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Previews

Caught Off Center: Rethinking the Requirements for Antibody Affinity Maturation

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Antibody affinity maturation involves selective survival of high affinity B cells and is thought to require the germinal center (GC) microenvironment. In this issue of *Immunity*, Di Niro et al. (2015) challenge this view, showing that low affinity B cells initiate Salmonella responses and affinity mature outside of GCs.

Antigen-specific B cell activation and affinity maturation are hallmarks of protective humoral immunity. A textbook rendition of these events (Figure 1A) posits that following infection or immunization, B cells whose receptors engage antigen with substantial affinity respond with activation and expansion. Within days, some of these activated B cells quickly differentiate to antibody secretion in the splenic extrafollicular regions, whereas other members of this initially activated cohort engage in cognate interactions with activated $CD4^+$ T cells and initiate germinal centers (GCs). These transient structures form at the T–B boundary in secondary lymphoid organs and exhibit unique architectural and cellular trafficking features. According to the cyclic reentry model, GC B cells undergo rounds of division and activation induced cell death (AICD)-mediated somatic hypermutation (SHM) in the GC dark zone. They then migrate to the GC light zone, acquire antigen sequestered on follicular dendritic cells, and subsequently process and present antigen to T follicular helper cells (Tfh). This cognate presentation interaction sustains both Tfh and GC B cell character and mediates GC B cell survival. Because high affinity B cells compete best for antigen, they are more likely to experience survival-promoting cognate Tfh interactions, and are hence selectively spared for further differentiation to antibody secreting plasma cells and memory B cells, or another round of mutation in the dark zone. Successive iterations of this process eventually lead to the dominance of affinity matured GC B cells, which can differentiate to yield memory B cells or antibody-secreting plasma cells. A substantial literature supports these general features of the GC reaction (reviewed in Allen et al., 2007; Kelsoe, 1996; Victora and Nussenzweig, 2012), fostering the view that GCs are a unique microanatomic niche that is essential to the precisely choreographed events needed for effective affinity maturation.

In their analyses of B cell responses to *Salmonella* Typhimurium (STm) infection, Di Niro et al. (2015) have made two unexpected observations challenging the notion that GCs are the sole microenvironment capable of supporting affinity maturation (Figure 1B). First, despite the lack of sustained GCs, the response nonetheless includes AICD-mediated SHM and displays affinity maturation. Second, their findings suggest that—at least in some circumstances—the threshold affinity for primary B cell activation may fall below detectable binding. Thus, while the STm response is B cell receptor (BCR) dependent, the B cells initially activated have far lower BCR avidity than anticipated by prevailing models.

Consistent with previous studies of STm infection (Cunningham et al., 2007), Di Niro et al. find that antibody-forming cells (AFCs) accumulate rapidly at extrafollicular sites, whereas GCs are delayed to 3 weeks post infection. Nonetheless, the AFC response produces class-switched antibody, particularly IgG2c. Surprisingly, analyses of Salmonella binding show that the majority of AFCs produce antibodies with undetectable binding activity for either major STm antigens or STm lysate. These findings raise the possibilities that this might reflect a polyclonal response driven by pattern-recognition receptors rather than BCR engagement and that affinity maturation may fail to occur normally. However, each of these potential explanations was systematically interrogated and ruled out. Thus, AFC accumulation was unaffected by the absence of Toll-like receptor-2 (TLR2), TLR4, or the TLR adaptor MyD88. Similarly, when the aggregate of antibody specificities was dissected by analyzing monoclonal antibodies from single cell-derived hybridomas, the findings with immune sera were corroborated: very few had measurable STm binding specificity, albeit some were LPS- or poly-specific. Finally, STm infection in the B1.8 BCR transgenic model, in which there is a single V_H gene but multiple V_L genes, showed a reduction in the AFC response. This reduction was exacerbated in $B1.8^{+/+}$ $J\kappa^{-/-}$ mice, which additionally express only λ and not κ V_L. Similar results were found in two models with restricted V_L repertoires, $J\kappa^{-/-}$ and $IgV\kappa8R^{+/+}$. These results demonstrate that responses to STm require a diverse BCR repertoire, supporting a role for BCR engagement in driving the response despite the apparently minimal selectivity.

Based on these results, Di Niro et al. hypothesized that the AFC response is initiated by very low affinity B cells, and that these undergo somatic mutation and affinity maturation despite the absence of GCs. Analyses of V_H and J_H usage by plasmablasts 7 or 21 days after infection show that although the repertoire did not skew to specific V_H families, it is uniformly less diverse than the preimmune repertoire, and the diversity decreased further between 7 and 21 days post infection. This limited diversity is antigen-dependent, because stimulation with CpG did not lead to a similar decrease in diversity. The plasmablast heavy-chain repertoire also displayed somatic mutation at both time points, with mutation increased at the later time point. These results demonstrate that the lack of detectable STm binding resulted from surprisingly low

