Published in final edited form as:

Immunol Rev. 2019 May ; 289(1): 84–100. doi:10.1111/imr.12758.

Compartmentalization of Dendritic Cell and T cell Interactions in the Lymph Node: Anatomy of T Cell Fate Decisions

Beatriz León[1,*] and **Frances E. Lund**[1,#]

[1]Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama, USA.

Summary:

Upon receiving cognate and co-stimulatory priming signals from antigen (Ag)-presenting dendritic cells (DCs) in secondary lymphoid tissues, naïve $CD4^+$ T cells differentiate into distinct effector and memory populations. These alternate cell fate decisions, which ultimately control the T cell functional attributes, are dictated by programming signals provided by Ag-bearing DCs and by other cells that are present in the microenvironment in which T cell priming occurs. We know that DCs can be subdivided into multiple populations and that the various DC subsets exhibit differential capacities to initiate development of the different $CD4^+$ T-helper populations. What is less well understood is why different sub-anatomic regions of secondary lymphoid tissues are colonized by distinct populations of Ag-presenting DCs and how the location of these DCs influences the type of T cell response that will be generated. Here we review how chemokine receptors and their ligands, which position allergen and nematode-activated DCs within different microdomains of secondary lymphoid tissues, contribute to the establishment of IL-4 committed follicular helper T (Tfh) and type 2 helper (Th2) cell responses.

Keywords

Dendritic Cells; CD4+ T cell priming; Chemokine receptors; Migration

INTRODUCTION

Dendritic cells (DCs) initiate T-cell activation and polarization by processing and presenting microorganism-derived peptides on Major Histocompatibility Complex (MHC) molecules (signal 1) and by providing potent co-stimulatory signals (signal 2) through engagement of CD28 expressed by the T cells (1, 2). These co-stimulatory signals, when combined with T cell receptor (TCR) recognition of the peptide-MHC complex (pMHC), result in activation, clonal expansion and survival of the peptide-specific T cells (2). DCs also provide additional cues, often referred to as “signal 3” (3), which are necessary for the full activation of naïve T cells and ultimately direct the effector fate of both $CD4^+$ and $CD8^+$ T cells. In fact, DCs that

[*]Address correspondence to:Beatriz León, Department of Microbiology, University of Alabama at Birmingham, 1825 University Blvd, SHEL 314, Birmingham, AL 35294-2182, Phone: 205-975-4806, bleon@uab.edu. [#] Address correspondence to:Department of Microbiology, University of Alabama at Birmingham, 1720 2nd Avenue South, 276 BBRB Box 11, Birmingham, AL 35294-2170, Phone: 205-934-9042, flund@uab.edu.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

provide these additional activation and developmental signals, often in the form of cytokines, dictate the differentiation of naïve $CD4^+$ T cells into the various effector T helper (Th) subsets (2). These subsets include: type 1 (Th1) cells, which are critical for resistance to intracellular viruses and bacteria (4); type 2 (Th2) cells, which mediate resistance to parasitic infections and susceptibility to allergic airway inflammation (5); and type 17 (Th17) cells, which play a pivotal role in defense against extracellular bacterial and fungal pathogens (6). DCs also provide signals that promote the development of inducible or peripheral regulatory T cells (pTreg), which maintain peripheral tolerance (7) and follicular helper T (Tfh) cells, which provide specialized help to B cells (8). Finally, DCs prime and activate $CD8^+$ T cell responses by processing and presenting intracellular antigens (Ag) derived from intracellular pathogens and by cross-presenting exogenous Ag in association with MHC class I (9).

Numerous subpopulations of DCs have been identified, based on their ontogeny, differentiation requirements and functional properties (10–13). DC subsets can be broadly subdivided into plasmacytoid DCs (pDCs), monocyte-derived DCs (mo-DCs) and conventional DCs (cDCs) (11, 14). pDCs, which arise from progenitors within the bone marrow and are recruited from the blood into peripheral and lymphoid tissues, are a major source of type I interferon (IFN) during viral infections (15). Monocyte-derived DCs, which are generated from circulating monocytes that differentiate into DCs upon entry into inflamed peripheral tissues and associated secondary lymphoid organs, also release inflammatory cytokines and chemokines during infection (16). Not surprisingly, given their ability to produce cytokines and chemokines, pDCs and mo-DCs play important roles in innate inflammatory responses (15, 16). However, these DC subsets are not typically considered be major contributors to T cell priming. Instead, that role is assumed by the cDCs, which are the primary APCs to initiate and shape T cell immune responses (12).

Since activation and differentiation of naïve T cell into effectors that are exquisitely tailored to respond to specific pathogens relies on encounters between the rare peptide-specific T cells with cDCs presenting the relevant peptide, the localization of both the cDCs and T cells must be highly orchestrated. cDCs are strategically located at sites of pathogen entry, such as mucosal surfaces like the skin, intestine and lungs, or at portals for blood-borne pathogens and Ags, such as the splenic marginal zone (1, 17). The DCs present in peripheral tissues continuously sample their surrounding environment for invading pathogens and, upon encountering pathogens and/or damage, the DCs are activated and migrate to secondary lymphoid organs, such as the regional lymph nodes (LNs). Once localized in the secondary lymphoid tissues, Ag-bearing cDCs specifically home to different niches or microenvironments within the lymphoid tissue, where the DCs can encounter and present Ags to the rare pathogen-specific T cells. The coordinated migration of the cDCs and T cells to different anatomic regions of the secondary lymphoid tissues is controlled by G protein-coupled chemoattractant/chemokine receptors, which are expressed by the DCs and T cells, as well as the ligands of these receptors, which are produced in a location-specific manner by other hematopoietic and non-hematopoietic cells (17). Importantly, these chemokine producing cells also provide other soluble and membrane-associated signals that can influence the nature and quality of the DC-T cell interactions in the different lymphoid tissue niches. Therefore, in addition to the three classical signals delivered by the DCs to naïve T

cells, other microenvironment or location-specific signals derived from the anatomic region of the lymphoid tissue in which the DCs and T cells co-localize may contribute to the fine-tuned control of T cell proliferation, differentiation and function *in vivo*. This review will highlight the mechanisms that control the migration and localization of cDCs and T cells in different microenvironmental niches within secondary lymphoid tissues and will dissect how crosstalk between DCs and T cells in these niches provide unique and specialized signals that ultimately shape the ensuing T cell response. Although DCs can initiate multiple flavors of both $CD4^+$ and $CD8^+$ T cell responses, this review will focus on the specific microenvironment-sensitive requirements for the priming of Th1, Th2, and IL-4 producing Tfh cells.

T cell priming in secondary lymphoid tissues – does one location fit all?

It is well established that activation and priming of naïve T cells occurs when the rare Ag-specific T cells encounter Ag-presenting cDCs within the confines of secondary lymphoid tissues (18, 19). It is also appreciated that the organization of secondary lymphoid tissues into distinct specialized cellular compartments and structures (20) facilitates these cognate encounters between Ag-presenting DCs and T cells. The functional importance of the T cell-DC encounters within a specific anatomic region of the secondary lymphoid tissues, referred to as the T cell zone, was first shown in homozygous *plt/plt* (paucity of lymph node T cell) mice, which lack the capacity to produce ligands of the CCR7 chemokine receptor in the T cell zone (21–23). The loss of these ligands in *plt/plt* mice not only impaired the recruitment of CCR7-expressing DCs and T cells to the T cell zone of the LN but also attenuated Th1 cell responses (24, 25). These data, along with studies using mice lacking CCR7 expression by DCs (26, 27), nicely demonstrate that CCR7-expressing DCs and T cells initiate T cell responses, particularly Th1 cell responses, in the T cell zone of secondary lymphoid tissues. Although this T cell priming model can be found in essentially all introductory immunology textbooks, a more in-depth analysis of *plt/plt* and CCR7 deficient mice reveal that some T cell responses remain intact even when T cells and DCs do not colocalize in the T cell zone. For example, $CD8^+$ T cell responses, particularly when primed in the spleen, are fully functional in *plt/plt* and CCR7-deficient mice following infection or immunization (25, 28, 29). Likewise, *plt/plt* mice do not show obvious defects in the induction of Th2 cell responses or Tfh cell responses, as they display normal Th2 cytokine production by $CD4^+$ T cells (30) and Ig class switch (28) after intestinal helminth and viral infection, respectively. Moreover, defective CCR7 signaling in lymphoid tissues, as seen in *plt/plt* and $Ccr7^{-/-}$ mice, can, in some experimental settings enhance Th2 cell responses and Tfh cell driven-B cell responses (31–33). These data, therefore, raise the intriguing possibility that the accepted model of T cell priming by DCs within the T cell zone of secondary lymphoid tissues may not reflect all the different niches in which DCs and T cells may interact in secondary lymphoid tissues. In the following sections we will review evidence supporting a modified model in which Ag-presenting cDCs, which localize within different microenvironmental niches in the LN (Figure 1) or spleen (Figure 2), exert distinct location-specific effects on $CD4^+$ T cells and thereby influence the differentiation and function of these T cells.

Conventional DC subsets.

The primary Ag-presenting DC subset, i.e. cDCs, is generated from blood circulating precursors (12). These cDC precursors either enter into peripheral tissues, such as the skin and mucosal surfaces, or exit the bloodstream directly into secondary lymphoid organs, like LNs and spleen (12). The precursors that migrate directly into the secondary lymphoid organs from the blood differentiate into resident DCs (rDCs), which will remain within the lymphoid tissue throughout their life span (12). On the other hand, the cDC precursors that migrate into peripheral tissues give rise to tissue-derived migratory DCs (mDCs). The mDCs, which have been described as the sentinels of the immune system, are triggered under both steady state and inflammatory conditions to leave the peripheral tissue and migrate through afferent lymphatics to the draining LN, where they will encounter and present Ags to naïve T cells (12). Both rDCs and mDCs can be further subdivided based on differential expression of fate-specifying IRF transcription factors. These subsets include the IRF8-expressing cDCs (cDC1 cells) and IRF4-expressing cDCs (cDC2 cells) (14). The $IRF4^+$ resident and migratory cDC2 cells share common developmental requirements (11, 14) and express CD11b while the $IRF8^+$ cDC1 cells lack CD11b and instead express CD103 (mDCs) and/or CD8α (rDCs) (11). Initially, the cDC1 cells were thought to mediate cross-presentation to $CD8^+$ T cells for immune defense against intracellular viruses and bacteria and for tumor surveillance (34–40). By contrast, the cDC2 cells were described as the primary Ag-presenting cells (APCs) for $CD4^+$ T cells (37, 41–43). However, as our current understanding of the division of labor between DCs subsets grows (12, 44), it is now clear that cDC1 and cDC2 cells display overlapping roles in $CD4^+$ and $CD8^+$ T cell activation, tolerance induction and differentiation. The distinct functional attributes of the cDC1 and cDC2 subsets are controlled, at least in part, by differences in (i) expression of the receptors that capture Ags, (ii) the signals that activate each subset, (iii) the timing of activation of each subset during the immune response and (iv) the capacity of each subset to respond to different migratory cues (12, 44–49). In the next sections we focus our attention on how the differential trafficking of each DC subset to different sub-anatomic regions of lymphoid tissues modulates $CD4^+$ T cell immunity.

CCR7 controls homing of migratory cDCs to LNs under steady state and in response to specific inflammatory stimuli.

