Micromanaging Memory with Immunoglobulin Microclusters

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Memory B cells generate rapid recall responses upon re-exposure to antigen. In this issue of *Immunity*, Liu et al. (2010b) show that for IgG1-expressing B cells the augmented memory response begins with the cytoplasmic tail.

In his account of the Plague of Athens in 430 BC, Thucydides commented that "the sick and the dying were tended by the pitying care of those who had recovered, because they knew the course of the disease and were themselves free from apprehensions. For no one was ever attacked a second time, or not with a fatal result." Substantial progress has since been made toward understanding the biological basis of this acquired specific immunity against reinfection with the same pathogen. In particular, memory T cells, memory B cells, and long-lived plasma cells have been characterized and their role in immunological memory elucidated (Ahmed and Gray, 1996). More recently, memory B cells have been extensively studied to determine the mechanisms by which they are able to generate the rapid, amplified responses required for protection. Thus, differential recruitment of activating and inhibitory coreceptors, protein tyrosine kinases, and phosphatases as well as other signaling modules have all been implicated in the enhanced signaling capabilities of the B cell receptor (BCR) expressed by memory B cells. Given that naive B cells express immunoglobulin M (IgM) and IgD and the majority of memory B cells express IgG, investigators have focused their attention on the cytoplasmic tail of the BCR, which has been shown by some, but not others, to exclude CD22 and prevent recruitment of SHP-1 phosphatase to the signalosome (Nitschke, 2009). The IgG cytoplasmic tail itself has also been reported to contain a conserved tyrosine residue that can be phosphorylated and recruit the adaptor Grb2 (Engels et al., 2009). Now, in the current issue of *Immunity*, Liu et al. (2010b) have applied state-of-the-art total internal reflection fluorescence (TIRF) microscopy and single-particle tracking to reveal another BCR-intrinsic mechanism for the enhanced memory B cell response. In a technical tour de force, the authors imaged and analyzed the earliest events following antigen triggering of the BCR and showed that BCR oligomerisation and microcluster growth was enhanced by a membrane proximal region of the IgG1 cytoplasmic tail not previously recognized to be involved in signaling.

The dynamics of early BCR activation have been extensively studied by the Batista and Pierce laboratories using high-resolution molecular imaging techniques including Förster resonance energy transfer (FRET), TIRF, and confocal microscopy (Harwood and Batista, 2010). This, combined with careful labeling of cells with fluorescent Fab fragments specific for the BCR, has enabled single-particle tracking of individual BCRs. Recent insights derived from these approaches include the role of actin and ezrin cytoskeletal networks in constraining BCR mobility and limiting tonic signals in the absence of antigen (Treanor et al., 2010) and differences in the dynamics of low- and high-affinity BCR aggregation in the presence of antigen (Liu et al., 2010a). The latter study by the Pierce laboratory employed the same tools and techniques as their study in this issue and, given the symmetries, it is interesting to consider all three papers in context of the advances they bring to our understanding of BCR signaling.

Liu and colleagues used antigen-loaded fluid planar lipid bilayers to mimic antigen-presenting cells and additionally used transformed B cell lines and primary B cells expressing yellow fluorescent protein (YFP) attached to the carboxy-terminus of Igα to directly visualize BCRs as they migrate into the contact area with the lipid bilayers. Thus, by swapping the cytoplasmic tail between IgM and IgG1 to generate chimeric BCRs they were able to analyze the contributions of the IgG1 cytoplasmic tail to BCR oligomerisation and microcluster formation. In the absence of antigen, IgG1 was found to be more mobile than IgM when the trajectories of individual BCRs were tracked and analyzed (Liu et al., 2010b). In contrast, Batista and colleagues found IgG to be less mobile than IgM under basal conditions (Treanor et al., 2010). These studies were performed on glass coverslips rather than planar lipid bilayers, and this might account for some of the discrepancy. Of note, the cytoplasmic domain of Igβ was reported to influence BCR diffusion dynamics of IgM in these studies (Treanor et al., 2010), and it is also possible that the addition of YFP to Igα has altered the baseline mobility of IgG1 and IgM. Nevertheless, if IgG1 BCRs are indeed highly mobile, then this may contribute to higher tonic signaling (Treanor et al., 2010) with implications for the long-term survival of memory B cells as well as lymphomas with a "memory B cell phenotype" such as diffuse large-cell lymphoma.

In a recent related study, Liu and colleagues used B cell lines transfected with mutant versions of the μ-B1-8 immunoglobulin heavy chain that differed by 50-fold in their affinity for the hapten 4-hydroxy-3-iodo-5-nitrophenyl (NIP) to show that BCR oligomerization and microcluster formation was affinity dependent (Liu et al., 2010a). These experiments provide a basis for translating the complex biophysical parameters generated by image analysis into the downstream readouts more familiar to B cell biologists.

