The molecular mechanisms controlling homing, differentiation and survival of peripheral lymphocytes

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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 (BM) and migrate to the spleen where they undergo further maturation events and 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 and survival in this compartment.

Our research objectives:

1. Regulation of immature, B cell splenic targeting.

B-cell development involves the ordered progression of a stem cell through a number of stages, ultimately resulting in a mature B cell. The first cells expressing IgM on their surface during this developmental process are the immature B cells, which leave the bone marrow for their final maturation in the spleen. These recirculating cells are sequestered and prevented from encountering foreign antigens present in lymph nodes or at sites of inflammation, prior to their splenic arrival; however, the mechanism controlling this phenomenon has not been fully elucidated. Our studies show that immature B cells can actively exclude themselves from antigen-enriched sites in an autocrine manner. This regulation of homing is mediated by two independent inhibitory pathways; both control cytoskeletal rearrangement required for promoting integrin-mediated adhesion and migration of B cells (Fig 1).

The first pathway involves the secretion of IFN-γ, which is transcribed and secreted at low levels by immature B cells. In our early studies, we followed the IFN-γ induced inhibitory pathway and showed that the activating Ly49D receptor, expressed at high levels on peripheral immature B cells, controls IFN-γ secretion. Activation of the Ly49D receptor triggers a signaling cascade that increases transcription of IL-12B and IL-18. These cytokines interact with their specific receptors, which are expressed at high and non-limiting levels on these immature B cells and induce IL-15 secretion. IL-12B, IL-18 and IL-15 enable the secretion of IFN-γ. Secretion of low levels of IFN-γ downregulates the ability of immature B cells to home to the lymph nodes or to sites of inflammation.

The second pathway is regulated by the chemokine receptor, CCR2, which is expressed on murine immature B cells and whose expression is downregulated in the mature stage. We showed that CCR2 is transcribed in immature B cells, while its message is dramatically down regulated at the mature stage. CCR2 deficiency results in upregulation of chemokine-induced actin polymerization, migration and homing to the lymph nodes of immature B cells. In addition, we demonstrated that CCR2 homing control is mediated by its ligand, CCL2 that is secreted by B cells and downregulates the CXCL12 signaling cascade. Thus, CCR2 and its ligand CCL2 negatively regulate homing of immature B cells.

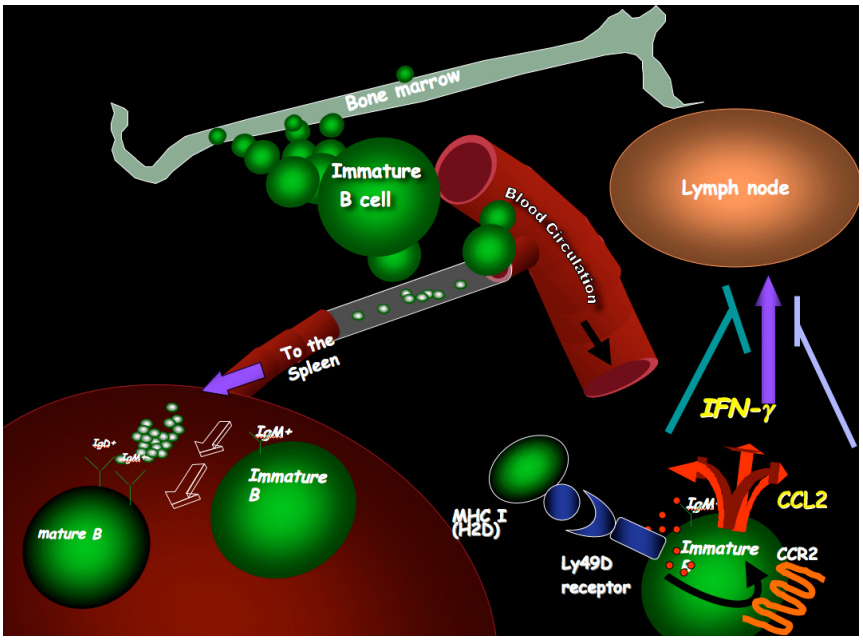

Fig. 1 *Immature B cells can actively exclude themselves from antigen-enriched sites. This homing regulation is mediated by two independent inhibitory pathways, both control cytoskeletal rearrangement required for promoting integrin-mediated adhesion and migration of B cells.*

The inhibitory pathways regulating immature B cell homing control T cell migration.