Migratory cDCs, i.e. mDCs, are strategically positioned at the body barriers such as the skin and mucosal sites, like the gastrointestinal and respiratory tracts (12). To ensure that the Ag-bearing mDCs and Ag-specific naïve T cells find one another, mDCs must undertake multiple migratory steps (Figure 1). First, the mDCs must leave the tissue and enter the afferent lymphatics (50–52). Second, the mDCs must transit through the afferent lymphatics and enter the LN (53, 54). Finally, the mDCs must traffic to the appropriate niche(s) within the LN where the DCs can interact with Ag-specific T cells (24, 53). The chemokine receptor CCR7 (52) and one of its ligands, CCL21, are critical regulators of the first migratory step and control entry of the CCR7-expressing mDCs into afferent lymphatic vessels under steady state and inflammatory conditions (55). CCL21 is encoded by two genes in mice (22), which can be distinguished by a single nucleotide change that results in a leucine to serine substitution at position 65 (CCL21-Leu and CCL21-Ser). Although both forms of CCL21 can be immobilized to extracellular matrix proteins through a C-terminal

heparin sulfate-binding domain (56–59) and can elicit equivalent chemotactic activity (60), the two forms of CCL21 are differentially expressed in lymphoid and nonlymphoid tissues (61). The CCL21-Leu chemokine, but not the CCL21-Ser chemokine, is constitutively expressed by lymphatic endothelial cells (22, 23, 25) and is immobilized on the basement membrane of the initial capillary lymphatic vessels. Thus, the CCL21-Leu form is required to promote transmigration and entry of the tissue mDCs into the afferent lymphatics and does so by facilitating the chemotaxis, adhesion and arrest of the mDCs (62, 63).

Once inside the lymphatic vessels, it has been long suggested that mDCs are passively and rapidly swept along the lymph flow and delivered into the subcapsular sinus of the LN (64). While it is true that intralymphatic migrating tissue mDCs can flow passively within the large collecting and afferent lymphatics that lead to the draining LN (62), more recent data indicate that DCs actively crawl, while in close contact with the lymphatic endothelium, from the initial capillaries toward the collecting vessels (62, 65). This migration is also dependent on CCR7 expression by the DCs (62, 65).

It is estimated that under steady state conditions approximately 5% of all $CD11c^+$ DCs residing in peripheral LNs are new arrivals from the tissues each day (66). Since mDCs require CCR7 to home to the draining LNs (50–52) and since CCL21-Leu is constitutively expressed by lymphatic vessels (61), the main factor influencing the migration of cDCs from the tissues to the draining LN is the regulated expression of CCR7 by the mDCs. CCR7 is expressed at low levels in immature DCs, however, its expression can be further induced under steady state and in the setting of inflammation (55). Although the mechanisms that trigger upregulation of CCR7 on resting cDCs under steady state conditions are not well understood (55), recent data reveal that a novel NF-κB-regulated gene network, which is driven by IκB kinase β (IKKβ), seems to control the expression of CCR7 and the steady-state accumulation of mDCs in draining LNs (67). These data therefore suggest that upregulation of CCR7 in tissue mDCs under homeostasic conditions is tightly controlled by a distinct NF-κB regulatory gene network specific to steady-state mDCs.

NF-κB signaling is also activated following inflammation (68) and inflammatory mediators produced upon infection also potently induce CCR7 upregulation by tissue mDCs (69-75). These CCR7-inducing stimuli include pathogen-associated molecular patterns (PAMPs), which are directly recognized by pattern-recognition receptors (PRR)s expressed on mDCs (69, 70, 75). In addition, inflammatory mediators such as tumor necrosis factor (TNF), interleukin 1 (IL-1), and eicosanoids can promote CCR7 expression on mDC (69-75). Thus, DCs that have been stimulated by microbial products and proinflammatory cytokines undergo phenotypic and functional changes that greatly augment their capacity to enter the afferent lymphatics and migrate to regional LN.

Homing of Th2-inducing mDCs to LNs requires cooperation between chemokine receptors.

Although CCR7 upregulation by mDCs under steady state and following exposure to stimuli that lead to Th1, Th17 and pTreg cell development is clearly important for the trafficking of these mDCs to LNs (55), mDCs can also respond to stimuli like parasitic infections and allergen exposure that do not induce high levels of CCR7 expression by the mDCs (30, 76). Despite the relatively low expression of CCR7 by these allergen and nematode-activated

mDCs, CCR7 still appears to play an important role in directing the migration of these cells to the draining LN as it is reported that migration of allergen-activated mDCs from the skin to the draining LN requires CCR7 (76). However, another G protein coupled chemokine receptor, CCR8, is also required for mDC emigration from the skin and, in fact, appears to augment the CCR7-induced migratory capacity of the mDCs (76). Similarly, sphingosine-1-phosphate (S1P), a lipid-signaling molecule that binds five G protein-coupled sphingosine-1-phosphate receptor subtypes ($S1PR_{1–5}$) (77), is required for mDC migration into the LN in the setting of Th2 immune responses (78). This study, which used an ovalbumin-induced Th2-driven murine asthma model, demonstrated that S1P signaling significantly enhances mDC migration from the lung to the draining mediastinal LN and potentiates the priming of allergen-specific Th2 cells within the LN (78). Interestingly, S1P signaling not only regulates DC trafficking but also modulates cytokine production by the DCs and ultimately influences the capacity of the DCs to induce a Th2 dominant response (79). Collectively, these data suggest that the trafficking of mDCs activated by Th2-inducing stimuli may require multiple receptors that play complementary and cooperative roles (Figure 1). Furthermore, the data predict that while CCR7 and its ligands can direct the emigration of mDCs from peripheral tissues under steady state and inflammatory conditions, these CCR7-directed signals are necessary but not sufficient to guide mDCs following exposure to stimuli that induce Th2 cell development. The requirement for additional, non-CCR7 cues to guide allergen and nematode-activated mDCs to the LN suggest that the stimuli that initiate Th2 cell responses do not optimally engage the regulatory networks that control CCR7 expression and signaling.

Anatomy and organization of the LN.

Tissue-derived mDCs reach the draining LN via afferent lymphatics in response to CCR7 signaling, alone or in combination with other chemoattractant signals. However, upon arrival to the LN, different mechanisms control how the mDCs will enter the LN and migrate to the appropriate niche. To better understand how these migratory steps are controlled, we will first review the anatomic features of LNs (Figure 1). In order to facilitate direct communication between Ags, professional APCs and Ag-specific B and T cells, LNs are organized into distinct specialized cellular compartments and structures (20). The LN can be subdivided into multiple distinct anatomic regions that extend from the edge of the LN to the innermost region of the tissue. These regions include: the subcapsular (or marginal) sinus, which lines the outer edge of the LN, the parenchyma, which includes the outer (cortex) and middle zones (paracortex) and the medulla, which includes the deep inner zone of the LN (20). While the subcapsular sinus is lined by specialized macrophage populations, the cortex, or outer zone, contains the B cell area where the B cells reside and are organized into compact and tightly packed B cell follicles. The paracortex is mostly populated by T cells and the medullary cords or inner zone contains macrophages, plasma cells, T cells and some DC populations. All these anatomic regions are maintained by a network of non-lymphoid stromal cells that not only provide structural support but also actively influence immune responses (80). Among the cells that are critical for generating this backbone are the fibroblastic reticular cells (FRCs) present in the T cell area (81), the marginal reticular cells (MRCs) that are enriched in the perifollicular region adjacent to B cell follicles (81) and the follicular dendritic cells (FDCs) that are present in the B cell follicles (82).

Entry of cells into the LN is broadly controlled by two different processes (Figure 1). Circulating B and T cells enter the LN through specialized high endothelial venules (HEV) that are located in the paracortex (20), while cells that are in tissues, like the mDCs, enter the LN through the afferent lymphatics (17) that empty into the subcapsular sinus, which is located at the outer edge of the LN, between the capsule and the cortex. Although entry of cells into the LN occurs by multiple mechanisms, cells exit the LN by the same pathway (20), which requires transiting through the LN via the cortical and medullary sinuses to the efferent lymphatics that are found in the central medullary region. Cells that enter the efferent lymphatics can eventually reach the blood stream and recirculate to other tissues.

Multiple chemokine receptors mediate entry of migratory cDCs to LN.

Once mDCs enter the LN subcapsular sinus through the afferent lymphatics, the cells must overcome the barrier imposed by the subcapsular sinus floor that is lined by LYVE-1-expressing lymphatic endothelial cells (83) and resident $CD169^+$ macrophages (84). Published data indicate that mDCs overcome this barrier in a CCR7-dependent manner by responding to CCL21, particularly, the CCL21-Ser form, which is expressed in the subcapsular sinus and paracortex or T cell zone of the LN (54). CCL21, which is a ligand for both CCR7 and the chemokine receptor ACKR4 (also commonly referred to as CCRL1 (85)), is bound, internalized and degraded by the CCRL1-expressing subcapsular sinus ceiling lymphatic endothelial cells (54). Since the subcapsular sinus floor lymphatic endothelial cells do not express CCRL1 (54), a CCL21 gradient across subcapsular sinus floor is established. This CCRL1-controlled CCL21 gradient is functionally important for the tissue-derived mDCs that upregulate CCR7 under steady state or following exposure to inflammatory PAMPs. For example, CCR7-expressing mDCs accumulate in the subcapsular sinus of CCRL1 deficient mice and are unable to penetrate into the parenchyma (54). Similarly, DCs accumulate in the subcapsular sinus rather than migrating to the LN parenchyma in the LN of mice that are unable to produce the CCR7 ligands that are normally expressed in lymphoid tissues (CCL21-Ser and CCL19) (24), due to a homozygous mutation (*plt/plt*) that disrupts these two genes but leave the CCL21-Leu gene intact (21–23).

Although the CCR7-CCRL1-CCL21 axis is clearly important for mDC entry into the LN tissue, DC intrinsic expression of CCR7 may not be sufficient to allow all types of mDCs to enter the LN parenchyma. As discussed earlier, mDCs responding to infections and allergens that induce Th2 cell responses express less CCR7 (30, 76) and require signals from additional chemokines, like CCL8 or S1P (76, 78), to enter the afferent lymphatics. Similarly, it was reported that the CCR8 ligand, CCL8, which is expressed by subcapsular sinus $CD169^+SIGN\text{-}R1^+$ macrophages, synergizes with CCL21 to promote the emigration of tissue mDCs out of the LN subcapsular sinus and into the LN parenchyma (76). Thus, while CCL21 plays an important role in the trafficking of most tissue-derived mDCs to the LN and into the LN parenchyma, there is a variable requirement for other migratory cues (Figure 1), which is most likely controlled by the pathogen and inflammatory stimuli delivered to the mDCs.

Differential chemokine receptor expression controls migratory cDC localization within the LN and impacts T cell fate decisions.

Upon entry into the LN parenchyma, tissue mDCs can move into the paracortex (T cell zone) to interact with incoming naïve T cells (Figure 1). This process is guided by two CCR7 ligands, CCL19 and CCL21-Ser, which are strongly expressed in and around HEVs by the HEV and FRCs (20). This CCR7-dependent process of moving tissue-derived mDCs into the T cell zone of the LN takes place under steady state and in response to pathogens that drive Th1 cell immunity. Under homeostatic conditions, CCR7-regulated migration of tissue mDCs to the T cell zone plays a dominant role in maintaining tolerance and immune homeostasis (55). By contrast, in the setting of infection with a Th1-inducing pathogen, CCR7-dependent migration of tissue-derived mDCs to the T cell zone of LN results in the activation and differentiation of Th1 effector cells (55).