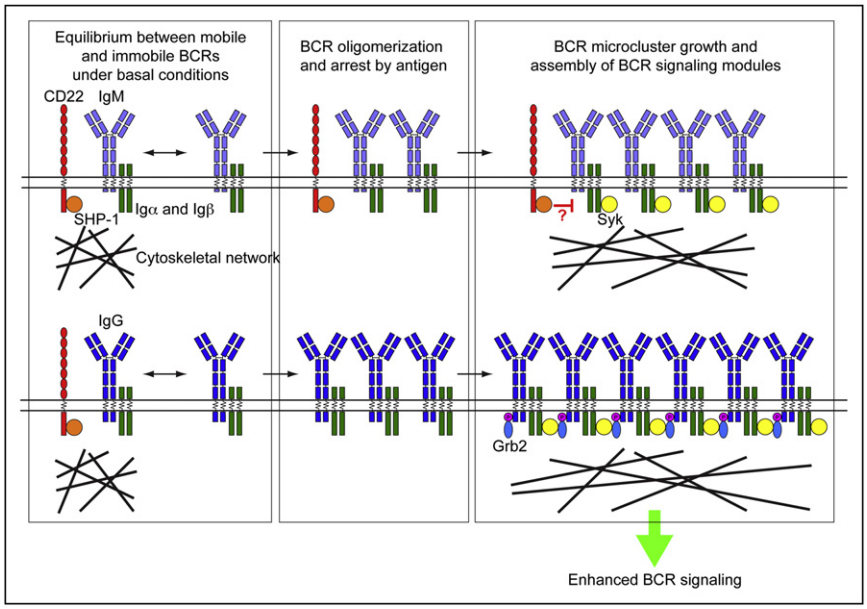

Figure 1. BCR-Intrinsic Mechanisms for Augmented Memory Responses of IgG-Expressing B Cells

Under basal conditions, individual BCRs are partitioned into mobile and immobile fractions trapped by the cytoskeletal network. Upon antigen engagement, previously mobile BCRs oligomerize. In the case of IgG1 this aggregation is more rapid than IgM BCRs. This begins an ordered rearrangement of the membrane cytoskeleon and assembly of BCR signaling modules. Immobilized IgG1-containing microclusters grow more rapidly and recruit more Syk kinase to generate larger calcium fluxes than IgM-containing microclusters. CD22 may recruit SHP-1 phosphatase to dampen signaling by IgM but not IgG BCRs. In addition, a conserved tyrosine in the cytoplasmic tail of IgG may be phosphorylated and recruit Grb2 to enhance signaling in response to soluble antigen.

Thus, the 50-fold affinity difference resulted in a 2- to 5-fold increase in the rate of microcluster growth and Syk phosphorylation and a less than 2-fold increase in calcium flux (Liu et al., 2010a). Remarkably, the differences observed between low- and high-affinity BCRs were similar in magnitude to those observed between IgG1 and IgM BCRs in the current study (Liu et al., 2010b). So what is the relative contribution of BCR affinity and immunoglobulin isotype to the enhanced signaling capabilities of memory B cells? In this regard, a direct comparison of the early activation events of high-affinity IgM and low-affinity IgG1 BCRs would be most informative. If the tail can in fact "wag the dog" as suggested by these data, then the implications are that immunoglobulin isotype switching can contribute a significant advantage to responding B cells even before they have undergone affinity maturation in the germinal center. This could, for example, explain the preferential expansion of IgG1 but not IgM plasmablasts in the early response to antigen (Chan et al., 2009).

One of the strengths of the system used by Liu et al. in the current study is the ability to engineer BCRs to express a range of mutant and chimeric receptors to test hypotheses. This has enabled, among other things, mutation to phenylalanine of the conserved tyrosine residue in the IgG and IgE cytoplasmic tail identified previously as essential for the phosphorylation-dependent recruitment of Grb2 (Engels et al., 2009). Intriguingly, the enhanced BCR oligomerization and microcluster growth observed for IgG1 was shown to be independent of this tyrosine residue (Liu et al., 2010b). The authors speculate that differences in the mode of antigen presentation (soluble antigen compared to membrane-bound antigen presented on a lipid bilayer) may have contributed to the different results. Thus, it is possible that a number of BCR-intrinsic mechanisms operate to augment memory B cell responses depending on the nature, affinity, and dose of the antigen (Figure 1). It must be remembered, however, there is more to a memory B cell than the expression of a switched immunoglobulin isotype and that in fact 15%–20% of human memory B cells express IgM (Seifert and Kuppers, 2009). Several BCR-extrinsic mechanisms have been proposed to account for the enhanced responsiveness of memory B cells including global changes in gene transcription profiles that reduce activation thresholds (Good et al., 2009). A challenge for future studies will be to dissect the relative contributions of BCR-intrinsic and BCR-extrinsic mechanisms to the rapid kinetics of memory B cell responses.

Another important focus for future studies will be the identification of the mechanisms by which the IgG1 cytoplasmic tail is able to accelerate the aggregation of BCR microclusters. A role for the membrane cytoskeleton in creating boundaries to the free diffusion of the BCR under basal conditions and its reorganization to facilitate the ordered assembly of BCR signaling microclusters has been demonstrated by Batista and colleagues (Treanor et al., 2010). One possibility, therefore, is that the IgG1 cytoplasmic tail also interacts with actin and ezrin networks in the same way as described for Igβ. Identification of the mechanisms by which the IgG1 cytoplasmic tail acts may indicate ways of selectively manipulating memory B cells that drive autoantibody responses or potentially malignancies derived from memory B cells.

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