibly delete key signaling molecules in MBCs at late time points after they are generated (with concurrent anti-CD40L to block further GC output). They show that either deletion of the BCR itself or the cytosolic signaling subunit CD79α leads to loss of antigen-specific PD-L2$^+$ MBC, consistent with prior studies. By taking an analogous approach to delete BAFFR after MBC generation, they reveal a clear requirement for BAFFR in this PD-L2$^+$ MBC population as well as roles for IKK2 and, to a lesser extent, IKK1, which mediate canonical and noncanonical NF-κB signaling respectively (see figure, panel B). BAFF blockade produces a similar loss of MBCs and impairs the classic recall response following immunization, and also results in a modest loss of antigen-specific lung-resident MBC following influenza infection. Lau et al. (2020) approach a similar problem from a different direction, capitalizing on adoptive transfer of swHEL BCR transgenic B cells in order to track two distinct populations of MBCs: low-affinity GC-independent MBCs and high-affinity GC-dependent MBCs. This approach serves to bypass abnormal lymphoid architecture and cell-extrinsic consequences of germline BAFF and BAFFR deficiency and reveals that GC B cells and affinity maturation are BAFF independent. In this study, both low- and high-affinity MBC populations are BAFFR

dependent, but generation of low-affinity IgM MBCs is considerably more sensitive to the supply of BAFF. Indeed, Lau et al. (2020) enhance BAFFR signaling by studying B cells that either lack TRAF3 or overexpress BAFFR, and observe disproportionate expansion of low-affinity IgM^+ MBCs.

Taken together, these studies overturn the prevailing paradigm that MBCs are BAFF independent. One way to reconcile the data reported previously with the present studies is to position B cell populations along a spectrum; naive B cells are most dependent on BAFF for survival, followed by GC-independent MBC, while high-affinity $PD\text{-}L2^+$ MBC are least dependent (see figure, panel A). Because BAFF depletion was used to study MBC and recall responses in prior studies, the naive B cell compartment was profoundly reduced (Benson et al., 2008; Scholz et al., 2008). Under such conditions, it is possible that MBCs exhibit a relative advantage compared with naive B cells and survive by compensating with other survival cues. By contrast, the use of coadoptive transfer and conditional genetic ablation in the new studies creates a more stringent, competitive setting in which to test the cell-intrinsic role of BAFFR in MBC survival without perturbing global homeostasis and the overall supply of BAFF. However, discrepancies between prior studies and anti-BAFF blockade experiments presented in Müller-Winkler et al. (2020)—which profoundly impair MBC survival and recall responses—are harder to explain. One possibility is that the BAFF depletion protocols used by Müller-Winkler et al. (2020) are more complete, and responses are assessed at later time points. If there is a gradient of sensitivity to and dependence on BAFF (see figure, panel A), then complete loss of BAFF, rather than partial depletion, might be necessary to unmask a role in MBC survival. Further delineation of the mechanism by which differential BAFF dependence is imposed across naive B cell and MBC populations will be important. Additional elaboration of the downstream signaling pathways that mediate MBC survival might identify unique factors that play a role in naive B cells and MBC subsets, and could in turn facilitate selective targeting of these populations in disease.

Studies of BAFF neutralization in humans have yielded highly variable findings. Administration of belimumab to SLE patients induces an early transient increase in the number of MBCs detected in peripheral blood, possibly as a result of mobilizing MBC from tissue niches before a return to homeostasis (Stohl et al., 2012). Consistent with a possible gradient of BAFF dependence across B cell populations, additional studies in humans report minimal effects of BAFF blockade on class-switched MBCs but eventual loss of unswitched MBC (Jacobi et al., 2010; Ramsköld et al., 2019; Stohl et al., 2012). This model, if validated in humans, would have important clinical implications; BAFF blockade may be more or less effective in individual SLE patients depending on whether naive B cells, low-affinity GC-independent MBCs, or classic GC MBCs harbor relevant autoreactive BCRs that are recruited into the plasmablast pool. Moreover, if BAFF blockade in patients is detrimental to specific subsets of MBCs, this would have important implications for susceptibility to certain infections and timing of booster vaccine doses. Indeed, responses to the seasonal flu vaccine (which is thought to be highly dependent upon preexisting MBC) are impaired in belimumab-treated patients (Chatham et al., 2012; Turner et al., 2020). The present two studies in mice suggest the need for further analysis of how BAFF blockade affects MBC survival and reactivation in humans.

Why, from a teleological standpoint, should MBCs be less dependent upon BAFF than naive B cells? Since naive B cells are constantly replenished, while MBCs are generated at much lower rates, it may be that MBCs need some competitive advantage relative to naive B cells in order to preserve an antigen-experienced memory compartment. Conversely, why pin MBC survival to a cell-extrinsic factor such as BAFF at all? Perhaps this serves to prevent the opposite outcome in which MBCs would overtake the peripheral B cell compartment entirely at the expense of naive B cells; BAFF may therefore serve to preserve memory while balancing the risks of "original antigenic sin," of particular concern for a species with a long lifespan.

References

Benson, M.J., et al. 2008. *J. Immunol.* https://doi.org/10.4049/jimmunol.180.6.3655

Cancro, M.P. 2009. *Nat. Rev. Immunol.* https://doi.org/10.1038/nri2621

Chatham, W.W., et al. 2012. *J. Rheumatol.* https://doi.org/10.3899/jrheum.111587

Jackson, S.W., and A. Davidson. 2019. *Immunol. Rev.* https://doi.org/10.1111/imr.12810

Jacobi, A.M., et al. 2010. *Arthritis Rheum.* https://doi.org/10.1002/art.27189

Jones, D.D., et al. 2015. *J. Immunol.* https://doi.org/10.4049/jimmunol.1501365

Lam, K.P., et al. 1997. *Cell.* https://doi.org/10.1016/S0092-8674(00)80373-6

Lau, A.W.Y., et al. 2020. *J. Exp. Med.* https://doi.org/10.1084/jem.20191167

Mesin, L., et al. 2020. *Cell.* https://doi.org/10.1016/j.cell.2019.11.032

Müller-Winkler, J., et al. 2020. *J. Exp. Med.* https://doi.org/10.1084/jem.20191393

Ramsköld, D., et al. 2019. *EBioMedicine.* https://doi.org/10.1016/j.ebiom.2018.12.035

Scholz, J.L., et al. 2008. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.0807841105

Srinivasan, L., et al. 2009. *Cell.* https://doi.org/10.1016/j.cell.2009.08.041

Stohl, W., et al. 2012. *Arthritis Rheum.* https://doi.org/10.1002/art.34400

Turner, J.S., et al. 2020. *Nature.* https://doi.org/10.1038/s41586-020-2711-0

Weisel, F., and M. Shlomchik. 2017. *Annu. Rev. Immunol.* https://doi.org/10.1146/annurev-immunol-041015-055531

Yu, X., et al. 2008. *Nature.* https://doi.org/10.1038/nature07231