Although tissue mDCs are typically depicted in textbooks as migrating to the T cell zone of the LN, we now know that DCs also localize in other anatomic sites within the LN. For example, immediately following exposure to stimuli that induce Th1 immunity, mDCs migrate to the T cell zone, in a CCR7 dependent fashion (18, 30, 86–88). However, later in the immune response, the mDCs migrate farther into the LN tissue and take up residence in the deep paracortex and inner medullary region (89), where they continue to interact with T cells that have also migrated to this region (Figure 1). Moreover, following allergen exposure or nematode infection, tissue-derived Ag-bearing mDCs are preferentially attracted to the border of the B cell follicles and T cell zone (T-B border) and to the area between B cell follicles (perifollicular region) rather than to the T cell zone (30, 90, 91). Interestingly, we found that the mDCs that induce Th2 cell development in response to nematode infection up-regulate the expression of the chemokine receptor CXCR5 (30). The ligand of CXCR5, CXCL13, which is constitutively produced by stromal-derived FDCs that underlie the B cell follicles, plays a crucial role in organizing the LN architecture by attracting CXCR5-expressing B cells to form the B cell follicles (82). Following nematode infection, the CXCR5-expressing tissue-derived mDCs become responsive to CXCL13 and migrate into the CXCL13-containing perifollicular areas of the LN (30). Although it is known that CCR7 can counteract CXCR5-induced chemotaxis (92), the mDCs responding to nematode infection do not move into the T cell area (30). This is likely due, at least in part, to the fact that tissue-derived mDCs that prime Th2 cell responses express lower levels of CCR7 (30, 43, 76). Moreover, the CXCL13 gradient in the perifollicular region may be enhanced following exposure to allergens and pathogens that drive Th2 cell responses. In support of this hypothesis, migration of the CXCR5-expressing tissue-derived mDCs to the perifollicular region is also controlled by Ag-activated lymphotoxin-α (LT)-producing B cells (30). LT, a member of the TNF family of cytokines, induces and boosts expression CXCL13 by the FDCs via a positive-feedback loop (93). Thus, in the absence of DC intrinsic CXCR5 expression, or B cells or B cell-derived LT, mDC localization in the perifollicular region of the LN is ablated and, most importantly, Th2 cell responses are compromised (30).

CXCR5 expression by the DCs may also play an important role in the development of Tfh cells as our data suggest that $CXCR5^+$ DCs are required for the development of $IL\text{-}4^+$ $CD4^+$

T cells expressing canonical Tfh markers, like BCL-6 and CXCR5 (30). Therefore, we speculate that the priming of both Th2 and some Tfh cell responses is initiated by tissue CXCR5$^+$ mDCs within perifollicular zone of secondary lymphoid organs (Figure 1). Consistent with this hypothesis, it is reported that priming of Tfh cells following peripheral immunization is initiated in the perifollicular zone (94). Moreover, DCs transduced with a CXCR5-containing retrovirus, which gain responsiveness to CXCL13 and localize to the perifollicular areas of the draining LN after subcutaneous injection, induce T cell-dependent B cell antibody responses in this location (95). Collectively, the evidence suggests that the paracortex or T cell area of the LN is a specialized microenvironment that contributes to the effective priming of Th1 cell responses by CCR7-expressing mDCs. By contrast, the specialized microenvironment of the perifollicular region may favor the priming of Th2 and some Tfh cells by peripheral tissue-derived mDCs that express lower levels of CCR7 and have upregulated CXCR5 and CCR8.

Positioning of migratory cDC1 and cDC2 cells in the LN.

Both subsets of mDCs, the CD103$^+$ cDC1 cells and the CD11b$^+$ cDC2 cells are found in LN. Interestingly, high CCR7 expression levels are most often observed in migratory cDC1 cells, both during steady state (96) and upon inflammation (43, 97, 98). cDC2 cells, on the other hand, seem to be more flexible in their capacity to modulate expression of CCR7 and other chemokine receptors (30, 76, 99). These data suggest that the migratory cDC2 subset may have the ability to home to either the T cell zone (86, 100) or the perifollicular region of the LN (30, 76) and that this capacity may be dictated by the kind of stimulus or microorganisms to which the cDC2 cells were exposed. In support of this concept, unpublished data from our labs show that, following nematode infection, the migratory CD11b$^+$ cDC2 cells express lower levels of CCR7 relative to the CD103$^+$ cDC1 cells and the cDC2 cells, but not cDC1 cells, upregulate CXCR5. This result is consistent with published data showing that tissue-derived migratory cDC2 cells preferentially induce Th2 cell responses in multiple mouse models, including parasitic infections, protease allergen skin immunizations and allergic airway disease induced by allergen extracts (43, 101–107). Migratory cDC2 cells are also responsible for priming of Tfh cells (108), which is initiated in the perifollicular zone of the LN (94) following peripheral immunization. Thus, the data supports a model in which Th1 cell responses are initiated largely by the migratory CCR7$^+$ cDC1 cells that localize in the T cell areas of the LN while the induction of Tfh and Th2 cell immunity is facilitated by interactions between the migratory cDC2 cells and T cells within the perifollicular region in close proximity to B cell follicles (Figure 1).

Positioning of resident cDC subsets in the spleen.

CD4$^+$ T cell responses to blood-borne Ags and pathogens are initiated in the spleen (109). The spleen (Figure 2), which acts as a filter for the blood, is organized into the red and white pulp regions that are separated by an interface called the marginal zone (MZ (109)). The splenic white pulp is similar to the LN parenchyma as it contains a T cell zone and B cell follicles that are colonized by circulating T cells and B cells that enter the spleen through the marginal zone (109). Similar to the LN, once in the spleen the CCR7-expressing T cells respond to CCL19 and CCL21 gradients and migrate to the T cell zone while the CXCR5$^+$ B cells migrate to the B cell follicles in response to a CXCL13 gradient produced by the

stromal FDCs (109). However, unlike LN where the tissue-derived Ags are transported by mDCs, in the spleen the resident cDCs take up and present Ags that arrive directly from the blood (109). The splenic rDCs, which arise from precursors that enter the spleen from circulation, are, along with the pDCs, the most prevalent DC population in the spleen (11). As in the LN, splenic rDCs can be subdivided into resident cDC1 and resident cDC2 populations and again, similar to the migratory cDC1 and cDC2 cells found in the LN, the two splenic resident cDC subsets preferentially occupy distinct areas within the white pulp (110, 111). In mice, resting splenic resident cDC1 cells, which are $CD8\alpha^{+}CD11b^{-}$, preferentially localize in the T cell zone of the white pulp. By contrast, the resting resident splenic cDC2 cells, which are $CD11b^{+}CD8\alpha^{-}$, are more flexible in their localization and can be found not only in the T cell zone but also in the marginal zone bridging channels (110, 111), which are located near the B cell follicles and connect the T cell zone of the white pulp to the marginal zone.

It is not known precisely how resident splenic cDC1 and cDC2 cells migrate from the marginal zone to the T cell zone in the white pulp, although published data suggest that intrasplenic migration of resident cDC1 cells requires CCR7 (Figure 2, (24). However, the requirement for CCR7 may not be absolute as others have found that trafficking of cDC1 cells to the T cell zone of the spleen can occur in the absence of CCR7 signaling (112). Somewhat more is known about the factors that control the localization of resident cDC2 cells in the marginal zone bridging channels (Figure 2). Splenic cDC2 cells express the G-protein coupled Epstein-Barr virus-induced receptor 2 (EBI2). The ligand of EBI2, 7α,25-dihydroxycholesterol (7α,25-OHC) (113, 114), is synthesized from cholesterol by enzymes that are expressed at the highest levels in the perifollicular regions, the interfollicular regions and the marginal zone bridging channels of the spleen and are repressed in the center of the B cell follicles (114). The EBI2-dependent positioning of resident cDC2 cells in the marginal zone bridging channels promotes maintenance of the cDC2 cells by facilitating signaling between the LTβR-expressing cDC2 cells and the LT-producing B cells that are located in close proximity (115, 116). This is reminiscent of the LN where the migratory $CXCR5^{+}$ cDC2 cells localize in a CXCL13-and LT-dependent fashion adjacent to LT-producing B cells (30). Thus, while the cues that position the splenic resident cDC2 cells and the LN migratory cDC2 cells adjacent to B cell follicles may be different, the maintenance of these DC populations is, in both cases, dependent on B cell-derived LT (30, 115, 116), suggesting that migratory and resident cDC2 cells in the LN and splenic perifollicular microenvironment may need to interact with B cells (Figures 1–2).

The preferential localization of resident cDC2 cells in the splenic marginal zone bridging channels under steady state conditions allows these cells to rapidly access blood-borne Ags. Resident cDC2 cells can upregulate CCR7 following immunization (114), which allows the cells to move from the bridging channels into the T cell zone (117–119). However, in some settings resident cDC2 cells integrate signals from both EBI2 and CCR7 ligands. These opposing signals, which are strong enough to facilitate exit from the bridging channels but are not strong enough to allow entry into the T cell zone, mediate the repositioning of the Ag-engaged cDC2 cells to the T-B border (114). Thus, while the intra-LN trafficking of the migratory cDC2 cells seems to depend on balanced responsiveness of the cells to CXCR5 and CCR7 ligands (Figure 1), the intra-splenic homing of resident cDC2 cells appears reliant

on the balanced responsiveness of these cells to EBI2 and CCR7 ligands (Figure 2). Since EBI2 can form heterodimers with CXCR5 and modulate CXCL13-mediated responses in B cells (120), it will be interesting to determine whether splenic resident cDC2 cells, like their migratory cDC2 LN counterparts, also express CXCR5 and/or CXCR5/EBI2 heterodimers and can respond to CXCL13.

Differential DC subset positioning within the spleen regulates programming of distinct CD4$^+$ T cell subsets.

As described above, the cDC1 and cDC2 subsets found in spleen and LN, can in some settings, distribute to anatomically distinct regions of the tissue. Moreover, in the LN, the localization of the migratory cDC1 and cDC2 cells influences the type of CD4$^+$ T cell response that will be generated (24, 30–33). Consistent with the LN data, splenic resident cDC1 and cDC2 cells also appear to preferentially initiate different types of T cell responses (Figure 2). Resident cDC1 cells control Th1 polarization in response to microbial stimuli, including LPS and *Toxoplasma gondii,* or to dead cell-associated Ags (39, 121). This fits with the finding that Th1 cells localize in the splenic T cell zone in a CCR7-dependent manner (122) and fits with LN data showing that Th1 cell responses are initiated in the CCL19/CCL21 expressing T cell zone (24). By contrast, splenic Th2 cells form rings around the B cell follicles (122), suggesting that splenic Th2 cell responses may be induced outside of the T cell zone. Moreover, mobilization of the resident cDC2 cells from marginal zone bridging channels to the T-B boundary is required for full-fledged Tfh cell differentiation and induction of antibody responses (113, 114, 123). Therefore, similar to the LN, the splenic T cell zone may be specialized for the generation of Th1 cell responses, whereas the perifollicular areas may favor Tfh and Th2 cell responses (Figure 2).

In summary, the collective data from LN (Figure 1) and spleen (Figure 2) supports a model in which both splenic resident cDC1 cells and LN migratory cDC1 cells express high levels of CCR7 and preferentially localize in the T cell zones where these cells initiate Th1 cell responses. By contrast, the LN migratory cDC2 cells and resident splenic cDC2 cells can either enter the T cell zone or can upregulate additional chemokine receptors that allow them to position themselves outside of the T cell zone, near B cell follicles. This more flexible migratory program, which is likely dictated by the initial stimuli the cDC2 cells encounter, facilitate cDC2 cell priming of Th2 and some Tfh cell immune responses in the perifollicular microenvironment.

Sequential encounters with DCs in different LN niches promote optimal Th1 cell development.

