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Affinity measured by microcluster

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Like T cell activation, B cell activation is driven by aggregation of B cell receptors (BCRs) into microclusters. New work suggests that the early dynamics of BCR mobility and microcluster formation "translate" BCR affinity for antigen into B cell responsiveness.

In the last few years, extensive progress has been made in understanding BCR triggering and early signaling (Harwood and Batista, 2010). This field has been chiefly driven by work from laboratories led by Facundo Batista and Susan Pierce. Batista posited that primary B cells might typically recognize ligands presented by other cells through a structure analogous to the immunological synapse of T cells (Grakoui et al., 1999; Batista et al., 2001). This conceptual advance and supporting evidence allowed them to leverage high-resolution imaging to visualize molecular dynamics and interactions in supported planar bilayer systems that permit introduction of selected, laterally mobile ligands at controlled concentrations (Fleire et al., 2006). The Pierce laboratory has used similar model systems with transformed B cells to study structural and signaling details that require extensive molecular engineering of the BCR itself (Tolar et al., 2009b). In this issue, Liu et al. continue the work of the Pierce laboratory to provide insight into how differences in BCR affinity for antigen (Ag) are read out through formation of submicron clusters.

BCR binding to cognate hapten antigens initiates a sequence of events that leads to B cell activation (Fig. 1). How this happens with monovalent ligands moving freely on a surface is distinct from the problem of how BCR aggregation is induced by multivalent particles; this distinction is nontrivial. McConnell developed the supported planar bilayer technology to understand the similar problem of how monovalent, laterally mobile IgE molecules confined to a target membrane surface could promote micron scale aggregation of and signaling by Fc receptors (Balakrishnan et al., 1982).

Previous work suggests that cryptic binding sites in the BCR allow association with other laterally diffusing BCRs to drive microcluster (MC) formation (Tolar et al., 2009a). Before antigen exposure, a proportion (~20–60%) of BCRs on the B cell surface are laterally mobile, but this varies by isotype (Treanor et al., 2010). IgM and IgG are more mobile than IgD. These differences seem to be related to the cytoplasmic and transmembrane domains, as substituting these IgM regions for corresponding segments from the MHC I protein increases motility. Restoring the cytoplasmic region back to IgM reduces the motility of the fusion protein back to the wild-type IgM levels. Ag binding leads to arrest of mobile BCRs, but this arrest does not occur through monovalent Ag binding alone. In the presence of low monovalent Ag concentrations, only 12% of BCRs bound to Ag actually arrest (Tolar et al., 2009a), suggesting that clustering with other BCRs (which are presumably also bound to Ag) is required for arrest.

The membrane-proximal C4 region of the Igμ chain mediates BCR clustering when Ag binding exposes a cryptic binding site (Tolar et al., 2009a). Deletion of Igμ C4 region and insertion of additional transmembrane mutations (C4+TM) ablates BCR clustering and arrest in response to monovalent Ag engagement. This is not a function of Ig chain length, as other truncations do not affect clustering or arrest. Interestingly, the C4+TM protein fragment expressed alone can cluster independently of Ag and recruit downstream signaling components. These findings led Tolar et al. (2009b) to propose a conformational change model of BCR triggering. It should be noted that polyvalent Ags, by nature, can aggregate and trigger BCRs, even in the absence of the C4 domain.

Formation of these small BCR MCs is essential for productive signaling in B cells (and in T cells). During the first 30 s after BCR triggering and clustering, the src-kinase, Lyn, phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAM) of Igα and Igβ, the signaling chains associated with Igμ. Technically rigorous fluorescence resonance energy transfer (FRET) experiments designed to measure interprotein distances showed that Ag binding leads to an initial spike in FRET between Igμ and the Igα–Igβ signaling complex, consistent with clustering. This FRET spike decays rapidly after 30 s, but only if ITAM phosphorylation occurs. Mutations of the ITAM tyrosine residues or treatment with Lyn inhibitors that prevent ITAM phosphorylation also prevent FRET reductions. Because the complex does not dissipate during phosphorylation, the drop in FRET is thought to be caused by an increase in interprotein distances, which is caused by "unpacking" of the Igα and Igβ chains. This finding mirrors recent findings in T cells concerning CD3ε unpacking in response to phosphorylation after T cell activation (Gil et al., 2002; Xu et al., 2008).

Lyn is biochemically defined as a lipid raft associated protein. Association

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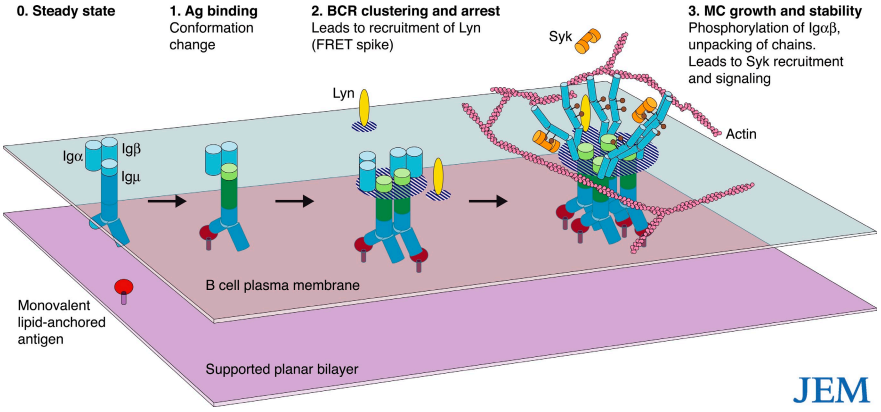