Our studies focus on the pathways that restrict homing of specific subsets of immune cells and thereby fine tune the immune response at specific lymphoid and peripheral tissues. We therefore wished to determine whether the inhibitory pathways described in B cells regulate T cell homing as well. We demonstrated that similarly low doses of IFN-γ downregulate integrin mediated- adhesion and migration of T cells and have a profound effect

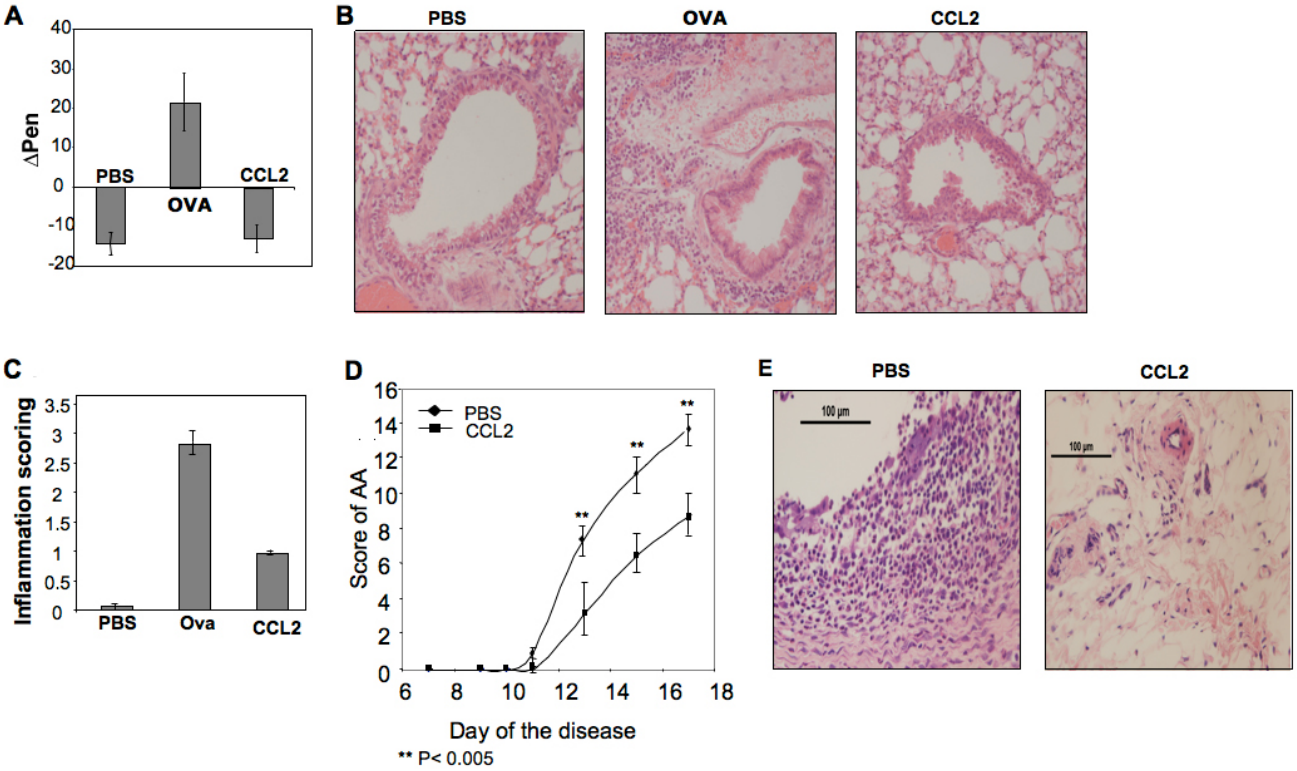

Fig. 2 *CCL2 plays an anti-inflammatory role in vivo (A) Control (PBS treated), OVA-primed mice (OVA) and OVA-primed mice i.p. injected with 60 ng CCL2 (CCL2) were analyzed for airway responsiveness on day 15. Values shown represent airway responsiveness in PBS treated control, OVA-primed mice, and OVA-primed mice treated with CCL2. The results represent an average of nine animals per treatment. (B) Lung histology in mice that received CCL2 treatment. Histologic features of representative control (PBS), ova-primed (OVA) and ova-primed animals treated with low dose CCL2 (CCL2) are shown. (C) The peribronchial and perivascular inflammatory infiltrates were assigned an inflammatory score of between 1-4 by a pathologist. The graph represents the average scores of nine animals from each treatment group. (D) Arthritis model: Adjuvant induced arthritis was induced, and rats immediately divided into two groups that were injected with low dose CCL2 (240 ng in 300 ml of PBS) or PBS. A disease score between 0 and 4 was assigned to each limb, based on the degree of joint inflammation, redness, and deformity; thus the maximum possible score for an individual animal was 16. The graph represents the average scores of nine animals in each group that were measured every day. (E) Joint histology of PBS or CCL2 treated mice.*

on their *in vivo* homing to the LN. Moreover, these low doses of IFN-γ have anti-inflammatory effects in an *in vivo* asthma model. Thus, in contrast to the pro-inflammatory effects of IFN-γ at relatively high concentrations, low dose IFN-γ appears to exert global suppressive effects on T cell trafficking and may have clinical application as an anti-inflammatory agent.

