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# The molecular mechanisms controlling development and function of immature B cells

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In order to fully mature and to participate in the humoral response, immature B cells first migrate into specific areas in the spleen, where they mature, while their arrival to other compartments is restricted.

**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.

**Homing of immature B cells**

Immature B cells are sequestered from encountering foreign antigens present in lymph nodes or sites of inflammation, prior to their final maturation in the spleen; however, the mechanism controlling this phenomenon has not been fully elucidated. Our studies show that immature B cells fail to home to the lymph nodes and can actively exclude themselves from antigen-enriched sites by down-regulating their integrin-mediated adhesion to the extracellular matrix protein, fibronectin. This homing regulation is mediated by interferon-γ, which is transcribed and secreted by immature B cells but dramatically downregulated in mature B cells. Perturbation of IFN-γ activity *in vivo* leads to the homing of immature B cells to the lymph nodes. This is the first example of autocrine regulation of immune cell migration to sites of foreign antigen presentation. We further analyzed the effect of IFN-γ on other members of the immune system. We demonstrated that similarly low doses of IFN-γ downregulate integrin mediated-adhesion and migration of naïve T and has a profound effect on the *in vivo* homing of naïve T cells to the LN, as well as the migration of effector Th2 cell to sites of inflammation in an asthma model (Fig. 1). Our goal is to follow IFN-γ induced homing regulation. Specifically we intend to: (i) Study the target adhesion step inhibited by IFN-γ in vivo (ii) Determine the IFN-γ controlled signaling pathway that regulates adhesion and migration of B cells on ECM integrin-ligands.

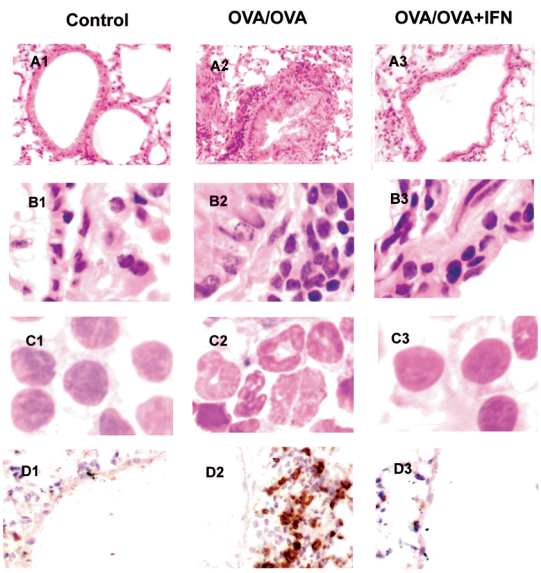


***Fig. 1*** *Lung histology in mice that received IFN-γ treatment. Histologic features of control, ova-primed (OVA/OVA) and ova-primed treated with IFN-γ (OVA/OVA+IFN). A1-3, x10; B1-3, x60; C1-3, BAL x100; D1-3 x20 CD3 cells.*

**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. Our preliminary studies provided initial insights into the role of invariant chain in B cell maturation. We have demonstrated that Ii regulates B cell maturation, and that its N-terminal domain is crucial for this process. Thus, we have dissected for the first time the chaperonin activity of Ii from its role in B cell maturation. Furthermore, we demonstrated that Ii initiates a signaling pathway that is mediated by modulation of the transcription factor NF-kB p65/RelA. Our recent studies have shown that Ii cytosolic domain is cleaved and released to the cytoplasm, a process which is essential for NF-kB activation

and B cell differentiation. We suggest that Ii mode of action shows a striking similarity to that of other membrane bound signaling proteins, which liberate their cytoplasmic domain to transmit a signal that results in the regulation of transcription (Fig. 2).

Our goals: 1. Characterize the pathway of B cell maturation controlled/regulated by Ii. To this end the following specific objectives will be addressed: (i) Isolation of Ii auxiliary proteins that participate in B cell maturation (ii) Characterization of the signaling pathway induced by Ii.

In order to analyze additional 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 Affymatrix 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. Our goal: (i) Further analyze the role of Id2 in immature B cell differentiation (ii) characterize additional genes that control differentiation of immature B cells.

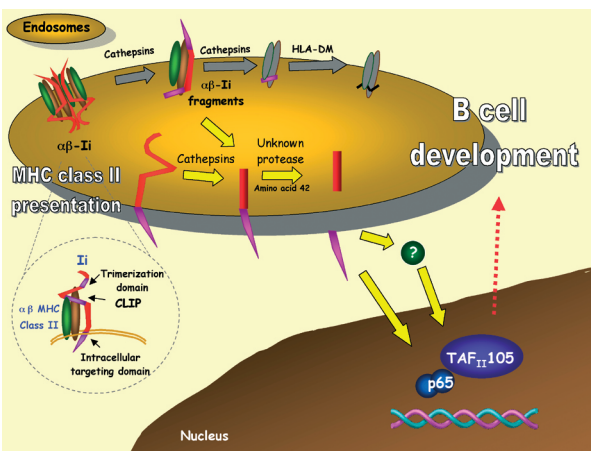


***Fig. 2*** *Schematic presentation of the invariant chain pathway in B cell differentiation.*

### *Selected Publications*


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