Differentiation of CD4$^+$ T cells into fully functional Th1 effectors requires long-term interactions between CD4$^+$ T cells and mDCs that deliver durable TCR/pMHCII engagement and strong CD28-CD80/86 co-stimulation (124). The integration of these signals induces the expression of the IL-12 receptor heterodimeric complex by the CD4$^+$ T cells. This, in turn, allows the T cells to efficiently sense IL-12 produced by the DCs (124) and induce expression of T-bet, the transcription factor that controls the Th1 regulatory gene network (4). As described earlier, published data show that CCR7-mediated signals in the LN are also critical for the initiation of Th1 cell responses (24). While there is an

appreciation for the important role that CCR7 and its ligands play in controlling the colocalization of $CD4^+$ T cells and DCs within the T cell area, there is also a growing awareness that the CCR7 ligands, CCL21 and CCL19, provide signals to the DCs and T cells that enhance T cell activation and Th1 cell polarization in the LN. For example, CCL19 and CCL21 signals enhance DC function by promoting endocytosis (125) and dendrite extension (126) and by inhibiting DC apoptosis (127). Moreover, CCL19 signals to $CCR7^+$ LPS-activated mDCs enhance Th1 cell development by facilitating up-regulation of the co-stimulatory molecules CD80 and CD86 on the DCs and inducing the DCs to produce IL-12 (128). Similarly, CCL21 delivers direct co-stimulatory signals to $CCR7^+$ T cells during early TCR activation, leading to enhanced proliferation of the T cells as well as IFNγ production and Th1 cell polarization (129, 130). Although the data are compelling that CCL19 and CCL21 mediated signals directly or indirectly support Th1 cell development, more recent data suggest that maximal IFNγ production by Th1 cells may be controlled by a two-step process (Figure 1) that requires movement of T cells from one niche to another within the LN (89). Based on the available data we speculate that in a first step, DCs and T cells co-localize in a CCR7-dependent manner in the T cell zone where the T cells and DCs each respond to CCL19 and CCL21 signals (128–130). In this niche, the T cells are programmed by the Ag-presenting mDCs to undergo initial activation and produce a rapid pulse of IFNγ. This burst in IFNγ can induce autocrine upregulation of the IFNγ-responsive *Cxcr3* gene and CXCR3 expression by the T cells (131). In a second step, the $CXCR3^+$ T cells are attracted to the deep paracortex and medullary region of the LN by mDCs in this region that are producing the CXCR3 ligand, CXCL10. It is only upon interaction with these medullary-localized mDCs that the T cells reach their full effector potential and acquire the capacity to produce maximal levels of IFNγ (89).

This requirement for the T cells and DCs to interact in two niches within the LN is consistent with reports showing that $CD4^+$ T cells undergoing Th1 cell commitment are activated by Ag-bearing DCs in two phases (124). The first phase, which results in the initial activation of the T cells, is characterized by transient T cell interactions with DCs in which T cells integrate the antigenic stimulus from each DC encounter (132–134). The second phase, which is important for optimal proliferation (124), increased responsiveness to polarizing cytokines (124) and full Th1 effector capacity with maximal IFNγ production (132, 135), is exemplified by stable and long-lasting interactions between the activated T cells and a single DC (132, 133). Given the requirement that developing Th1 cells undergo two types of temporally distinct interactions with DCs, and the fact that developing Th1 cells migrate sequentially to different niches within the LN, it is possible that the movement of the T cells between these niches facilitates these two different types of encounters with DCs (Figure 1). Consistent with this idea, it has been hypothesized that only transient T cell interactions with DCs can be supported within the T cell zone, since the pro-migratory signals mediated by CCR7 are reported to outcompete the TCR-triggered stop signals (136), Thus, while engagement of CCR7 by its ligands may promote the initial activation, proliferation and early IFNγ production by $CD4^+$ T cells (129, 130), these CCR7 signals may interfere with the formation of the strong and durable T-DC interactions that are required for maximal effector function. However, if the activated T cells up-regulate CXCR3 (89) and down-modulate expression of CCR7 (137), the cells can migrate deeper in the LN paracortex and

medullary regions where the activated T cells can interact with Ag-bearing CXCL10-producing DCs that localize in these areas (89). Importantly, the CXCL10 produced by DCs seems to be important, not just for attracting the activated T cells to this LN niche, but also for directing the formation of long-lasting interactions between the DCs and T cells (89). These long-lasting interactions may be mediated by CXCL10 attracting the activated T cells away from the influence of CCR7 ligands in the T cell area and thereby minimizing the CCR7-mediated competition with TCR-triggered stop signals. Alternatively, the CXCL10 produced by the mDCs that are present in the deep paracortex and inner medullary areas may enhance interactions that increase synapse formation between the DCs and T cells, thereby facilitating stable and productive communication between DCs and T cells that will ultimately lead to efficient T cell IFNγ production.

Although we understand how CXCL10 may direct activated $CXCR3^+$ T cells from the T cell area to the deeper medullary region of the LN, the migration of mDCs between these two regions is less well described. Tissue-derived $CD103^+$ cDC1 cells are reported to express high levels of CCR7 and to localize in the T cell areas of the LN (97, 98, 138), but only transiently at the beginning of the immune response (46, 138). By contrast, migratory $CD11b^+$ cDC2 cells traffic from the peripheral sites to the LN, both at the beginning and during the later stages of the immune response. The migratory $CD11b^+$ cDC2 cells are, as described earlier, more flexible in where they can localize within the LN, and, depending on how the cells were activated, can migrate to the perifollicular region (30, 90), the T cell area (30, 138) or even to the deep paracortex and medullary regions (139). During the later stages of the response it is the migratory $CD11b^+$ cDC2 cells that are reported to produce CXCR3 ligands in the medullary region of the LN (89). While we still do not know how these migratory cDC2 cells move into the inner zone of the LN, one possibility is that the migratory cDC2 cells may lose their responsiveness to the CCR7 ligands produced in the T cell area (140), which would potentially allow these cells to be attracted by chemoattractants produced in the inner medullary region of the LN. Interestingly, the lipid signaling molecule S1P is expressed at higher concentrations in the medullary region than in the T cell zone (141) and DCs express S1P receptors (78, 142). Thus, it is possible that DC migration into the inner medullary region of the LN could be controlled by the S1P/S1PR axis. Regardless, the data suggest a model in which $CD103^+$ and $CD11b^+$ migratory cDCs home to the draining LN in two temporally distinct waves and mediate different functional processes (Figure 1). We speculate that migratory $CD103^+$ cDC1 cells, expressing high levels of CCR7 and producing abundant IL-12 (12), rapidly migrate to the T cell areas of the LN to initiate T cell activation and early IFNγ production and CXCR3 upregulation by the T cells. By contrast, the $CD11b^+$ cDC2 cells that also migrate to the LN in the second wave may preferentially localize within the deeper paracortex and medullary regions where they produce CXCL10, recruit the already activated $CXCR3^+$ T cells and promote differentiation of fully functional IFNγ-producing Th1 effectors.

Th2 cell development requires CXCR5.

CCR7 and the CCR7 ligands, CCL21 and CCL19, provide location-specifying instructions and co-stimulatory cues that are critical for the development of Th1 cells (128–130). However, as discussed earlier, some $CD4^+$ T cell responses develop and are maintained

outside of the lymphoid tissue T cell zone. In fact, Th2 cell responses are not impaired in animals that lack CCR7 signals in lymphoid tissues and, in some cases, these animals make an exacerbated Th2 cell response (30–33). Recent data reveal that induction of Th2 cell responses is driven preferentially by the tissue-derived migratory $CD11b^+$ cDC2 subset (43, 102–107) and we find that CXCR5 is upregulated specifically within the migratory $CD11b^+$ cDC2 subset (unpublished) and is not expressed by the $CD103^+$ cDC1 subset following nematode infection. These $CXCR5^+$ cDC2 cells, which express lower levels of CCR7 compared to the cDC1 cells (43, 96–98), accumulate in the subcapsular sinus and perifollicular areas of the LN and are not found in appreciable numbers in the T cell zone (30). We further showed that the $CXCR5^+$ mDCs co-localize with T cells in the perifollicular region following nematode infection and that the induction of Th2 cell responses to this infection is dependent on $CD4^+$ T cell intrinsic expression of CXCR5 (Figure 1, (30)). These CXCR5-expressing Th2 precursors continue to express low levels of CCR7 and can thus potentially respond to CXCL13, which is found in B cell follicles, and CCL19, which is present in the T cell zone. This opposing responsiveness to these two chemokines likely prevents the entry of the Th2 precursors into both the B cell follicle and the T cell zone, resulting in the positioning of the Ag-engaged T cells in the perifollicular region of the LN (92). In support of this, multiple studies show that Th2 cells preferentially accumulate at the interface of the T-B cell border (30, 122, 143). These data therefore suggest that co-localization of migratory $CD11b^+$ cDC2 cells and responding $CD4^+$ T cells specifically within the LN perifollicular region is necessary for the establishment of Th2 cell responses. This idea is supported by our data showing that shifting the co-localization of the DCs and T cells from the perifollicular areas to the T cell zone, by eliminating CXCR5 expression by DCs and $CD4^+$ T cells, results in severely impaired Th2 cell responses following nematode infection (30). Thus, the early encounter and interaction of Ag-bearing migratory $CD11b^+$ cDC2 cells and Ag-specific $CD4^+$ T cells in the perifollicular areas, which is mediated by CXCR5 signaling in both cells, is an important determinant of Th2 cell commitment (Figure 1).

The perifollicular microenvironment supports Th2 cell development.

The data showing that Th2 cell differentiation is dependent on location-specific signals received outside of the T cell zone gives rise to multiple predictions. First, CCR7 ligand-directed co-stimulatory signals will not be critical for the APCs that initiate Th2 cell development and the $CD4^+$ T cells that interact with these DCs. Second, DCs and T cells that interact in the perifollicular region of the LN may not receive T cell zone-specific signals that would normally repress Th2 cell development. Third, Th2 lineage commitment may be actively initiated by perifollicular region-specific signals. While there is much left to learn, the available data suggests that Th2 cell responses may be shaped by all three of these mechanisms. It is clear that the initial priming of Th2 cell development in most settings requires Ag presentation by DCs, particularly the Ag-presenting migratory $CD11b^+$ cDC2 cells (43, 102–107), rather than by other MHCII-expressing cells (43, 144, 145). Although there is abundant *in vitro* data showing that IL-4 stimulation directs expression of the Th2 lineage-specifying transcription factor GATA-3 in activated $CD4^+$ T cells (5), there is no compelling evidence to support a model in which the $CD11b^+$ cDC2 subset, or indeed any other DC subset, provides IL-4 to the developing Th2 cells. In fact, $CD4^+$ T cells with

impaired IL-4 receptor signaling can still develop into Th2 cells *in vivo* (146). Thus, while IL-4 may be critical for the expansion and maintenance of Th2 cells, perhaps by initiating an autocrine positive feedback loop that results in the enhanced expression of GATA-3 and stabilization of Th2 phenotype (147), it is unlikely that IL-4 plays an early role in Th2 commitment.

If DCs are not providing IL-4 in the perifollicular region of the LN, then how might Th2 cell development be induced in this unique niche? Several studies suggest that Th2 cell differentiation may occur by a default pathway (147, 148), in which $CD4^+$ T cells are programmed to differentiate into the Th2 lineage when activated by DCs that deliver weak TCR and co-stimulatory signals in the absence of producing polarizing cytokines, like IL-12 (124, 148). This model predicts that the delivery of a strong CD28 co-stimulatory signal and the presence of IL-12 at the site of contact between T cells and Ag-presenting DCs blocks the default differentiation of activated T cells into Th2 cells and instead actively initiates Th1 cell commitment (124, 148, 149). Thus, optimal Th2 cell development is likely to be initiated in niches that do not support strong CD28-CD80/CD86 co-stimulatory interactions and IL-12 signaling. As discussed earlier, $CXCR5^+$ DCs that migrate to the perifollicular areas of the LN, rather than in the T cell areas, will not be exposed to CCL19 and thus will not respond to CCL19 by maximally upregulating the co-stimulatory molecules CD80 and CD86 and secreting large quantities of IL-12 (128). Therefore, the perifollicular positioning of the DCs and $CD4^+$ T cells could potentially prevent the mDCs from attaining a Th1 cell priming phenotype and thereby allow the default Th2 cell differentiation program to proceed. Alternatively, it is possible that other cell types that are present in the perifollicular microenvironment will provide additional distinct, niche-specific signals that further inhibit the capacity of DCs to induce Th1 cell differentiation. For example, B cells that are co-localized with mDCs in the perifollicular region may prevent the mDCs from initiating Th1 cell development by producing IL-10, which can inhibit DC IL-12 production (150–154).

