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INSIGHTS

New twists in humoral immune regulation by SLAM family receptors

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SLAM family receptors are involved in humoral immune regulation. In this issue of *JEM*, Zhong et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20200756) provide evidence that these receptors collectively suppress germinal center reaction but promote production of antigen-specific antibodies.

Antibody production by B lymphocytes is crucial for host defense. Antigen-activated B cells develop into germinal center (GC) B cells to undergo somatic hypermutation and be subjected to affinity-based selection before giving rise to affinity-matured memory B cells and plasma cells. T follicular helper cells (Tfh cells) are a specialized helper subset in promoting GC formation and positive selection of affinity-matured GC B cells. The follicular subset of regulatory T cells (Tfr cells) counterbalance Tfh effects and constrain GC formation and expansion. A main route for delivery of Tfh help to GC B cells is through immunological synapse, a physical platform that depends on GC B cell–mediated antigen presentation to trigger T cell receptors and facilitates exchange of both soluble (e.g., cytokines) and membrane-bound (e.g., CD40 ligand) signals. The GC reaction is regulated by multiple receptors and ligands expressed by B cells and T cells, including members of the signaling lymphocytic activation molecule (SLAM) family (Cannons et al., 2011). Using a mouse model engineered to lack seven SLAMF genes on chromosome 1, Veillette and colleagues in this issue of *JEM* report that SLAMF molecules as a group exert a net suppressive effect on GC formation and expansion while promoting plasma cell generation and antibody production (Zhong et al., 2021).

All SLAMF proteins are transmembrane molecules, and six of them contain immunoreceptor tyrosine-based switch motifs (ITSMs) or immunoreceptor tyrosine-based inhibitory motifs and can signal by associating with SLAM-associated protein (SAP; Schwartzberg et al., 2009). In mice, SAP is highly expressed in T cells but normally not in B cells. SLAMF molecules, expressed by T cells, B cells, and many other immune cells, are the only group of transmembrane receptors that SAP binds to with its SH2 domain. After SAP-deficient mice were found to be relatively normal in mounting T cell responses but almost completely unable to mount any significant GC responses (Cannons et al., 2004; Crotty et al., 2003; Davidson et al., 2004; Hron et al., 2004), it was naturally assumed that one or more SLAMF proteins would be responsible for promoting the GC response in a SAP-dependent manner. As SAP was later found to be specifically required in T cells for productive antigen-specific interactions with B cells but not with antigen-carrying dendritic cells (Qi et al., 2008), it became even more appealing a hypothesis that a SLAMF molecule(s) collaborates with SAP to promote stable T–B synapse and help delivery and thus the GC response (Qi, 2012).

The road to hunt SAP-collaborating SLAMF molecules that promote the GC response has proved twisty, as no single

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SLAMF molecule seems to be able to explain in full the GC defect caused by a SAP deficiency. For example, SLAM knockout mice were found to mount a relatively normal GC response, albeit with defective IL-4 production by Tfh cells (McCausland et al., 2007). Unlike SAP-deficient mice, CD84 knockout mice showed a partial GC defect in response to protein immunization (Cannons et al., 2010). Perhaps the most interesting case was with Ly108, as even though Ly108 ablation did not appreciably impair the GC response, it remarkably rescued the GC defect caused by a SAP deficiency (Kageyama et al., 2012). This latter phenomenon is

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further explained by the observation that Ly108 can recruit SHP-1 through its cytoplasmic ITSM motif, particularly in the absence of SAP, and inhibits proximal T cell receptor signaling, leading to destabilization of antigen-specific T–B interactions (Chu et al., 2014; Kageyama et al., 2012; Zhao et al., 2012). It thus emerges that different SLAMF molecules may play a positive or negative role regulating antigen-specific T–B interactions, while the same SLAMF molecule may simultaneously play both a positive and a negative role depending the precise context of intracellular signaling in T cells. In this context, whether some SLAMF receptors (SFRs) would antagonize or synergize with other SFRs in regulating the GC response becomes even more interesting a question but has been difficult to address, partly because of technical hurdles in creating compounded knockout of two or more *Slamf* genes closely positioned on the same chromosome within ∼400 kb.

By targeted disruption of *Slamf1*, *Slamf5*, and *Slamf6* genes in embryonic stem cells, Terhorst group showed that, in such triple knockout mice, plasma cell formation and antigen-specific antibody titers were enhanced without discernable changes in GCs, suggesting that these three SFRs are synergistically inhibitory to humoral immunity (Wang et al., 2015). Using a CRISPR/Cas9 approach to disable *Slamf1* through *Slamf7* individually, Dong group reported the first SLAMF KO strain that does not express these seven SFRs but still has most of the intronic and intergenic chromosome regions intact (Chen et al., 2016). They found that, unlike SAP-deficient mice, animals lacking seven SFRs can mount a normal GC response, even with a slightly increased proportion of Tfh cells (Chen et al., 2017b). These authors did not analyze antibody responses.

