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REVIEW

The bone marrow perisinusoidal niche for recirculating B cells and the positive selection of bone marrow-derived B lymphocytes

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A unique 'second' niche for follicular B cells has been described in the extravascular compartment of the bone marrow surrounding vascular sinusoids. The occupancy of this niche by B cells presumably evolved to facilitate humoral immune responses to blood-borne pathogens. B cells appear to be sustained in this niche by bone marrow dendritic cells and are lost from this compartment in certain mutant mice. We discuss here what is known regarding the mechanisms of entry and egress of B cells from the perisinusoidal niche and also consider the function of the bone marrow as a secondary lymphoid organ. Although immature B cells can mature into follicular B cells in this niche as well as in the spleen, the lineage commitment event that accompanies positive selection of B cells occurs only in the spleen.
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B cells in adult vertebrates develop initially in the bone marrow. About two-thirds of surface immunoglobulin M (IgM)-expressing immature B cells that have survived tolerance-related checkpoints exit the bone marrow and migrate to the spleen, whereas the remaining immature B cells continue to mature in the bone marrow itself[1,2] (Figure 1). In the spleen, immature B cells pass through two transitional stages to give rise to long-lived $IgM^{hi}IgD^{hi}$ follicular type II B cells (FO-II) that can differentiate either into $IgD^{hi}IgM^{lo}$ follicular type I B cells (FO-I) or into marginal zone (MZ) precursor B cells and MZ B cells.[3] In the bone marrow, immature B cells differentiate through the same transitional stages to yield FO-II and FO-I B cells, but MZ precursor and MZ B cells do not develop at this site or at any site outside the spleen.

Upon maturation, naive follicular B cells acquire the ability to recirculate, traveling from one secondary lymphoid organ to another in search of antigen. In conventional secondary lymphoid organs such as the spleen, lymph nodes and Peyer's patches, these B cells are drawn into well-defined B-cell areas or follicles by CXCL13 secreted by follicular dendritic cells. The presence of IgD^{hi} B cells in the bone marrow, often referred to as recirculating B cells, has been recognized for decades[4,5] but only recently has it been established that these B cells are actually extravascular in location, recirculate freely and that they occupy a unique perisinusoidal niche in the bone marrow, clustered around vascular sinusoids in the bone marrow.[6] Although follicular B cells are supported in the conventional follicular niche by follicular dendritic cells secreting the tumor necrosis factor family member B cell activating factor of the TNF family (BAFF), the survival of these very same B cells when they reside in the bone marrow perisinusoidal niche is mediated not by follicular dendritic cells (FDCs) and BAFF but by bone marrow dendritic cells that secrete macrophage migration inhibitory factor.[7] Functional studies indicate that these follicular phenotype B cells that cluster around bone marrow vascular sinusoids mediate T-independent immune responses against blood-borne microbes. These perisinusoidal B cells, however, failed to express activation-induced deaminase even as they were activated by a blood-borne Gram-negative pathogen.[6]

There are a number of unanswered questions regarding B cells in this niche that we will consider in this review. How do naive B cells home to the niche and what governs their exit from this location? Why is there a selective loss of follicular B cells in this niche in certain mutant mice even as they are preserved in the conventional follicular niche? What are the mechanisms involved in retaining mature B cells around bone marrow sinusoids? Why do these cells fail to activate activation-induced deaminase in response to blood-borne pathogens? Should the bone marrow be considered a bona fide secondary lymphoid organ? Does positive selection of B cell occur in this niche? Are the bone marrow and thymus truly analogous in the context of B- and T-lymphocyte development?