Sequential interactions between T cells and Ag-presenting DCs and B cells in the perifollicular microenvironment support Th2 cell development.

It has been well documented both *in vitro* and *in vivo* that TCR signal strength can influence Th2 cell development. High affinity agonists or high dose peptide stimulations favor development of IFNγ-producing Th1 cells whereas stimulation with low dose peptide or weakly agonistic ligands favors development of IL-4-, IL-5-, and IL-13-producing Th2 cells (155–158). *In vivo* experiments reveal that $CD4^+$ T cells undergoing Th1 cell polarization show longer periods of T cell-DC interaction and increased TCR-associated signaling compared to T cells undergoing Th2 cell commitment (124). These differences in TCR signal strength and duration of TCR engagement are important determinants controlling $CD4^+$ T cell polarization as full Th1 cell induction and suppression of Th2 cell differentiation program requires increased TCR signal intensity (124). While these *in vivo* data using immunization protocols are compelling, it is not at all clear how signal strength is controlled *in vivo* in response to Th1 or Th2-inducing pathogens since it seems unlikely that the peptide/MHCII ligands from these different pathogens that are recognized by the TCRs are globally different in their affinity and abundance.

One way in which APCs can regulate TCR signaling duration independently of the affinity or abundance of pMHCII complexes is by modulating the formation or maintenance of stable conjugates between the T cell and APC. As one example, the Signaling Lymphocytic Activation Molecule (SLAM) and SLAM-associated protein (SAP) complex can regulate the quality of T cell-APC interactions (159). Upon TCR ligation, the SLAM-SAP complex redistributes to the immunological synapse in T cells (160, 161) and brings protein kinase C-theta (PKC-θ), a kinase that is constitutively associated with SAP, to the immune synapse (161). Active PKC-θ can attenuate T cell arrest signals (162) and thereby terminate stable synaptic interactions between DCs and T cells. Thus, activation of PKC-θ following antigenic stimulation and engagement of the SLAM-SAP signaling cascade (163) (160, 161), results in brief interactions between DCs and T cells. This translates into weaker signals being delivered to the T cells – and conditions that favor Th2 cell development (164). Consistent with this, T cells from PKC-θ and SAP-deficient mice exhibit defective induction of GATA-3 following TCR engagement and poorly differentiate into IL-4-producing Th2 cells (160, 161, 165, 166). By contrast, overexpression of SAP in T cells increases sustained recruitment of SAP-associated PKC-θ to the immune synapse and elevated IL-4 production after TCR plus SLAM-mediated stimulation (160). Taken together, these results suggest that SLAM engagement in T cells facilitates Th2 cell commitment by amplifying PKC-θ recruitment to the immunologic synapse, which will destabilize the APC-T cell contacts and reduce the strength of the Ag-dependent signals delivered to the T cells by the APCs.

Interestingly, SLAM is more highly expressed by B cells compared to DCs (167) and a selective defect in T cell communication with B cells, but not with DCs, has been reported in the absence of SLAM-SAP signaling (167). Therefore, it is possible that B cells provide this unique SLAM-dependent co-stimulatory signal that is required for Th2 cell differentiation and that co-localization of the CXCR5-expressing B cells, T cells and DCs in the perifollicular region allows for these B cell-controlled signals to be delivered to the T cells. Consistent with this, several studies show that Th2 cell responses are impaired in B cell deficient mice, transiently B cell depleted mice or in mice selectively MHC II deficient in B cells (30, 143, 151, 152, 168–172). Moreover, others have reported that Ag presentation solely by DCs does not induce optimal Th2 cell development (173, 174), suggesting that additional APCs are required to support the development or maintenance of Th2 cells. Therefore, we propose that optimal Th2 cell development, like Th1 cell development, requires sequential encounters with different APCs and that, in the case of Th2 induction, T cells require sequential and cooperative interactions with DCs and B cells (Figure 1). In our model $CXCR5^+$ DCs that migrate to the perifollicular region of the LN will provide the initial antigenic stimulation that is required to initiate the Th2 cell differentiation program in naïve $CD4^+$ T cells. This initial activation will facilitate retention of the CXCR5-expressing $CD4^+$ T cells in this niche, which will allow for later encounters with Ag-presenting B cells (30) that provide additional signals to support full Th2 cell differentiation.

Development of IL-4-producing Tfh cells is initiated in the perifollicular region of the LN.

Our model (Figure 1) suggests that both Th1 and Th2 cell development are supported by sequential encounters between Ag-specific $CD4^+$ T cells and different populations of APCs. In the case of Th1 cell development, we propose that T cells first interact with cDC1 cells in

the T cell zone and then cDC2 cells in the medullary region of the LN. In the case of Th2 cell development, we propose that the T cells interact first with $CXCR5^+$ cDC2 cells in the perifollicular region and then with the B cells in the same anatomic site. This model also fits well with Tfh cell development, which is known to require interactions with Ag-presenting DCs and later cognate encounters between Ag-presenting B cells and the developing Tfh cells (90). Furthermore, there are a number of reports demonstrating that development of Th2 and IL-4-producing Tfh (Tfh2 cells) cells is reliant on similar co-stimulatory signals (8, 143, 172, 175, 176). For example, as described above, SLAM-SAP-controlled T-B cell interactions are not only required for Th2 cell differentiation (160, 161, 165) but are also necessary for the generation of IL-4-producing Tfh cells (167, 177). Given these similarities, we recently examined the developmental relationship between Th2 and Tfh2 cells. Using a mouse model of house dust mite (HDM) allergen exposure, we showed that initial intranasal sensitization with repetitive low dose HDM induces the differentiation and expansion of IL-4-producing Tfh2 cells in the LN (90, 101). Following HDM challenge, the IL-4-committed Tfh2 cells rapidly differentiate into effector Th2 cells that home to the lung and produce Th2 cytokines (90, 101). Consistent with the idea that IL-4-committed Tfh2 cells may serve as a transitional intermediate that can be stimulated to undergo full effector Th2 cell differentiation, it is reported that Th2 cell responses are impaired in mice lacking Tfh cells (178).

If the IL-4 producing Tfh2 cells are indeed precursors of Th2 cells, then we predicted that Tfh2 cell development is likely to occur in the perifollicular environment (Figure 1). Consistent with this hypothesis, we find that the priming of IL-4-producing Tfh2 cells after allergic airway sensitization to HDM occurs at the border of the B cell follicle where the T cells first interact with lung mDCs and then with B cells (90, 101). Moreover, our data in the nematode infection model (30) indicate that the CXCR5-expressing migratory $CD11b^+$ cDC2 subset is required for both Th2 and Tfh2 cell development. These data fit well with published reports showing that the $CD11b^+$ migratory cDC2 cells are the principal DC subset that captures and transports HDM-derived Ags from the lung into the LN for presentation to T cells (43) and that $CD11b^+$ migratory cDC2 cells are essential for the efficient priming of Tfh cell responses after intranasal immunization (108). Similarly, the spleen resident $CD11b^+$ cDC2 cells, which localize in the marginal zone bridging channels and perifollicular areas near B cells, are reported to initiate Tfh cell responses (123). This again suggests a model in which sequential Ag presentation to the T cells by DCs and then B cells may be necessary for Tfh cell differentiation in the spleen (Figure 2). However, while the perifollicular positioning of the migratory CXCR5-expressing $CD11b^+$ cDC2 cells in the LN is controlled by the countervailing activities of CXCR5 and CCR7, the homing of the resident $CD11b^+$ cDC2 cells to the T-B cell border in the spleen is, as discussed earlier, controlled by the opposing activities of the G-protein-coupled receptor EBI2 and CCR7 (114). Taken together, the data support a model in which both Th2 and IL-4-committed Tfh2 cell responses develop in the lymphoid tissue perifollicular areas and likely require initial priming by $CD11b^+$ migratory cDC2 cells, followed by subsequent interactions with Ag-presenting B cells that are also present within this microenvironmental niche (Figures 1–2). In addition, we speculate that sequential Ag presentation and specific co-stimulation first by DCs and then by B cells in the perifollicular region induces the development of a “type-2”

precursor that can give rise to Tfh2 and Th2 cells, with sustained interactions between B cells and the type-2 precursor reinforcing Tfh cell programming and transient interactions with B cells favoring Th2 cell differentiation.

Unanswered questions and future directions.

Our model predicts that sequential interactions between T cells and distinct populations of APCs that are present in different regions of the LN regulate the type of T cell response that will be generated. We propose that Th2 and Tfh2 cells develop from a common precursor that is initially primed by $CD11b^+$ cDC2 cells in the perifollicular area of secondary lymphoid tissues and then further programmed by B cells also present in this niche. Finally, we postulate that Th2 cells can develop from previously formed Tfh2 cells. While each of these predictions are supported by experimental data, there is much still to be learned. For example, other studies reported that high dose allergen exposure induces lung-localized, $CD4^+$ resident memory (Trm) cells that produce Th2 cytokines within the lung microenvironment (179) and that this response does not depend on B cell help. Given the requirement for B cells in Tfh cell development (176), this would argue that Th2 cells can develop independently of a Tfh cell intermediate (179, 180). By contrast, it is reported that exposure to lower and repetitive doses of intranasal allergens induces allergen-specific LN $CD4^+$ T cells with characteristics of Tfh cells that appear to be reservoirs of Ag-specific memory $CD4^+$ T cells in the LN that can significantly contribute to the secondary Th2 cell response (90, 101, 180). Therefore, it is possible that the Ag dose may influence whether Th2 cell development proceeds through a memory cell-like Tfh intermediate. In the future it will be interesting to determine whether the type-2 $CD4^+$ Trm cells are induced by $CD103^+$ migratory cDC1 cells, rather than by $CD11b^+$ migratory cDC2 cells, as it has been described for the generation of $CD8^+$ Trm cells (181). If so, it will be important to assess whether Ag dose differentially targets the different migratory DC subsets and to assess whether the type-2 $CD4^+$ Trm cells are induced in the T cell zone or in the perifollicular area.

While type-2 Tfh and Th2 cells seem to preferentially localize in the perifollicular area, this region is not limited to supporting IL-4 committed T cell responses as other T cell subsets can migrate to this specific location. For example, NKT cells are constitutively localized in the perifollicular area (182) and $CD8^+$ central memory T cells (Tcm) migrate away from the T cell zone to both the perifollicular region and medulla (183, 184) following exposure to antigens and pathogens that arrive in the LN through the lymph. Interestingly, migration of the $CD8^+$ Tcm cells out of the T cell zone is controlled by CXCR3 and the CXCR3 ligands, CXCL9, which is preferentially localized in the perifollicular region (89, 183, 184) and CXCL10, which is most highly expressed in the medulla of the LN (89). Tcm cells that move to the perifollicular region rapidly produce IFNγ due, at least in part, to immediate exposure to cytokine and chemokine producing innate cells and Ag presenting DCs at the site of pathogen entry (182). Thus, the data suggest that the perifollicular region is not only able to support Th2/Tfh2 responses but can, at least in some settings, promote the differentiation of $CD8^+$ Tcm cells into IFNγ-producing secondary effector cells.

