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INSIGHTS

Sending positive signals and good (calcium) vibes

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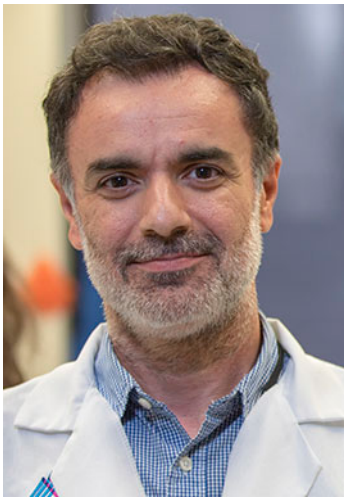
In this issue of *JEM*, Yada et al. (https://doi.org/10.1084/jem.20222178) demonstrate that effective antibody affinity selection in germinal centers relies on the store-operated calcium entry (SOCE) component of the B cell receptor (BCR) signaling network. Therefore, active BCR signaling is as relevant to positive selection as the function of BCRs as endocytic receptors, answering a question that had puzzled experts for a while. These findings transform our understanding of the mechanisms supporting adaptive immune responses (to vaccines, for example) and have important implications for interpreting the genomics and pathogenesis of germinal center–derived B cell lymphomas.

B cell development depends on the effective assembly of a functional antigen receptor (Torres et al., 1996), which provides indispensable "tonic" survival signals and endows B cells with the capacity to capture and internalize antigens for their presentation to T cells. Such duality of the B cell receptor (BCR) as both a source of intracellular signals and an endocytic receptor is crucial for B cell homeostasis and function (McShane and Malinova, 2022). However, a series of studies implied that BCR signaling per se is silenced in B cells transiting the germinal center, suggesting that B cells may not always rely on this signaling pathway (Khalil et al., 2012). Later studies questioned this view, showing that germinal center B cells can indeed signal through their BCR and do so during normal germinal center responses (Mueller et al., 2015). The question remained whether BCR signaling plays any role in the control of affinity maturation and B cell dynamics in germinal centers (Victora and Nussenzweig, 2022).

Yada and colleagues now report that survival signals triggered by calcium influx upon BCR engagement are required for optimal selection, affinity maturation, and maintenance of germinal center B cells following antigen capture. According to their study, *Stim1/2* null B cells, unable to uptake Ca^{2+} via store-operated calcium entry (SOCE), are progressively outcompeted by wildtype B cells in germinal centers, fail to undergo affinity maturation, and exhibit decreased survival and increased apoptosis. The authors attribute these effects to an inability to upregulate the expression of *Bcl2a1*, a gene controlled by NFAT in response to BCR activation and Ca^{2+} signaling. These effects are especially pronounced in B cells with high-affinity BCRs and alter the cellular output of germinal centers, with a relatively higher abundance of memory cells than plasma cells. This finding is consistent with the enrichment of high-affinity BCR repertoires in the plasma cell pool (Ise and Kurosaki, 2021).

This study demonstrates that BCR signaling is necessary for affinity-based positive selection, beyond the contributions of T cell help and endocytic activity of the BCR. Yada et al. (2024) propose that survival signals triggered by BCR signaling facilitate positive selection as they allow cells to wait for help signals from T cells. A similar conclusion was put forward in a contemporary study by Nussenzweig and colleagues (Chen et al., 2023), who investigated the role of BCR signaling during positive selection by targeting the Bruton Tyrosine Kinase (BTK). BTK lies further upstream in the BCR signaling cascade than SOCE (Tanaka and Baba, 2020). Collectively, these two complementary studies convincingly demonstrate that BCR signaling plays a crucial role in the biology of the germinal center.



Insights from David Dominguez-Sola.

The results of Yada et al. (2024) may come as a surprise because this group also showed in the past that *Stim1/2* null B cells mount normal antibody responses in vivo (Matsumoto et al., 2011). The defects in positive selection, germinal center maintenance, and affinity maturation described in the new study are only revealed in competitive settings with *Stim1/2* null and wildtype B cells, particularly in the context

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of high-affinity, antigen-specific transgenic BCRs (B1-8^{high}). As Yada et al. (2024) discuss, competitive settings were also key to finding that germinal center B cell survival is impacted by relatively lower BCR affinities (Schwickert et al., 2011). Such manipulations may reduce the strength of signals that, emanating from the BCR, translate into diverging signaling cascades, including SOCE Ca^{2+} influx. Relative disparities in signaling strength may be better appreciated in competitive settings, where mixtures of cells with high and low strengths can be found together, or during polyclonal responses in wildtype mice, where antigens engage a diverse pool of B cells with different BCRs and affinities.