In addition, we reported that CCL2 (at picomolar (pM) levels) renders both murine and human T cells defective in their ability to develop CCR7 triggered LFA-1-mediated adhesion strengthening to endothelial ICAM-1 both *in vitro* and *in vivo*. Such impaired homing of lymphocytes to peripheral lymph nodes results in attenuated progression of both asthma and adjuvant-arthritis (Fig 2). Thus, picomolar levels of circulating CCL2 can exert global suppressive effects on T cell trafficking and differentiation within peripheral lymph nodes and may be clinically beneficial as an anti-inflammatory agent.

2. The mechanisms regulating B cell differentiation and survival in the spleen.

CD74 (invariant chain; Ii) is a non-polymorphic type II integral membrane protein; it has a short N-terminal cytoplasmic tail of 28 amino acids (aa), followed by a single 24 aa transmembrane region and an approximately 150 aa lumenal domain. The CD74 chain was thought to function mainly as an MHC class II chaperone. However, CD74 was recently shown to have an additional role as an accessory signaling molecule. CD74 was reported to be a high-affinity binding protein for the proinflammatory cytokine, macrophage migration-inhibitory factor (MIF), providing further evidence for its role in signal transduction pathways. Our studies demonstrated that CD74 regulates B cell differentiation by inducing a pathway leading to the activation of transcription mediated by the NF-κB p65/RelA homodimer and its coactivator, TAFII105 in CD74 transfected 293 cells and in B cells. NF-κB is activated by the intracellular domain of CD74 (CD74-ICD), which is liberated from the membrane. Thus,

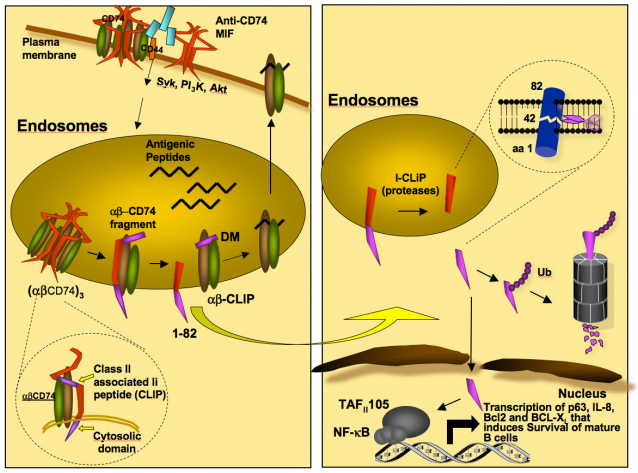

Fig. 3 Schematic representation of the CD74 induced signaling pathway regulating B cell survival.

the behavior of CD74 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 CD74 as a chaperone and as a signaling molecule are intertwined.