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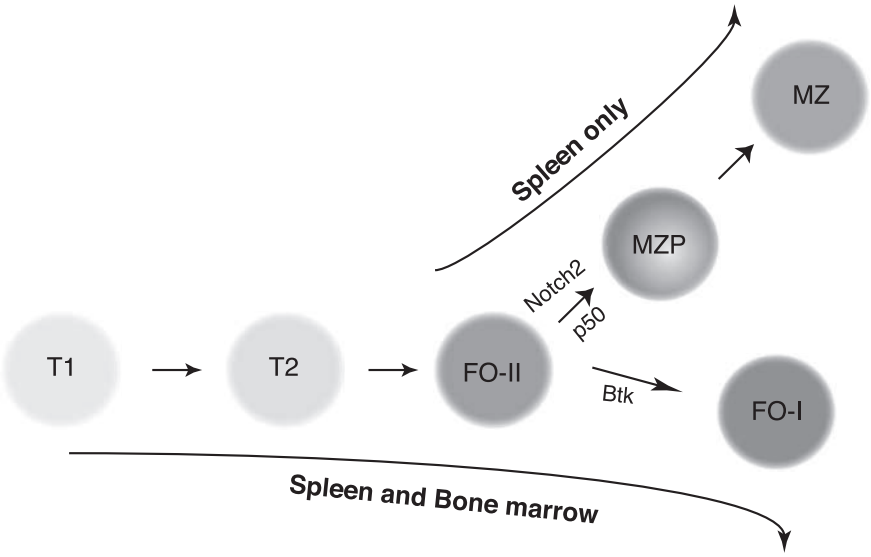

Figure 1 B-cell maturation occurs simultaneously in the spleen and bone marrow. FO-II and FO-I B cells develop both in the spleen and the bone marrow but MZP and MZ B cells develop only in the spleen. See text for details. FO-I, follicular type I B cells; FO-II, follicular type II B cells; MZP, marginal zone precursor.

EGRESS OF B CELLS FROM THE BONE MARROW AND ENTRY INTO THIS COMPARTMENT

Little is known as to how B cells at the immature B-cell stage leave the bone marrow parenchyma to enter the circulation, nor is there any clear knowledge as to how recirculating mature B cells leave the bone marrow compartment. There is a role for signaling from the B-cell receptor in the egress of immature B cells from the bone marrow as revealed from an analysis of mb-1 mutant mice in which immature B cells fail to exit the bone marrow.[8] How exactly does B cell receptor (BCR) signaling regulate egress from the bone marrow? Egress from the thymus and lymph nodes depends on a gradient of sphingosine-1 phosphate (S1P) between the blood and the lymphoid organ that is primarily sensed by $S1P_1$, the major S1P receptor in lymphocytes.[9,10] Such a mechanism also presumably operates in the egress of B cells from the bone marrow as $S1P_1$ deficiency and treatment with FTY720 result in an accumulation of B cells in the bone marrow.[11] Does the BCR perhaps regulate the cell surface expression of a receptor for S1P? Are the blood levels of S1P higher than those in the bone marrow? The answers to these questions are not known at present.

Xid mice harbor a point mutation in Btk, and peripheral follicular type I B cells fail to accumulate in these mice. The loss of these cells is sensed in a poorly understood manner and this results in the accelerated emigration of immature B cells from the bone marrow to the periphery.[12] It appears therefore that in addition to a role for the BCR in driving emigration, some mechanism exists to sense follicular B-cell loss in the periphery that results in a feedback acceleration of immature B-cell emigration from the bone marrow compartment. The nature of the presumptive humoral factor that might enhance B-cell emigration from the bone marrow remains unknown.

Nothing is known as to how recirculating B cells enter the bone marrow compartment. No selectins or integrins have as yet been implicated in the homing process. The chemokine CXCR4 is abundant in the bone marrow parenchyma but has not been implicated in the homing of recirculating B cells. A case has been made for the possible role of the sialic acid-binding inhibitory Siglec, CD22, in the homing of B cells to the bone marrow. This will be discussed in the next section.

