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INSIGHTS

Cbls boost B cells

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T cell regulation of antibody-mediated immunity is critical for health. In this issue of *JEM*, Li et al. (https://doi.org/10.1084/jem.20191537) identify the Cbl family of E3 ubiquitin ligases as B cell–intrinsic gatekeepers of T cell–dependent humoral immunity.

An enduring antibody response after vaccination or infection can generate protective immunity that prevents subsequent infections. Indeed, harnessing the power of antibody-mediated immunity through vaccination has had a major impact on limiting the disease burden associated with infections worldwide; only good sanitation systems have had a greater impact on human health (Doherty et al., 2016; Greenwood, 2014). However, when antibodies are directed against nonpathogen antigens, they can cause autoimmune disease, allergies, and the rejection of transplanted organs (Crotty, 2019; Gowthaman et al., 2019; Linterman et al., 2009; Qureshi et al., 2019). Because T cell–dependent humoral immunity plays a significant role in both health and disease, understanding how these responses are generated can inform the development of the next generation of vaccines and new therapeutics for antibody-driven disease.

To elicit T cell help, B cells must first capture antigen via their B cell receptors, internalize and degrade it, and present the resulting peptide fragments via MHC class II to CD4$^+$ helper T cells. Cognate interactions enable the delivery of costimulation and cytokines from the T cell to the B cell, which facilitate class switch recombination and differentiation (Vinuesa et al., 2016). After receiving T cell help, B cells can differentiate into short-lived antibody-secreting cells, memory B cells, or migrate into the B cell follicle to initiate the germinal center response. Once the germinal center is established, it divides into two functionally distinct poles: the light zone and the dark zone. Within the dark zone, germinal center B cells proliferate and undergo somatic hypermutation of the genes encoding their B cell receptors. Then they exit the cell cycle and migrate to the light zone, where they test the function of their newly mutated B cell receptors by attempting to bind antigen held on the surface of follicular dendritic cells. B cells with functional B cell receptors will rip antigen from the surface of the follicular dendritic cells and process and present this to a specialized subset of CD4$^+$ T cells, follicular helper T (Tfh) cells. Germinal center B cells that present the most antigen to Tfh cells receive positive selection signals and can either reenter the dark zone for further proliferation and mutation or exit the germinal center as memory B cells or antibody-secreting plasma cells (Silva-Cayetano and Linterman, 2020). Therefore, the development of robust humoral immunity requires T cell help at two distinct phases: early after activation, to direct the B cell response; and within the germinal center, to facilitate positive selection of B cell clones.

Regulation of the B cell receptor and the subsequent processing of antigen is critical for the development of T-dependent immune responses. The Casitas B-lineage lymphoma (Cbl) proteins belong to the superfamily of the RING finger-containing E3 ubiquitin ligases, and are key regulators of lymphocyte antigen receptor signaling. Cbl proteins facilitate the ubiquitinylation of antigen receptors and their accessory proteins; monoubiquitin promotes sorting of these proteins into internal vesicles facilitating lysosomal degradation. Two family members, Cbl and Cbl-b, are expressed in the hematopoietic compartment, are highly structurally similar, and can exhibit functional redundancy in vivo (Duan et al., 2004). To understand the role that Cbl and Cbl-b play in B cells during T-dependent immune responses, Li et al. (2020) generated mice in which all B cells lack these ubiquitin ligases. In the absence of both Cbl family members, naive B cells have reduced B cell receptor internalization, impaired lysosomal degradation of antigen, and poor presentation of peptides on MHC class II. This results in an inability to engage with and activate CD4$^+$ T cells, resulting in a failure to mount a germinal center and