Figure 1. Modeling the steps of BCR triggering and early B cell activation on supported planar bilayers. To study early B cell signaling, supported planar bilayers (light purple) are loaded with monovalent antigens (large red circles) with or without adhesion molecules such as ICAM-1, which can freely diffuse laterally along the bilayer membrane. The B cell membrane (light blue) is shown from the cytoplasmic side. At steady state (without Ag, Step 0), BCR complexes are inactive and can migrate in the plasma membrane. Upon binding Ag (Step 1), the Igμ chain undergoes a conformational change (green). This leads to BCR clustering (Step 2), arrest of the complex, and recruitment of Lyn (yellow), possibly through changes in the lipid microenvironment (striped membrane region). Lyn phosphorylates the Igα and Igβ chains (small red circles) leading to an unfolding of the chains, which recruits Syk (orange) to the microcluster (Step 3). The microcluster grows in size and stability through actions of Syk signaling and interactions with the actin cytoskeleton.

of BCR MC with Lyn occurs transiently during the first 30 s after Ag encounter. Recruitment of the src-kinase Lyn is thought to be mediated by changes in the BCR lipid microenvironment, which may enrich raft-like proteins (Sohn et al., 2008). Initial MC formation is not dependent on Syk, but Syk recruitment to MCs requires ITAM phosphorylation as Syk docks onto ITAMs via its SH2 domain (Bradshaw, 2010). Syk signals in peripheral MC until they reach the central supramolecular activation cluster, where MC deactivation and degradation occurs.

It is unclear what role BCR affinity for Ag plays in early BCR cell signaling. In terms of the final outcome for the cell, it is clear from numerous studies that B cells with BCRs having higher affinity for Ag outcompete clones with lower affinity BCRs during the course of an immune response (McHeyzer-Williams and McHeyzer-Williams, 2005). A higher affinity BCR manifests many advantages for the B cell; more Ag uptake leads to more Ag for presentation, and better T cell help. To determine if B cells can differentiate between high- and low-affinity antigens without T cell intervention, Liu et al. (2010) tested the influence of BCR affinity on early B cell signaling. Using BCRs having high and low affinity for 4-hydroxy-3-iodo-5-nitrophenyl (Shih et al., 2002b), the authors were able to compare strong and weak stimuli. Using the tools developed in their previous studies, they showed that BCR affinity directs BCR mobility, as well as rates of MC formation and growth. These findings show that from the earliest stages, BCR affinity enhances B cell function. In addition, these data provide a mechanism explaining how MC formation can measure Ag quality for B cells.

A second major point of this paper is the breakdown of MC formation into early (nucleating) and late (growing) stages based on high-rate imaging. The first stage (occurring in the first 30 s) is Syk-independent, whereas the later stage is Syk dependent. Similarly, inhibitors of microtubule polymerization blocked the late but not early stages of MC growth. However, it appears that both stages of MC development are dependent on actin dynamics. It will be interesting to see what role cytoskeletal motor proteins play in these stages.

Actin plays a complicated role in BCR triggering and MC formation. In the steady state, immobile BCRs seem to be corralled within regions enriched with cortical actin and ezrin membrane tethers (Treanor et al., 2010). In previous studies, Tolar et al. (2005) did not see an effect of depolymerizing actin on the FRET profile of BCR triggering. However, Liu et al. (2010) show that the G-actin–sequestering drug latrunculin B seems to arrest early and late stages of MC growth. A recent paper (Treanor et al., 2010) showed that B cell activation could be induced even in the absence of Ag by disrupting the actin cytoskeleton with the depolymerizing drugs latrunculin A or cytochalasin D, or with the actin-stabilizing drug jasplakinolide; all drugs led to reduced cortical actin content. These drugs were capable of inducing calcium flux and ERK phosphorylation in a manner dependent on Vav and phospholipase C-γ, suggesting that this signaling may be caused by release of immobilized BCRs. Actin plays a critical role in stability and signaling from TCR MCs (Campi et al., 2005). The actin cytoskeleton simultaneously promotes the single-molecule sensitivity of the TCR, while acting to restrain triggering of the BCR so that many ligands are needed to make a microcluster. The differing roles of F-actin in T and B cells are aligned with the challenges of generating MHC-peptide complexes, which requires that a B cell take up ~500 protein molecules to make one MHC–peptide complex (Dadaglio et al., 1997).

Another important aspect of the data presented by Liu et al. (2010) is the finding that B cells expressing low-affinity BCRs still show some level of BCR triggering and MC formation, although the response is weaker and slower than those of high-affinity BCRs. As with their previous studies, these measurements were made in a monovalent Ag system. In vivo, B cells expressing the same low-affinity BCR described here are capable of mounting an immune response, but only in the absence of higher affinity clones, and perhaps only in response to polyvalent Ags (Shih et al., 2002a). It may also be interesting to see how, under low-affinity interactions, BCR clustering and arrest regulate Lyn

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recruitment. However, under weak stimuli, these processes may be extremely transient and difficult to measure by imaging. Lastly, these data are important for understanding how B cells quantify affinity, which may be useful for developing better vaccines targeting weak antigens.

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