MUTATIONS THAT LEAD TO A SELECTIVE LOSS OF RECIRCULATING B CELLS FROM THE BONE MARROW WHILE PRESERVING B CELLS IN THE FOLLICULAR NICHE

In a number of gene-targeted mice, follicular B cells are present in normal or near-normal levels in the spleen and lymph nodes, but are largely absent in the bone marrow. This phenotype is often seen in knockout mice lacking known inhibitors of B-cell receptor signaling. Examples include mutant mice lacking the heterochromatin-binding nuclear protein known as Aiolos,[13,14] mice lacking the inhibitory Siglec, CD22,[15–17] and mice lacking the CD72 inhibitory receptor.[18] In all these knockout mice, BCR cross-linking results in an enhanced flux of intracellular calcium, MZ B-cell development is defective and the mice develop autoantibodies or go on to exhibit frank autoimmunity. We consider the specific loss of perisinusoidal B cells as one of the *in vivo* features of enhanced BCR signaling. We speculate that enhanced BCR signaling in mature B cells in the bone marrow generates an exaggerated version of the same, but poorly characterized, egress signals that allow the BCR-dependent exit of immature B cells from the bone marrow. Presumably, in mutant mice with enhanced constitutive BCR signaling, B cells reside very briefly in the bone marrow, egressing this compartment in a relatively short time after they arrive because of strong signals from the BCR that instruct them to leave. In contrast to mutants in which recirculating B cells fail to accumulate exclusively in the bone marrow, in mice lacking both Rac1 and Rac2, recirculation of follicular B cells is completely abrogated; although immature B cells in these mice still home from the bone marrow to the spleen and mature into 'recirculating' B cells, these cells are unable to subsequently home to lymph nodes and the bone marrow.[19]

A distinct role for CD22 on B cells and α2–6 sialic acid-containing ligands on the endothelial surface has been suggested as a mechanism by which recirculating B cells home to the bone marrow.[20–22] It has been suggested that the density of α2–6-linked sialic acid-containing

ligands is particularly high on the bone marrow endothelium and that these ligands contribute to recognition by CD22 on B cells and the subsequent entry of these cells into the parenchyma. Although such a mechanism remains a possibility, given the general inability of B cells with enhanced BCR signaling to accumulate in the bone marrow, we suspect that the major reason for the loss of perisinusoidal B cells in *Cd22*$^{-/-}$ mice is the enhanced strength of BCR signaling in mutant B cells.

IS THE BONE MARROW A SECONDARY LYMPHOID ORGAN?

Mature recirculating B cells can be activated by intravenously injected *Salmonella typhimurium* to generate PNA^{hi}Fas^{hi} B cells in the bone marrow even in mice that lack all secondary lymphoid organs.[6] In addition, they can go on to generate IgM-secreting plasma cells as revealed in ELISPOT assays.[1,6] These activation events occur in mice that completely lack T cells.[6] By these criteria, the bone marrow is a site for T-independent antibody responses. Trafficking of B cells to the perisinusoidal bone marrow niche potentially evolved to permit all follicular B cells, and not just splenic MZ B cells, to participate in the generation of humoral antibody responses to blood-borne pathogens.

The bone marrow appears to provide an environment that is suited for the long-term survival of certain terminally differentiated lymphocytes. It is the compartment to which germinal center-derived long-lived plasma cells home and where they receive trophic signals through the BCMA receptor for long-term survival.[23] The bone marrow is also a major reservoir of memory CD8$^+$ T cells that have been described as being capable of responding more vigorously here than at other anatomical sites.[24–27] Apart from being a home for naive follicular B cells, the bone marrow is also a site at which human IgM and IgD memory cells appear to accumulate.[28,29] Whether these cells are derived from the *in situ* activation of naive perisinusoidal B cells in the bone marrow is unclear. Although most multiple myelomas probably represent the clonal outgrowth of cells that are derived from germinal centers and that have homed to the bone marrow, some IgM myelomas may potentially have arisen from the transformation of activated and terminally differentiated perisinusoidal B cells.