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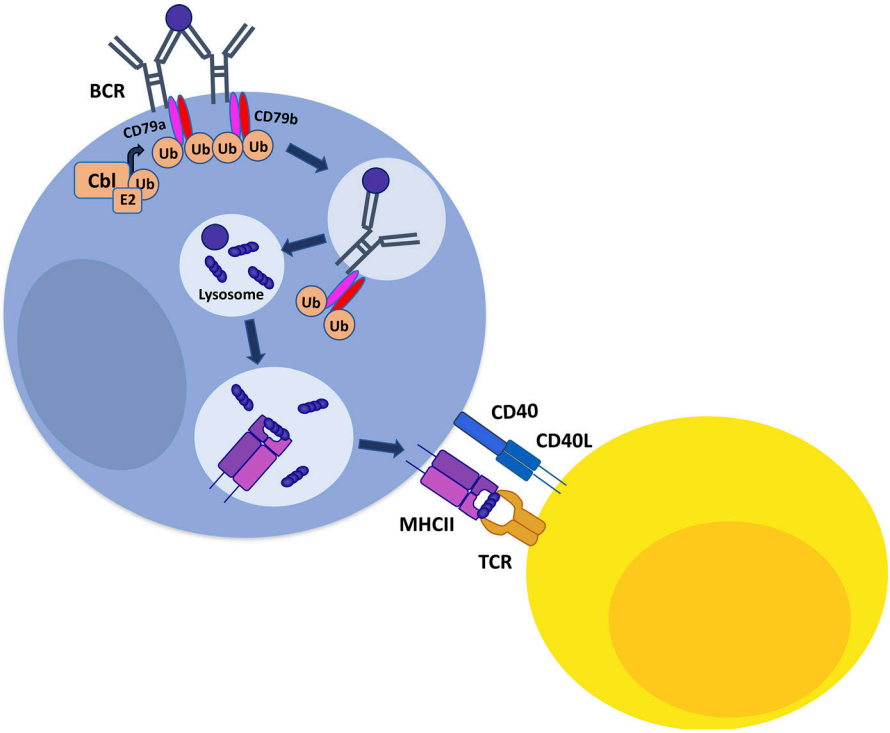

After ligation of the B cell receptor (BCR) by antigen, Cbl and Cbl-b interact with E2-containing proteins to ubiquitinate CD79a and CD79b, resulting in receptor internalization and lysosomal degradation of antigen into peptide. Peptides are loaded onto MHC class II and transported to the cell surface, and activated CD4$^+$ T cells that are specific for the presented antigen bind peptide MHCII via their TCR and provide costimulatory signals to the B cell, e.g., via CD40L.

develop antibody responses upon immunization or infection. Mechanistically, the Cbl proteins ubiquitinate the B cell receptor–associated proteins CD79a and CD79b, enabling internalization of the B cell receptor and the subsequent lysosomal processing of antigen. This work demonstrates that the ubiquitination activity of the Cbl proteins is required for B cells to access T cell help—without this, the germinal center cannot form, and B cells cannot differentiate into short-lived antibody-secreting cells. Whether pre-germinal center memory B cell differentiation is affected is not directly addressed in this study. However, antibody titers following secondary infection with intestinal parasitic helminth *Heligmosomoides polygyrus bakeri* are reduced, as is clearance of the worm, indicating memory B cell formation is impaired (Mesin et al., 2020). One interesting aspect of this study is that, despite high levels of Cbl and Cbl-b protein in germinal center B cells (Li et al., 2018), these ubiquitin ligases do not regulate antigen presentation in these cells. This demonstrates that antigen presentation to T cells is distinctly regulated in different B cell states and highlights that the mechanism of antigen presentation in germinal center B cells remains incompletely understood.

The absence of a role for the Cbl family in antigen presentation by germinal center B cells is curious, as, like naive B cells, these cells express CD79. Germinal center B cells have attenuated antigen receptor signaling upon ligation, including reduced phosphorylation of the CD79 proteins (Shlomchik et al., 2019). It is possible that this impaired posttranslational modification upon B cell receptor triggering limits the ability of the Cbls to associate with CD79. This could be direct, or due to impaired interactions of CD79 with other scaffold proteins, such as the tyrosine kinase Syk, which can link the B cell receptor to Cbl-b (Katkere et al., 2012). Despite this, the ongoing germinal center response requires antigen presentation to Tfh cells for its maintenance and output. This study indicates that antigen processing and presentation by germinal center B cells must occur via an alternative, Cbl-independent, mechanism.

The B cell–intrinsic ability of the Cbl family to dampen B cell responses prompts consideration of these proteins as putative therapeutic targets for antibody-mediated disease. However, Cbl and Cbl-b are widely expressed through the hematopoietic system, and loss of their ubiquitination activity in T cells can promote antitumor T cell immunity but also T cell–mediated autoimmunity. Likewise, a lack of Cbl proteins in myeloid cells and natural killer cells causes a proinflammatory phenotype (Duan et al., 2004; Li et al., 2019). This indicates that the role of Cbls in B cells as factors that promote, rather than limit, immune responses is unusual in the hematopoietic system, and therefore requires that any attempts to use this pathway to alter antibody responses must be exquisitely targeted to B cells.

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