If the perifollicular region of the LN can facilitate IFNγ-driven responses by CD8 T cells, can it also, under some circumstances, promote the development of IFNγ competent $CD4^+$

Th cells? In particular, we are intrigued by the IFNγ-competent peripheral Th (Tph) (185) and follicular Tfh cells (Tfh1 cells) (186), which are localized at the T-B border or in the B cell follicle and are found in the settings of vaccination, infection and chronic inflammation. Given that the CXCR3 ligand, CXCL9, can be expressed in the perifollicular region (89), and that CXCR3 is expressed by pre-Th1, Th1, Tph and Tfh1 cells, it is tempting to apply our sequential two step model of Th2 and Tfh2 development to Th1 and Tph or Tfh1 development (Figure 1). In this model, pre-Th1 cells, located in the T cell zone interact in a transient fashion with Ag-presenting DCs, upregulate CXCR3 and then either migrate into the deep LN medullary region in response to CXCL10 or transit to the perifollicular T-B border in response to CXCL9. Those pre-Th1 cells that migrate into the perifollicular region will have the opportunity to interact with B cells present in this region, thereby allowing for full commitment of the pre-Th1 cells to Tfh1 cells. However, it is also possible that Tph and Tfh1 cells arise from CD4 T cells that are first primed by resident DCs, which are reported to localize in the medullary interfollicular regions (187), and then sustained through ongoing interactions with Ag-presenting B cells. In the future, it will be important to develop model systems to directly test these two possibilities.

Conclusions.

Although the data for Th1 cell development supports the textbook model depicting the initial priming of $CD4^+$ T cells in the T cell zone of the LN or spleen, the model does not accurately capture the variable locations and full range of sequential T cell and APC interactions in the LN. DCs are the clearly the principal inducers of distinct functional subsets of $CD4^+$ T cells, however the ultimate T cell differentiation decisions are dictated by the integration of multiple cues in the lymphoid microenvironment in which $CD4^+$ T cells are first activated. These niche-specific cues affect the function and T cell stimulatory capacity of the DC subsets that present Ags in the secondary lymphoid tissues and provide the T cells with additional and unique differentiation signals. We propose that sequential interactions between T cells and different populations of APCs is a common feature of T cell differentiation, regardless of the type of T cell response that will ultimately be generated. In the case of Th1 cell differentiation, T cells may migrate between different anatomic regions of the secondary lymphoid tissues to interact with different DC subsets that can provide distinct types of co-stimulatory cytokine/chemokine signals. In the case of Th2 and Tfh2 cells the sequential interactions with APCs are facilitated by moving the DC-T cell interactions away from the T cell zone. This movement may prevent inappropriate or overly vigorous co-stimulation by allowing the T cells to interact with another type of APC, the B cell. In the future it will be important to understand the specific signals that can be imparted (or not delivered) within these different lymphoid tissue microenvironments and to address how the microenvironment shapes that functionality of the DCs, that localize to these sites.

Acknowledgements.

Financial support for the work described here was provided by the US National Institutes of Health (NIH) grants NIH/NIAID 1R01AI104725 (to FEL) and NIH/NIAID 1R01AI116584 (to BL) and by funding from the University of Alabama at Birmingham (to FEL and BL).

References

1. Mildner A, Jung S. Development and function of dendritic cell subsets. Immunity.2014;40:642–656. [PubMed: 24837101]
2. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol. 2003;3:984–993. [PubMed: 14647480]
3. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell.2001;106:263–266. [PubMed: 11509174]
4. Murphy KM, Ouyang W, Farrar JD, et al. Signaling and transcription in T helper development. Annu Rev Immunol.2000;18:451–494. [PubMed: 10837066]
5. Walker JA, McKenzie ANJ. TH2 cell development and function. Nat Rev Immunol.2018;18:121–133. [PubMed: 29082915]
6. Sandquist I, Kolls J. Update on regulation and effector functions of Th17 cells F1000Res. 2018;7:205. [PubMed: 29527301]
7. Audiger C, Lesage S. BIM determines the number of merocytic dendritic cells, a cell type that breaks immune tolerance. Immunol Cell Biol.2018;96:1008–1017. [PubMed: 29754432]
8. Ballesteros-Tato A, Randall TD. Priming of T follicular helper cells by dendritic cells. Immunol Cell Biol.2014;92:22–27. [PubMed: 24145854]
9. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol.2012;12:557–569. [PubMed: 22790179]
10. Collin M, Ginhoux F. Human dendritic cells. Semin Cell Dev Biol.2018.
11. Guilliams M, Dutertre CA, Scott CL, et al. Unsupervised High-Dimensional Analysis Aligns Dendritic Cells across Tissues and Species. Immunity.2016;45:669–684. [PubMed: 27637149]
12. Merad M, Sathe P, Helft J, Miller J, Mortha A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annu Rev Immunol. 2013;31:563–604. [PubMed: 23516985]
13. Granot T, Senda T, Carpenter DJ, et al. Dendritic Cells Display Subset and Tissue-Specific Maturation Dynamics over Human Life. Immunity.2017;46:504–515. [PubMed: 28329707]
14. Guilliams M, Ginhoux F, Jakubzick C, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol.2014;14:571–578. [PubMed: 25033907]
15. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. Nat Immunol. 2004;5:1219–1226. [PubMed: 15549123]
16. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. Nat Rev Immunol.2017;17:349–362. [PubMed: 28436425]
17. Worbs T, Hammerschmidt SI, Förster R. Dendritic cell migration in health and disease. Nature Reviews Immunology.2017;17:30–48.
18. Ingulli E, Mondino A, Khoruts A, Jenkins MK. In vivo detection of dendritic cell antigen presentation to CD4(+) T cells. The Journal of experimental medicine.1997;185:2133–2141. [PubMed: 9182685]
19. Tang HL, Cyster JG. Chemokine Up-regulation and activated T cell attraction by maturing dendritic cells. Science.1999;284:819–822. [PubMed: 10221917]
20. Willard-Mack CL. Normal structure, function, and histology of lymph nodes. Toxicol Pathol. 2006;34:409–424. [PubMed: 17067937]
21. Luther SA, Tang HL, Hyman PL, Farr AG, Cyster JG. Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. Proc Natl Acad Sci U S A.2000;97:12694–12699. [PubMed: 11070085]
22. Nakano H, Gunn MD. Gene duplications at the chemokine locus on mouse chromosome 4: multiple strain-specific haplotypes and the deletion of secondary lymphoid-organ chemokine and EBI-1 ligand chemokine genes in the plt mutation. Journal of immunology (Baltimore, Md : 1950).2001;166:361–369.

23. Vassileva G, Soto H, Zlotnik A, et al. The reduced expression of 6Ckine in the plt mouse results from the deletion of one of two 6Ckine genes. The Journal of experimental medicine. 1999;190:1183–1188. [PubMed: 10523616]
24. Gunn MD, Kyuwa S, Tam C, et al. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. The Journal of experimental medicine.1999;189:451–460. [PubMed: 9927507]
25. Mori S, Nakano H, Aritomi K, et al. Mice lacking expression of the chemokines CCL21-ser and CCL19 (plt mice) demonstrate delayed but enhanced T cell immune responses. The Journal of experimental medicine.2001;193:207–218. [PubMed: 11148224]
26. Ato M, Stager S, Engwerda CR, Kaye PM. Defective CCR7 expression on dendritic cells contributes to the development of visceral leishmaniasis. Nat Immunol.2002;3:1185–1191. [PubMed: 12436111]
27. Basu A, Chakrabarti G, Saha A, Bandyopadhyay S. Modulation of CD11C+ splenic dendritic cell functions in murine visceral leishmaniasis: correlation with parasite replication in the spleen. Immunology.2000;99:305–313. [PubMed: 10692051]
28. Junt T, Nakano H, Dumrese T, et al. Antiviral immune responses in the absence of organized lymphoid T cell zones in plt/plt mice. Journal of immunology (Baltimore, Md : 1950). 2002;168:6032–6040.
29. Junt T, Scandella E, Forster R, et al. Impact of CCR7 on priming and distribution of antiviral effector and memory CTL. Journal of immunology (Baltimore, Md : 1950).2004;173:6684–6693.
30. Leon B, Ballesteros-Tato A, Browning JL, et al. Regulation of T(H)2 development by CXCR5+ dendritic cells and lymphotoxin-expressing B cells. Nat Immunol.2012;13:681–690. [PubMed: 22634865]
31. Xu B, Aoyama K, Kusumoto M, et al. Lack of lymphoid chemokines CCL19 and CCL21 enhances allergic airway inflammation in mice. Int Immunol.2007;19:775–784. [PubMed: 17513879]
32. Moschovakis GL, Bubke A, Dittrich-Breiholz O, et al. Deficient CCR7 signaling promotes TH2 polarization and B-cell activation in vivo. Eur J Immunol.2012;42:48–57. [PubMed: 21969271]
33. Grinnan D, Sung SS, Dougherty JA, et al. Enhanced allergen-induced airway inflammation in paucity of lymph node T cell (plt) mutant mice. J Allergy Clin Immunol.2006;118:1234–1241. [PubMed: 17157652]
34. Maldonado-Lopez R, De Smedt T, Michel P, et al. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. The Journal of experimental medicine.1999;189:587–592. [PubMed: 9927520]
35. Hildner K, Edelson BT, Purtha WE, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science.2008;322:1097–1100. [PubMed: 19008445]
36. Allan RS, Smith CM, Belz GT, et al. Epidermal viral immunity induced by CD8alpha+ dendritic cells but not by Langerhans cells. Science.2003;301:1925–1928. [PubMed: 14512632]
37. Pooley JL, Heath WR, Shortman K. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. Journal of immunology (Baltimore, Md : 1950).2001;166:5327–5330.
38. Desch AN, Randolph GJ, Murphy K, et al. CD103+ pulmonary dendritic cells preferentially acquire and present apoptotic cell-associated antigen. The Journal of experimental medicine. 2011;208:1789–1797. [PubMed: 21859845]
39. Sancho D, Joffre OP, Keller AM, et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature.2009;458:899–903. [PubMed: 19219027]
40. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(−) dendritic cells cross-prime cytotoxic T cells in vivo. The Journal of experimental medicine.2000;192:1685–1696. [PubMed: 11120766]
41. Dudziak D, Kamphorst AO, Heidkamp GF, et al. Differential antigen processing by dendritic cell subsets in vivo. Science.2007;315:107–111. [PubMed: 17204652]
42. Schlitzer A, McGovern N, Teo P, et al. IRF4 transcription factor-dependent CD11b+ dendritic cells in human and mouse control mucosal IL-17 cytokine responses. Immunity.2013;38:970–983. [PubMed: 23706669]