CD4$^+$ T cells have been demonstrated to reside in the vicinity of perisinusoidal B cells and dendritic cells in the bone marrow.[7] It is unclear, however, how B cells and T cells are anatomically organized in this compartment. One possible reason for the failure to observe the expression of activation-induced deaminase in PNA^{hi} B cells in the bone marrow after mice are challenged intravenously with *S. typhimurium*[6] is that the bone marrow might not contain adjacent and anatomically organized B- and T-cell areas. In addition, there is no evidence for the presence of follicular dendritic cells in the bone marrow. Although the issue of the lymphoid anatomy of the bone marrow requires to be further addressed, the failure so far to demonstrate adjacent T- and B-cell zones in the bone marrow suggests that this organ does not fit the mould of representing a conventional secondary lymphoid organ. It remains an unusual site that is in part a primary lymphoid organ, in part an organ with the ability to mount a subset of adaptive immune responses and in part is the home of certain terminally differentiated long-lived lymphoid populations.

Positive selection in the B lineage: is the bone marrow truly analogous to the thymus in a functional sense?

It has been argued that the recirculation of mature T cells to the thymus might facilitate the positive selection of T cells.[30] Should a similar function be considered for recirculating B cells in the bone marrow? Although positive selection in T cells occurs in the thymic cortex and is tightly linked to CD4$^+$ versus CD8$^+$ T-cell lineage commitment, the site of positive selection in the B lineage is unclear and lineage commitment into follicular and MZ B-cell subsets occurs only in the spleen.

Some of the uncertainty about the role of the BCR in positively selecting follicular and MZ B cells has arisen from the failure to discriminate between FO-II B cells and FO-I B cells (Figure 1). FO-II B cells are long-lived recirculating post-transitional B cells that apparently do not require self-antigen-driven BCR signaling or Btk for their generation. Constitutive ligand-independent BCR signals and BAFF may be sufficient to generate FO-II B cells. In contrast, FO-I B cells require relatively strong self-antigen-driven BCR signals delivered through Btk for their development.

Immature B cells mature through the transitional T1 and T2 stages. T2 cells can develop in the spleen and bone marrow and represent cells that have very recently acquired a recirculatory phenotype. A T2 cell that is exposed to a relatively high-avidity self-antigen in the bone marrow or the spleen may never pass through the FO-II compartment but may rapidly differentiate into an FO-I B cell either in the bone marrow or in the spleen. A developing T2 cell that does not have a receptor with a relatively strong affinity for any self-structure may differentiate into an FO-II B cell. If the MZ B-cell compartment is not yet 'full', an FO-II B cell may presumably respond to weaker self-antigens, Notch ligands and BAFF to differentiate into an MZ precursor B cell before migrating to the MZ and developing into an MZ B cell. FO-II B cells are therefore functional recirculating follicular B cells that develop in a self-antigen-independent manner but may be capable of rapidly repleting the MZ B-cell compartment when it is depleted by certain blood-borne pathogens. Interestingly, although it has been suggested that BAFF is not required for the survival of recirculating B cells in the bone marrow,[7] clearly, BAFF contributes to the development and/or survival of T2, FO-II and FO-I cells.[31] As these populations can mature in the bone marrow, it is assumed that either BAFF must be available in the bone marrow and contributes to maturation at this site, or that peripheral B-cell maturation requires BAFF only in the spleen and lymph nodes, and that the small numbers of follicular B cells seen in *Baff*−/− mice might have matured preferentially in the bone marrow.

The thymus is anatomically designed to facilitate positive selection and mediate a key linked lineage commitment event. Positive selection of B cells may be initiated in the bone marrow or the spleen and FO-I B cells may be generated at either site. The lineage commitment event linked to the positive selection of B cells of the B-2 lineage occurs exclusively in the spleen presumably because of the availability of appropriate ligands only at this site for MZ B-cell differentiation. The bone marrow is not fully equipped to mediate all the events that are crucial during peripheral B-cell maturation. This compartment is therefore both a primary and a secondary lymphoid organ, but can be distinguished from other secondary lymphoid organs by its ability to mediate a distinct subset of effector immune functions during host defense.

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