The nature of the signal transmitted by CD74 was only recently revealed. We showed that stimulation of CD74 using anti-CD74 antibody or its natural ligand MIF, leads to an induction of a signaling cascade resulting in NF-κB activation, entry of the stimulated cells into the S phase, elevation of DNA synthesis and cell division. NF-κB activation also upregulates the expression of p53-related TAp63 proteins. TAp63 then binds and transactivates the Bcl-2 gene and induces the production of Bcl-2 protein, thereby providing the cells with increased survival capacity. Thus, these studies therefore demonstrate that surface CD74 functions as a survival receptor. The CD74/NF-κB/TAp63 axis defines a novel anti-apoptotic pathway in mature B cells, resulting in the shaping of both the B cell repertoire and the immune response (Fig 3).

Previous studies have shown that chronic lymphocytic leukemia B lymphocytes (B-CLL) express relatively large amounts of CD74 mRNA relative to normal B cells. We therefore analyzed the molecular mechanism regulated by CD74 in B-CLL cells. Our studies showed that activation of cell surface CD74, expressed at high levels from an early stage of the disease, by its natural ligand MIF initiates a signaling cascade that contributes to tumor progression. This pathway induces NF-κB activation, resulting in the secretion of interleukin 8, which in turn promotes cell survival. Blocking of this pathway leads to decreased cell survival (Fig 4). These findings could form the basis of novel therapeutic strategies aimed at blocking the CD74 induced, IL-8 dependent survival pathway.

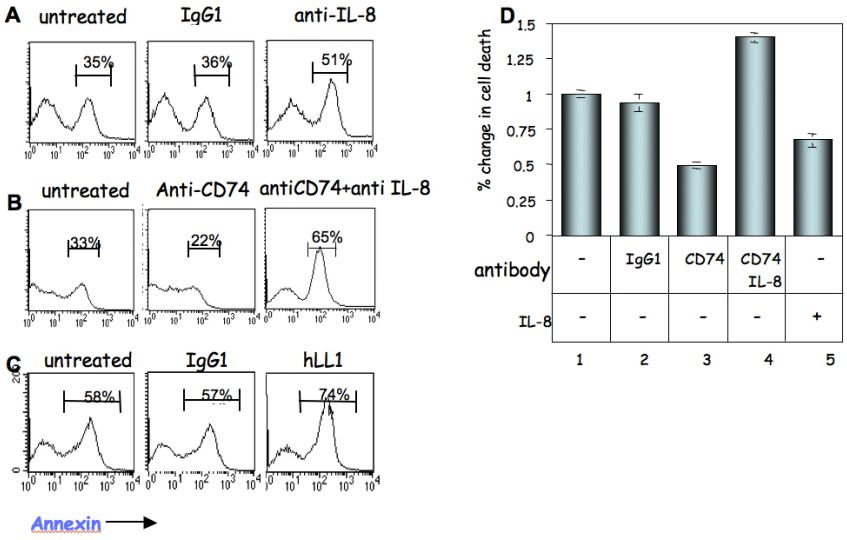

Fig. 4 IL-8 secreted following CD74 stimulation regulates B-CLL cell survival.
(A-C) B-CLL cells were incubated in the presence or absence of the activating anti-CD74 antibody (B), anti-IL-8 (A, B) or a control antibody (c-jun; A) for 48 h or with the inhibitory antibody (hLL1) versus control antibody (C). Cells were stained for Annexin V and analyzed by FACS. The results presented are representative of seven B-CLL patients. (D) B-CLL cells were incubated in the presence or absence of a control antibody (c-jun), anti-CD74, anti-IL-8 or IL-8 for 48 h. Cell death was analyzed by ELISA. The graph shows the results of one experiment, representative of four.

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