Against this backdrop came in the new twist reported by Veillette and colleagues (Zhong et al., 2021). These authors created another compounded SFR KO strain by deleting the continuous chromosome segment of ∼400 kb that contains seven SLAMF genes, *Slamf1* through *Slamf7* (Chen et al., 2017a). Without any immunization, these SFR KO mice exhibited a 70% reduction in serum IgG1. After immunization with model antigen NP-OVA (4-hydroxy-3-nitrophenyl–OVA) or hepatitis B vaccine HBsAg or after infection with an enteric nematode, antibody responses in SFR KO mice were clearly impaired, being lower in magnitude, affinity, and protective potentials to counter reinfection. Surprisingly, however, these mutant mice actually generated more, not less, GCs, while at the same time produced fewer memory B cells and plasma cells in the spleen and bone marrow. These authors also tested a triple knockout strain that lacks SLAMF1, SLAMF5, and SLAMF6 receptors and found that such animals exhibited not defects in GCs but a similar defect in serum antibodies to that seen in SFR KO mice. This is in sharp contrast to the enhanced antibody response reported with the other triple knockout strain by Terhorst group (Wang et al., 2015).

To pinpoint the cell compartment in which SFRs are required for normal GC expansion, these authors transferred SFR KO T cells into T cell–deficient mice and SFR KO B cells into B cell–deficient mice. In both cases, they observed GC exaggeration, suggesting SFRs are required in both T and B cell compartments for a normal GC response. To investigate potential mechanisms in greater details, they explored the possibility that SFR KO B cells would inherently expand more. They did observe an increased number of $BrdU^+$ GC B cells, although the $BrdU^+$ fraction in GCs, a better indicator of intrinsic proliferative capacity, was not demonstrably increased. They showed that the GC dark zone, which is the most proliferative compartment, was disproportionately enlarged in SFR KO mice and that SFR KO B cells would expand more when activated in vitro. Using a Nur77 reporter strain, they provided evidence that T cell receptor signaling, but not B cell receptor signaling, may be enhanced in vivo in the absence of the seven SFRs, a finding in line with the observed GC exaggeration. By intracellular staining, they showed that expression of pro-survival proteins such as BCL2 was reduced in SFR KO GC B cells, implying these cells were more prone to die, a possible explanation for the contradiction between GC exaggeration and reduction in plasma cells and antibody production. In support of this latter notion, transgenic BCL2 overexpression rescues the antibody defect in SFR KO mice.

By providing arguably the first evidence that SFRs as a whole are critical for normal antibody responses and several hints as to how these receptors might function in this context, the current study reminds us much is still to be learned and surprises would still come striking from a receptor family that has been investigated for more than two decades.

In terms of apparently conflicting results with regards to GC expansion (Chen et al., 2017b), as Veillette and colleagues pointed out, higher antigen doses used in the Dong study might have obscured the detrimental effect of compounded SLAMF ablation. It is more difficult to reconcile the marked difference in terms of antibody titers observed with two lines of triple knockout mice lacking SLAMF1, SLAMF5, and SLAMF6 (Wang et al., 2015). One important factor to bear in mind is the potentially subtle but significant difference in the noncoding region of the *Slam* locus as introduced by various gene-targeting methods. For example, in addition to deletion of coding sequences for the seven SFRs, the mutant strain used in the current study also lacks regulatory DNA elements that may exist in the 400-kb chromosome 1 region, whereas this would not be an issue with the mutant strain created by Dong group (Chen et al., 2017b).

While the current study offers some plausible explanations for the interesting contradiction between GC exaggeration and reduction in plasma cells and serum antibodies, many uncertainties remain. For example, it is not clear how BCL2 expression in GCs would depend on SFRs, particularly when normal GC B cells express BCL2 at a very low level. Given clearly enhanced expansion of SFR KO B cells after in vitro stimulation through the B cell receptor (BCR) and CD40 receptor, it should not be ruled out that SFRs may regulate BCR signaling, particularly when it is difficult to ascertain whether sensitivity of the Nur77 reporter system is sufficient for measuring strengths of antigen signals systematically dampened in GCs (Khalil et al., 2012). The reason why GCs in SFR KO mice harbor more mutations is not clear. Despite increased mutation loads, affinities of serum antigen-specific antibodies are actually reduced, suggesting impaired affinity-based selection in SFR KO mice. In addition to the possibility that SFR KO B cells may proliferate more, die more, and accumulate to a greater extent without leaving the GC, it seems possible that SFRs could more specifically regulate affinity selection, GC exit,

and/or GC development of plasma cells, areas worth of future investigation. Consistent with the reduced antibody affinity, GCs in SFR KO mice also contained smaller fractions of antigen-specific B cells. This is reminiscent of GCs devoid of Tfr cells (Linterman et al., 2011). It is possible that Tfr functions are impaired in the absence of SFRs, even though the Tfr frequency is increased in SFR KO mice. Finally, because SFRs are expressed by cells other than T and B cells, it will be important in the future to more precisely dissect contributions of different cell compartments in determining the complex phenotype of SFR KO mice.

Those uncertainties notwithstanding, the current report by Veillette and colleagues has surely shone a new light on the old question: how multiple SFRs, antagonistically or synergistically, regulate the GC response and humoral immunity. Fresh thoughts are needed on this twisty road toward deeper understanding.

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