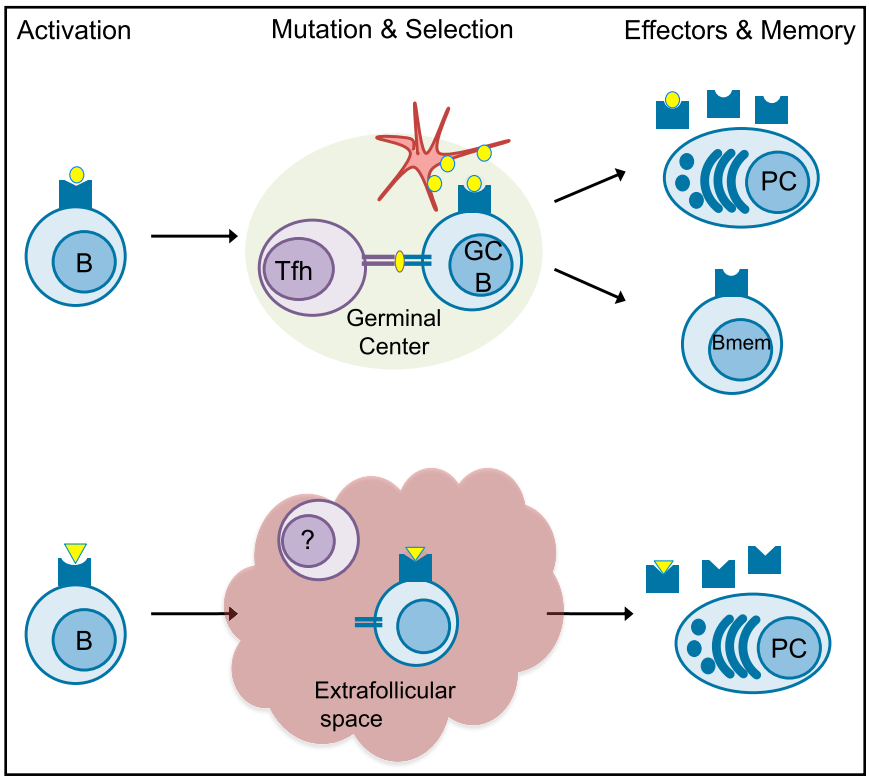

Figure 1. Alternative Routes and Locales for Antibody Affinity Maturation
Top: In a conventional T cell-dependent response, B cells activated by avid BCR engagement receive cognate T cell help and form GCs, where their interactions with FDCs and Tfh enable rounds of somatic hypermutation and selection that yield high-affinity plasma and memory B cells.
Bottom: The response to STm initiates with comparatively low affinity B cells, which undergo SHM and affinity maturation in the absence of GCs.

affinity of the initially responding B cells, rather than lack of BCR involvement per se. Moreover, it indicates that SHM was active and cumulative in these responses. To determine whether the B cells with mutated BCRs had undergone concerted affinity maturation, the effects of mutations on affinity were measured directly; immunoglobulin (Ig) genes from hybridomas with STm specificity were cloned and V regions expressed in germline and mutated form, including intermediates between germline and fully mutated. Removal of mutations led to marked decreases in affinity, demonstrating that despite the low initial affinities, the mutated BCRs were, in fact, affinity matured.

The low affinity receptors of initially responding B cells, as well as the emergence of somatically mutated, affinity matured plasmablasts in the absence of a GC reaction, are unexpected findings, raising the question of how this response differs from those used to establish accepted norms. The relatively unselective initial response, while clearly involving the BCR, likely reflects additional signals that either modulate the necessary BCR signaling threshold or directly enhance BCR signaling per se. These might reflect inflammatory cytokines or costimulators delivered by third party cells, contributions from pattern-recognition receptors not assessed in these experiments, or superantigenic properties of STm. Alternatively, STm may preferentially recruit B cells from subsets with intrinsically different tonic and threshold signaling properties, such as the marginal zone (MZ) or B1 pools. These possibilities are not mutually exclusive and are amenable to experimental interrogation.

A more intriguing question is how affinity maturation, albeit at a lower register, occurs without the facilitating architecture of GCs. One possibility is that concerted selection can proceed through either of two fundamentally different mechanisms, one initiated by and reliant upon classical cognate T cell help, and the other largely T-independent. Consistent with this possibility, most detailed studies of affinity maturation have employed obligate T dependent antigens—often adjuvanted hapten carrier conjugates—whereas STm responses are at least partially T cell independent. This might predict that in responses initiated by low affinity BCR engagement and devoid of conventional cognate T help, affinity maturation is driven by BCR occupation per se, rather than through a stepwise mechanism involving antigen capture and subsequent selection via cognate antigen presentation. In this regard, it might be worthwhile to establish whether the damped BCR signaling reported in GC B cells (Khalil et al., 2012) is not observed in the B cell response to STm. Alternatively, other forms of help, as might be delivered by NKT cells through nonconventional cognate interactions and inflammatory cytokines, might play a role in mediating selective survival.

Regardless of their underlying mechanisms, these findings in toto suggest that certain classes of pathogens elicit humoral responses whose properties differ substantially from those predicted by traditional immunization models and prompt reassessment of currently held concepts of specificity, affinity maturation, and immunological memory. Establishing the host-pathogen interactions that provoke such responses, as well as the underlying cellular and molecular mechanisms involved, should yield valuable insights into pathogenesis, intervention, and prophylaxis. Thus, B cells may be caught off center in some pathogen responses, but not caught off guard, placing canonical and noncanonical routes to affinity maturation on equal footing.

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