43. Plantinga M, Guilliams M, Vanheerswynghels M, et al. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. Immunity.2013;38:322–335. [PubMed: 23352232]
44. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nat Immunol.2015;16:343–353. [PubMed: 25789684]
45. Chung Y, Chang JH, Kweon MN, Rennert PD, Kang CY. CD8alpha-11b+ dendritic cells but not CD8alpha+ dendritic cells mediate cross-tolerance toward intestinal antigens. Blood. 2005;106:201–206. [PubMed: 15774618]
46. Ballesteros-Tato A, Leon B, Lund FE, Randall TD. Temporal changes in dendritic cell subsets, cross-priming and costimulation via CD70 control CD8(+) T cell responses to influenza. Nat Immunol.2010;11:216–224. [PubMed: 20098442]
47. Kretschmer K, Apostolou I, Hawiger D, et al. Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol.2005;6:1219–1227. [PubMed: 16244650]
48. Fukaya T, Murakami R, Takagi H, et al. Conditional ablation of CD205+ conventional dendritic cells impacts the regulation of T-cell immunity and homeostasis in vivo. Proc Natl Acad Sci U S A.2012;109:11288–11293. [PubMed: 22736794]
49. Kim TS, Gorski SA, Hahn S, Murphy KM, Braciale TJ. Distinct dendritic cell subsets dictate the fate decision between effector and memory CD8(+) T cell differentiation by a CD24-dependent mechanism. Immunity.2014;40:400–413. [PubMed: 24631155]
50. Forster R, Schubel A, Breitfeld D, et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell.1999;99:23–33. [PubMed: 10520991]
51. MartIn-Fontecha A, Sebastiani S, Hopken UE, et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. The Journal of experimental medicine.2003;198:615–621. [PubMed: 12925677]
52. Ohl L, Mohaupt M, Czeloth N, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity.2004;21:279–288. [PubMed: 15308107]
53. Braun A, Worbs T, Moschovakis GL, et al. Afferent lymph-derived T cells and DCs use different chemokine receptor CCR7-dependent routes for entry into the lymph node and intranodal migration. Nat Immunol.2011;12:879–887. [PubMed: 21841786]
54. Ulvmar MH, Werth K, Braun A, et al. The atypical chemokine receptor CCRL1 shapes functional CCL21 gradients in lymph nodes. Nat Immunol.2014;15:623–630. [PubMed: 24813163]
55. Forster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol.2008;8:362–371. [PubMed: 18379575]
56. Schumann K, Lammermann T, Bruckner M, et al. Immobilized chemokine fields and soluble chemokine gradients cooperatively shape migration patterns of dendritic cells. Immunity. 2010;32:703–713. [PubMed: 20471289]
57. Patel DD, Koopmann W, Imai T, et al. Chemokines have diverse abilities to form solid phase gradients. Clin Immunol.2001;99:43–52. [PubMed: 11286540]
58. Yang BG, Tanaka T, Jang MH, et al. Binding of lymphoid chemokines to collagen IV that accumulates in the basal lamina of high endothelial venules: its implications in lymphocyte trafficking. Journal of immunology (Baltimore, Md : 1950).2007;179:4376–4382.
59. Bax M, van Vliet SJ, Litjens M, Garcia-Vallejo JJ, van Kooyk Y. Interaction of polysialic acid with CCL21 regulates the migratory capacity of human dendritic cells. PLoS One.2009;4:e6987. [PubMed: 19750015]
60. Kellermann SA, Hudak S, Oldham ER, Liu YJ, McEvoy LM. The CC chemokine receptor-7 ligands 6Ckine and macrophage inflammatory protein-3 beta are potent chemoattractants for in vitro- and in vivo-derived dendritic cells. Journal of immunology (Baltimore, Md : 1950). 1999;162:3859–3864.
61. Lo JC, Chin RK, Lee Y, et al. Differential regulation of CCL21 in lymphoid/nonlymphoid tissues for effectively attracting T cells to peripheral tissues. J Clin Invest.2003;112:1495–1505. [PubMed: 14617751]

62. Tal O, Lim HY, Gurevich I, et al. DC mobilization from the skin requires docking to immobilized CCL21 on lymphatic endothelium and intralymphatic crawling. The Journal of experimental medicine.2011;208:2141–2153. [PubMed: 21930767]
63. Weber M, Hauschild R, Schwarz J, et al. Interstitial dendritic cell guidance by haptotactic chemokine gradients. Science.2013;339:328–332. [PubMed: 23329049]
64. Alvarez D, Vollmann EH, von Andrian UH. Mechanisms and consequences of dendritic cell migration. Immunity.2008;29:325–342. [PubMed: 18799141]
65. Russo E, Teijeira A, Vaahtomeri K, et al. Intralymphatic CCL21 Promotes Tissue Egress of Dendritic Cells through Afferent Lymphatic Vessels. Cell Rep.2016;14:1723–1734. [PubMed: 26876174]
66. Tomura M, Yoshida N, Tanaka J, et al. Monitoring cellular movement in vivo with photoconvertible fluorescence protein “Kaede” transgenic mice. Proc Natl Acad Sci U S A. 2008;105:10871–10876. [PubMed: 18663225]
67. Baratin M, Foray C, Demaria O, et al. Homeostatic NF-kappaB Signaling in Steady-State Migratory Dendritic Cells Regulates Immune Homeostasis and Tolerance. Immunity.2015;42:627–639. [PubMed: 25862089]
68. Dev A, Iyer S, Razani B, Cheng G. NF-kappaB and innate immunity. Curr Top Microbiol Immunol.2011;349:115–143. [PubMed: 20848362]
69. Hegde S, Pahne J, Smola-Hess S. Novel immunosuppressive properties of interleukin-6 in dendritic cells: inhibition of NF-kappaB binding activity and CCR7 expression. FASEB J.2004;18:1439–1441. [PubMed: 15247147]
70. Ritter U, Wiede F, Mielenz D, et al. Analysis of the CCR7 expression on murine bone marrow-derived and spleen dendritic cells. J Leukoc Biol.2004;76:472–476. [PubMed: 15197229]
71. Del Prete A, Shao WH, Mitola S, et al. Regulation of dendritic cell migration and adaptive immune response by leukotriene B4 receptors: a role for LTB4 in up-regulation of CCR7 expression and function. Blood.2007;109:626–631. [PubMed: 16985179]
72. Scandella E, Men Y, Gillessen S, Forster R, Groettrup M. Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. Blood. 2002;100:1354–1361. [PubMed: 12149218]
73. Luft T, Jefford M, Luetjens P, et al. Functionally distinct dendritic cell (DC) populations induced by physiologic stimuli: prostaglandin E(2) regulates the migratory capacity of specific DC subsets. Blood.2002;100:1362–1372. [PubMed: 12149219]
74. Sallusto F, Palermo B, Lenig D, et al. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol.1999;29:1617–1625. [PubMed: 10359116]
75. Sozzani S, Allavena P, D’Amico G, et al. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. Journal of immunology (Baltimore, Md : 1950).1998;161:1083–1086.
76. Sokol CL, Camire RB, Jones MC, Luster AD. The Chemokine Receptor CCR8 Promotes the Migration of Dendritic Cells into the Lymph Node Parenchyma to Initiate the Allergic Immune Response. Immunity.2018;49:449–463 e446. [PubMed: 30170811]
77. Baeyens A, Fang V, Chen C, Schwab SR. Exit Strategies: S1P Signaling and T Cell Migration. Trends Immunol.2015;36:778–787. [PubMed: 26596799]
78. Idzko M, Hammad H, van Nimwegen M, et al. Local application of FTY720 to the lung abrogates experimental asthma by altering dendritic cell function. J Clin Invest.2006;116:2935–2944. [PubMed: 17080194]
79. Idzko M, Panther E, Corinti S, et al. Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses. FASEB J.2002;16:625–627. [PubMed: 11919175]
80. Chang JE, Turley SJ. Stromal infrastructure of the lymph node and coordination of immunity. Trends Immunol.2015;36:30–39. [PubMed: 25499856]
81. Katakai T, Hara T, Lee JH, et al. A novel reticular stromal structure in lymph node cortex: an immuno-platform for interactions among dendritic cells, T cells and B cells. Int Immunol. 2004;16:1133–1142. [PubMed: 15237106]

82. Cyster JG, Ansel KM, Reif K, et al. Follicular stromal cells and lymphocyte homing to follicles. Immunol Rev.2000;176:181–193. [PubMed: 11043777]

83. Bai Y, Wu B, Terada N, et al. Histological study and LYVE-1 immunolocalization of mouse mesenteric lymph nodes with “In Vivo Cryotechnique”. Acta Histochem Cytochem.2011;44:81–90. [PubMed: 21614169]

84. Phan TG, Grigorova I, Okada T, Cyster JG. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. Nat Immunol.2007;8:992–1000. [PubMed: 17660822]

85. Gosling J, Dairaghi DJ, Wang Y, et al. Cutting edge: identification of a novel chemokine receptor that binds dendritic cell- and T cell-active chemokines including ELC, SLC, and TECK. Journal of immunology (Baltimore, Md : 1950).2000;164:2851–2856.

86. Lindquist RL, Shakhar G, Dudziak D, et al. Visualizing dendritic cell networks in vivo. Nat Immunol.2004;5:1243–1250. [PubMed: 15543150]

87. Kumamoto Y, Denda-Nagai K, Aida S, Higashi N, Irimura T. MGL2 Dermal dendritic cells are sufficient to initiate contact hypersensitivity in vivo. PLoS One.2009;4:e5619. [PubMed: 19440334]

88. Clatworthy MR, Aronin CE, Mathews RJ, et al. Immune complexes stimulate CCR7-dependent dendritic cell migration to lymph nodes. Nat Med.2014;20:1458–1463. [PubMed: 25384086]

89. Groom JR, Richmond J, Murooka TT, et al. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. Immunity.2012;37:1091–1103. [PubMed: 23123063]

90. Ballesteros-Tato A, Randall TD, Lund FE, et al. T Follicular Helper Cell Plasticity Shapes Pathogenic T Helper 2 Cell-Mediated Immunity to Inhaled House Dust Mite. Immunity. 2016;44:259–273. [PubMed: 26825674]

91. Saeki H, Wu MT, Olasz E, Hwang ST. A migratory population of skin-derived dendritic cells expresses CXCR5, responds to B lymphocyte chemoattractant in vitro, and co-localizes to B cell zones in lymph nodes in vivo. Eur J Immunol.2000;30:2808–2814. [PubMed: 11069061]

92. Haynes NM, Allen CD, Lesley R, et al. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. Journal of immunology (Baltimore, Md : 1950).2007;179:5099–5108.

93. Mebius RE. Organogenesis of lymphoid tissues. Nat Rev Immunol.2003;3:292–303. [PubMed: 12669020]

94. Kerfoot SM, Yaari G, Patel JR, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity.2011;34:947–960. [PubMed: 21636295]

95. Wu MT, Hwang ST. CXCR5-transduced bone marrow-derived dendritic cells traffic to B cell zones of lymph nodes and modify antigen-specific immune responses. Journal of immunology (Baltimore, Md : 1950).2002;168:5096–5102.

