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# The Molecular Mechanisms Regulating Homing and Differentiation of Immature B cells

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In order to mature to antibody secreting cells that perform in the humoral immune response, B cells undergo an ordered differentiation process. In the first step stem cells differentiate in the bone marrow to immature B cells. These immature B cells leave the bone marrow and migrate to the spleen to undergo further maturation events to gain their antigen responsiveness. The transition from immature to mature B cells in the spleen is characterized by a series of changes in the properties of these cells. Although differentiation of immature B cells is a key event in the immune response, at present little is known about the molecular mechanisms regulating their targeting to the spleen and their differentiation in this compartment.

**Our research objectives:**

1. Follow the mechanisms controlling homing of immature B cells to the spleen.
2. Determine the mechanisms regulating immature B cell differentiation in the spleen.

**1. Homing of immature B cells to the spleen:**

Encounter with antigens at the immature developmental stage would lead to the death of immature B cells and elimination of effective clones due to the negative selection process. We therefore hypothesized the existence of pathways regulating homing of immature B cells to antigen- enriched sites like the peripheral lymph nodes and sites of inflammation. Indeed, we have discovered that by secreting low levels of the cytokine interferon gamma (IFN-γ), that was considered until recently to be an inflammatory cytokine secreted by T cells, immature B cells autocrinely down-regulate their homing to antigen enriched sites, such as the lymph nodes and sites of inflammation. Regulation results in their targeting to the spleen, where differentiation to mature cells occur. IFN-γ transmits a signal that

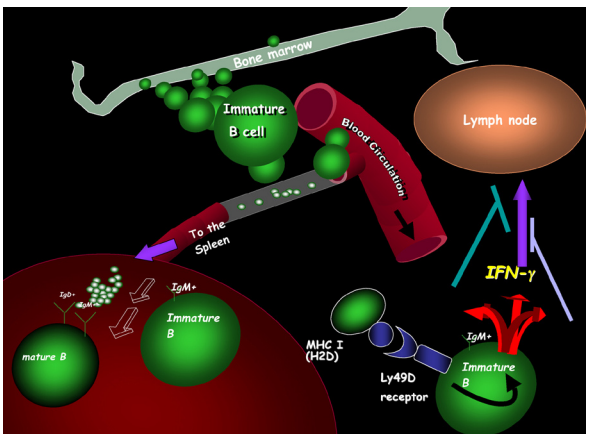


**Fig. 1** *B cells circulate in the periphery before their final arrival to the spleen for their maturation. Ly49D expressed on these immature cells recognizes MHC class I on various peripheral tissues resulting in constant low level secretion of IFN-γ. This constant recognition and IFN-γ secretion prevent immature B cells cytoskeleton rearrangement and their homing to antigen-enriched sites and target them to their maturation compartment in the spleen. After maturation, B cells downregulate their Ly49D expression and this downregulation terminates the IFN-γ secretion.*

results in the inhibition of actin polymerization and the extensive reorganization of the actin-based cytoskeleton, which is required for inducible integrin-mediated adhesion. We next showed that the MHC class I receptor, Ly49D, is expressed on immature B cells and is down regulated during maturation. Activation of this receptor leads to elevation in IFN-γ transcription and translation, thereby resulting in reduced ability of B cells to polymerize actin in response to chemokine stimulation. Moreover, we showed that MHC class I blockade inhibits the ability of immature B cells to transcribe the IFN-γ gene, and results in the rescue of cytoskeletal rearrangement. Thus, Ly49D, which is expressed on immature B cells, recognizes MHC class I on

peripheral tissues. This interaction induces secretion of low levels of IFN-γ by immature B cells, thereby downregulating their homing to the lymph nodes or to sites of inflammation (Fig 1). Moreover, we have shown that such low concentrations of IFN-γ have an anti-inflammatory effect, inhibiting migration of B and T cells.

**2. The mechanisms regulating B cell differentiation in the spleen:**

This final maturation step is crucial for B cells to become responsive to antigens and to participate in the immune response. Invariant chain (Ii) is an MHC class II chaperone, which was recently found to play a role in the differentiation of B cells in the spleen. We identified the Ii cytosolic domain as the segment involved in B cells differentiation by induction of NF-κB transcription activity. To follow the mechanism by which Ii transmits the signal to NF-κB in the nucleus, we analyzed the localization of the Ii cytosolic domain (Ii-Cy). These studies showed that the Ii cytoplasmic segment is released into the cytoplasm in transfected 293 cells and in primary B cells. This cleavage is essential for NF-κB activation and B cell differentiation. Amino acids 42-44 were found crucial in the Ii intramembrane cleavage event, as their mutation completely blocked the proteolytic release of the Ii cytosolic fragment. Our studies suggest that the behavior of Ii shows remarkable similarities to the function of a recently described family of proteins whose activity is activated by intramembrane proteolysis (RIP) and suggest that the roles of Ii as a chaperone and as a signaling molecule are intertwined (Fig 2).

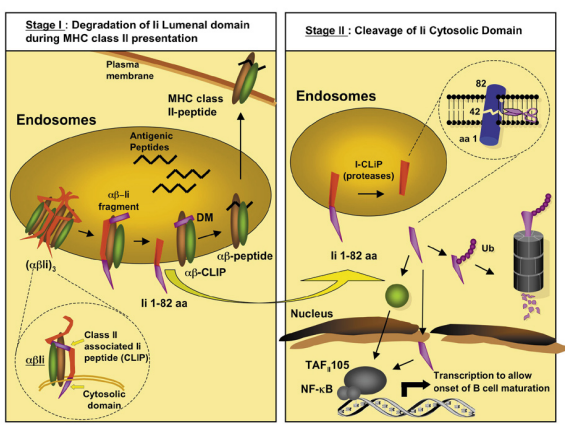


**Fig. 2** *Model for activation of signal transduction through Ii. The 1-82 Ii fragment is formed during the MHC class II biosynthetic and expression pathway (stage I). Upon receiving a signal, immature B cells release the 1-42 amino acid cytosolic domain by cleavage within the transmembrane region (Stage II). The released cytosolic fragment then induces NF-κB activity either directly (1) or by activating another protein or pathway that can induce NF-κB (2); the fragment is then degraded.*

In addition, in order to analyze molecules that regulate the differentiation of immature to mature B cells, we looked for genes that are differentially expressed in these cells. We compared relative mRNA levels between the immature and mature populations by screening mouse DNA chip arrays using the Affymetrix genechip expression analysis system. Several genes have been raised in this screen and we chose to further analyze the role of the inhibitor of DNA binding, Id2. Id2 is a HLH protein that lacks a DNA-binding region and therefore inhibits basic HLH (bHLH) functions in a dominant negative manner. Our studies show that Id2 expression is downregulated during differentiation of immature B cells to mature cells. Mice lacking Id2 exhibit an extensive maturation of peripheral B cell indicating that Id2 is a regulator of the final stages of B cell development occurring in the spleen.

***Selected Publications***


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***Acknowledgements:***

I.S. is the incumbent of the Alvin and Gertrude Levine Career Development Chair of Cancer Research. The Israel Science Foundation; Minerva foundation; Teva Pharmaceuticals; Israel cancer foundation; Abisch-Frenkel foundation.