Sensing BCR strength is already hardwired in the biology of B cells—it is used, for example, to determine if B cells are autoreactive during the establishment of central tolerance (Nemazee, 2017). In naïve B cells, BCR signaling strength is encoded in quantitatively distinct patterns of intracellular Ca^{2+} signaling (peak amplitude, steady-state concentration, and spike frequency), then decoded by specific pathways (NF-κB, NFAT, and mTORC1) to drive cell survival and fate (Berry et al., 2020). When the BCR fails to engage STIM-dependent Ca^{2+} entry, cells fail to activate the expression of anti-apoptotic genes (i.e., *Bcl-xL* or *Bcl2a1*; Berry et al., 2020), which play a crucial role in the phenotypes described by Yada et al. (2024). Ca^{2+} entry also modifies the activities of mTORC1 and MYC, preparing cells for cell-cycle entry and proliferation. Ca^{2+} signaling in *Stim1/2* null cells, like in Yada et al. (2024)'s experiments, is likely quantitatively similar to the low-strength signals emanating from low-affinity BCRs. These B cells would only have a fair chance of capturing T cell help if all neighboring cells had equal affinities or signaled through the BCR with equivalent strengths.

Importantly, CD40 (or TLR9) costimulation can circumvent the requirement for SOCE during this process, and rescue *Stim1/2* null naïve B cells after antigen capture (Berry et al., 2020). And although the quality of signaling crosstalk between CD40 and BCR seems to differ significantly between germinal center B cells and naïve B cells (Luo et al., 2018), it could be argued that in certain circumstances, second signals may be able to rescue cells with meager BCR signal strengths. Data from Nussenzweig and colleagues (Chen et al., 2023) showed that enforcing T cell help could not rescue the fate of cells with inactive BTK, but BTK inactivation resets BCR signaling to zero (Berry et al., 2020; Chen et al., 2023) and may not be equivalent to losing SOCE—in this case, some residual signaling, like that provided by the PI3K arm of the BCR and coreceptors, may still remain.

Considering such crosstalk seems important because B cells integrate inputs from disparate receptors to decide their fate. Specifically, during T-dependent antigen responses B cell activation relies on two distinct signals: first from the BCR and subsequently from T helper cells. Once the first signal is received, B cells enter a "primed" state, waiting around for signals from T cells. This not-yet-well-understood primed state coincides with metabolic changes and increased mitochondrial function (Chen et al., 2023; Akkaya et al., 2018), and it opens a limited time window during which B cells can receive additional signals before they undergo cell death. Sometimes, pattern recognition receptors such as Toll-like receptors (TLRs) may substitute for T cell help and provide that second signal (Rawlings et al., 2012). It is also possible that additional immune cues, known or still unknown, may influence the quality or duration of the primed state induced by BCR signaling—one would suggest, for example, complement signaling through C3aR/C5aR receptors (Cumpelik et al., 2021). The quality of the first BCR signal, the nature of the second signal, and synergies between these two can then modulate and fine-tune key functional features of the B cell response and its outcomes.

The above considerations are relevant to disease settings, particularly during B cell lymphomagenesis. Certain subsets of B cell non-Hodgkin lymphoma (B-NHL) cases display genetic or non-genetic alterations that lead to increased BCR signaling. In paradigmatic cases such as Activate B cell–like Diffuse Large B cell lymphomas (ABC-DLBCL), this translates to a chronic signaling state that resembles active signaling from antigen-engaged BCRs (Davis et al., 2010). These states, crucial for lymphoma survival and pathogenesis (Young et al., 2015; Schmitz et al., 2018), seemed difficult to interpret in the context of the biology of germinal centers, where BCR signaling was believed to be silenced (Khalil et al., 2012; Victora and Nussenzweig, 2022). However, the new research by Yada et al (2024) and others (Chen et al., 2023) calls for a reinterpretation of existing B-NHL data. In fact, "chronic" active BCR signaling, as found in these tumors, could be an expression of a prolonged primed state. In this scenario, mutant cells could linger around for prolonged periods of time waiting for second signals, which, in some cases, could be completely stochastic, spurious, or even provided by additional mutations—unrelated to antigen affinities. Excessive signaling could make these cells also sensitive to T-independent second signals, which are normally not captured in germinal centers. We know, for example, that increases in BTK activity also sensitize B cells to TLR stimulation. Additionally, although BCR and TLR are largely distinct pathways, they interact through shared signaling molecules such as STAT3 via DOCK8-MyD88-Pyk2, a signaling module that is recurrently mutated in cluster 5/MCD DLBCL (Schmitz et al., 2018; Chapuy et al., 2018). These mutations can enhance proximal BCR signaling (Mandato et al., 2023), which may suffice to extend that "primed" state. Finally, CARD11 mutations in a fraction of ABC-DLBCL (Lenz et al., 2008) are known to drive increased expression of *BCL2A1* (Decombis et al., 2023), the anti-apoptotic factor engaged by SOCE and reinforced by CD40 stimulation (Basso et al., 2004) that Yada et al. (2024) showed can rescue high-affinity *Stim1/2* null cells. Thus, predictably, the mutational repertoire of B-NHLs may be peppered with genes encoding for previously unknown second signals. A careful revision of the genetics of B-NHLs, in light of the studies by Yada et al. (2024) and others, may lead to unexpected insights into the pathogenesis of these malignancies.

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