96. Park K, Mikulski Z, Seo GY, et al. The transcription factor NR4A3 controls CD103+ dendritic cell migration. J Clin Invest.2016;126:4603–4615. [PubMed: 27820700]

97. Roberts EW, Broz ML, Binnewies M, et al. Critical Role for CD103(+)/CD141(+) Dendritic Cells Bearing CCR7 for Tumor Antigen Trafficking and Priming of T Cell Immunity in Melanoma. Cancer Cell.2016;30:324–336. [PubMed: 27424807]

98. Kandasamy M, Ying PC, Ho AW, et al. Complement mediated signaling on pulmonary CD103(+) dendritic cells is critical for their migratory function in response to influenza infection. PLoS Pathog.2013;9:e1003115. [PubMed: 23326231]

99. Nakano H, Burgents JE, Nakano K, et al. Migratory properties of pulmonary dendritic cells are determined by their developmental lineage. Mucosal Immunol.2013;6:678–691. [PubMed: 23168837]

100. Kissenpfennig A, Henri S, Dubois B, et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity.2005;22:643–654. [PubMed: 15894281]

101. León B T Cells in Allergic Asthma: Key Players Beyond the Th2 Pathway. Current Allergy and Asthma Reports.2017;17. [PubMed: 28290015]

102. Mesnil C, Sabatel CM, Marichal T, et al. Resident CD11b(+)Ly6C(−) lung dendritic cells are responsible for allergic airway sensitization to house dust mite in mice. PLoS One. 2012;7:e53242. [PubMed: 23300898]
103. Williams JW, Tjota MY, Clay BS, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. Nat Commun.2013;4:2990. [PubMed: 24356538]
104. Deckers J, Sichien D, Plantinga M, et al. Epicutaneous sensitization to house dust mite allergen requires interferon regulatory factor 4-dependent dermal dendritic cells. J Allergy Clin Immunol. 2017;140:1364–1377 e1362. [PubMed: 28189772]
105. Gao Y, Nish S, Jiang R, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. Immunity.2013;39:722–732. [PubMed: 24076050]
106. Kumamoto Y, Linehan M, Weinstein JS, et al. CD301b(+) dermal dendritic cells drive T helper 2 cell-mediated immunity. Immunity.2013;39:733–743. [PubMed: 24076051]
107. Tussiwand R, Everts B, Grajales-Reyes GE, et al. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. Immunity.2015;42:916–928. [PubMed: 25992862]
108. Krishnaswamy JK, Gowthaman U, Zhang B, et al. Migratory CD11b(+) conventional dendritic cells induce T follicular helper cell-dependent antibody responses. Sci Immunol.2017;2.
109. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. Immunity. 2013;39:806–818. [PubMed: 24238338]
110. Leenen PJ, Radosevic K, Voerman JS, et al. Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover. Journal of immunology (Baltimore, Md : 1950).1998;160:2166–2173.
111. Agger R, Witmer-Pack M, Romani N, et al. Two populations of splenic dendritic cells detected with M342, a new monoclonal to an intracellular antigen of interdigitating dendritic cells and some B lymphocytes. J Leukoc Biol.1992;52:34–42. [PubMed: 1379295]
112. Czeloth N, Schippers A, Wagner N, et al. Sphingosine-1 phosphate signaling regulates positioning of dendritic cells within the spleen. Journal of immunology (Baltimore, Md : 1950). 2007;179:5855–5863.
113. Gatto D, Wood K, Caminschi I, et al. The chemotactic receptor EBI2 regulates the homeostasis, localization and immunological function of splenic dendritic cells. Nat Immunol.2013;14:446–453. [PubMed: 23502855]
114. Yi T, Cyster JG. EBI2-mediated bridging channel positioning supports splenic dendritic cell homeostasis and particulate antigen capture. Elife.2013;2:e00757. [PubMed: 23682316]
115. Kabashima K, Banks TA, Ansel KM, et al. Intrinsic lymphotoxin-beta receptor requirement for homeostasis of lymphoid tissue dendritic cells. Immunity.2005;22:439–450. [PubMed: 15845449]
116. Wang YG, Kim KD, Wang J, Yu P, Fu YX. Stimulating lymphotoxin beta receptor on the dendritic cells is critical for their homeostasis and expansion. Journal of immunology (Baltimore, Md : 1950).2005;175:6997–7002.
117. De Becker G, Moulin V, Pajak B, et al. The adjuvant monophosphoryl lipid A increases the function of antigen-presenting cells. Int Immunol.2000;12:807–815. [PubMed: 10837408]
118. De Trez C, Pajak B, Brait M, et al. TLR4 and Toll-IL-1 receptor domain-containing adapter-inducing IFN-beta, but not MyD88, regulate Escherichia coli-induced dendritic cell maturation and apoptosis in vivo. Journal of immunology (Baltimore, Md : 1950).2005;175:839–846.
119. Calabro S, Liu D, Gallman A, et al. Differential Intrasplenic Migration of Dendritic Cell Subsets Tailors Adaptive Immunity. Cell Rep.2016;16:2472–2485. [PubMed: 27545885]
120. Barroso R, Martinez Munoz L, Barrondo S, et al. EBI2 regulates CXCL13-mediated responses by heterodimerization with CXCR5. FASEB J.2012;26:4841–4854. [PubMed: 22913878]
121. Reis e Sousa C, Hieny S, Scharton-Kersten T, et al. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. The Journal of experimental medicine.1997;186:1819–1829. [PubMed: 9382881]
122. Randolph DA, Huang G, Carruthers CJ, Bromley LE, Chaplin DD. The role of CCR7 in TH1 and TH2 cell localization and delivery of B cell help in vivo. Science.1999;286:2159–2162. [PubMed: 10591648]

123. Li J, Lu E, Yi T, Cyster JG. EBI2 augments Tfh cell fate by promoting interaction with IL-2-quenching dendritic cells. Nature.2016;533:110–114. [PubMed: 27147029]

124. VanPanhuys N, Klauschen F, Germain R. T-Cell-Receptor-Dependent Signal Intensity Dominantly Controls CD4+T Cell Polarization InVivo. Immunity.2014;41:63–74. [PubMed: 24981853]

125. Yanagawa Y, Onoe K. CCR7 ligands induce rapid endocytosis in mature dendritic cells with concomitant up-regulation of Cdc42 and Rac activities. Blood.2003;101:4923–4929. [PubMed: 12609829]

126. Yanagawa Y, Onoe K. CCL19 induces rapid dendritic extension of murine dendritic cells. Blood. 2002;100:1948–1956. [PubMed: 12200351]

127. Sanchez-Sanchez N, Riol-Blanco L, de la Rosa G, et al. Chemokine receptor CCR7 induces intracellular signaling that inhibits apoptosis of mature dendritic cells. Blood.2004;104:619–625. [PubMed: 15059845]

128. Marsland BJ, Battig P, Bauer M, et al. CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. Immunity.2005;22:493–505. [PubMed: 15845453]

129. Gollmer K, Asperti-Boursin F, Tanaka Y, et al. CCL21 mediates CD4+ T-cell costimulation via a DOCK2/Rac-dependent pathway. Blood.2009;114:580–588. [PubMed: 19451552]

130. Flanagan K, Moroziewicz D, Kwak H, Horig H, Kaufman HL. The lymphoid chemokine CCL21 costimulates naive T cell expansion and Th1 polarization of non-regulatory CD4+ T cells. Cell Immunol.2004;231:75–84. [PubMed: 15919372]

131. Nakajima C, Mukai T, Yamaguchi N, et al. Induction of the chemokine receptor CXCR3 on TCR-stimulated T cells: dependence on the release from persistent TCR-triggering and requirement for IFN-gamma stimulation. Eur J Immunol.2002;32:1792–1801. [PubMed: 12115663]

132. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature.2004;427:154–159. [PubMed: 14712275]

133. Miller MJ, Hejazi AS, Wei SH, Cahalan MD, Parker I. T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. Proc Natl Acad Sci U S A.2004;101:998–1003. [PubMed: 14722354]

134. Hugues S, Fetler L, Bonifaz L, et al. Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity. Nat Immunol.2004;5:1235–1242. [PubMed: 15516925]

135. Henrickson SE, Perro M, Loughhead SM, et al. Antigen availability determines CD8(+) T cell-dendritic cell interaction kinetics and memory fate decisions. Immunity.2013;39:496–507. [PubMed: 24054328]

136. Dustin ML. Stop and go traffic to tune T cell responses. Immunity.2004;21:305–314. [PubMed: 15357942]

137. Bjorkdahl O, Barber KA, Brett SJ, et al. Characterization of CC-chemokine receptor 7 expression on murine T cells in lymphoid tissues. Immunology.2003;110:170–179. [PubMed: 14511230]

138. Itano AA, McSorley SJ, Reinhardt RL, et al. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. Immunity.2003;19:47–57. [PubMed: 12871638]

139. Groom JR. Moving to the suburbs: T-cell positioning within lymph nodes during activation and memory. Immunology and Cell Biology.2015;93:330–336. [PubMed: 25753266]

140. Byers MA, Calloway PA, Shannon L, et al. Arrestin 3 mediates endocytosis of CCR7 following ligation of CCL19 but not CCL21. Journal of immunology (Baltimore, Md : 1950). 2008;181:4723–4732.

141. Fang V, Chaluvadi VS, Ramos-Perez WD, et al. Gradients of the signaling lipid S1P in lymph nodes position natural killer cells and regulate their interferon-gamma response. Nat Immunol. 2017;18:15–25. [PubMed: 27841869]

142. Rathinasamy A, Czeloth N, Pabst O, Forster R, Bernhardt G. The origin and maturity of dendritic cells determine the pattern of sphingosine 1-phosphate receptors expressed and required for efficient migration. Journal of immunology (Baltimore, Md : 1950).2010;185:4072–4081.

143. Liu Q, Liu Z, Rozo CT, et al. The role of B cells in the development of CD4 effector T cells during a polarized Th2 immune response. Journal of immunology (Baltimore, Md : 1950). 2007;179:3821–3830.

144. Phythian-Adams AT, Cook PC, Lundie RJ, et al. CD11c depletion severely disrupts Th2 induction and development in vivo. The Journal of experimental medicine.2010;207:2089–2096. [PubMed: 20819926]

145. Hammad H, Plantinga M, Deswarte K, et al. Inflammatory dendritic cells--not basophils--are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. The Journal of experimental medicine.2010;207:2097–2111. [PubMed: 20819925]

146. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol.2010;10:225–235. [PubMed: 20336151]

147. Zheng W, Flavell RA. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell.1997;89:587–596. [PubMed: 9160750]

148. Zhu J, Jankovic D, Oler AJ, et al. The transcription factor T-bet is induced by multiple pathways and prevents an endogenous Th2 cell program during Th1 cell responses. Immunity. 2012;37:660–673. [PubMed: 23041064]

149. Pulecio J, Petrovic J, Prete F, et al. Cdc42-mediated MTOC polarization in dendritic cells controls targeted delivery of cytokines at the immune synapse. The Journal of experimental medicine. 2010;207:2719–2732. [PubMed: 21059854]

150. Moulin V, Andris F, Thielemans K, et al. B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. The Journal of experimental medicine.2000;192:475–482. [PubMed: 10952717]

151. Leon B, Ballesteros-Tato A, Lund FE. Dendritic cells and B cells: unexpected partners in Th2 development. Journal of immunology (Baltimore, Md : 1950).2014;193:1531–1537.

152. Leon B, Ballesteros-Tato A, Misra RS, Wojciechowski W, Lund FE. Unraveling effector functions of B cells during infection: the hidden world beyond antibody production. Infect Disord Drug Targets.2012;12:213–221. [PubMed: 22394173]

153. Ronet C, Hauyon-La Torre Y, Revaz-Breton M, et al. Regulatory B cells shape the development of Th2 immune responses in BALB/c mice infected with Leishmania major through IL-10 production. Journal of immunology (Baltimore, Md : 1950).2010;184:886–894.

154. Blackwell NM, Else KJ. B cells and antibodies are required for resistance to the parasitic gastrointestinal nematode Trichuris muris. Infect Immun.2001;69:3860–3868. [PubMed: 11349052]

155. Constant S, Pfeiffer C, Woodard A, Pasqualini T, Bottomly K. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. The Journal of experimental medicine.1995;182:1591–1596. [PubMed: 7595230]

156. Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A. The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. The Journal of experimental medicine.1995;182:1579–1584. [PubMed: 7595228]

157. Milner JD, Fazilleau N, McHeyzer-Williams M, Paul W. Cutting edge: lack of high affinity competition for peptide in polyclonal CD4+ responses unmasks IL-4 production. Journal of immunology (Baltimore, Md : 1950).2010;184:6569–6573.

158. Yamane H, Zhu J, Paul WE. Independent roles for IL-2 and GATA-3 in stimulating naive CD4+ T cells to generate a Th2-inducing cytokine environment. The Journal of experimental medicine. 2005;202:793–804. [PubMed: 16172258]

159. Brezar V, Tu WJ, Seddiki N. PKC-Theta in Regulatory and Effector T-cell Functions. Front Immunol.2015;6:530. [PubMed: 26528291]

160. Cannons JL, Yu LJ, Hill B, et al. SAP regulates T(H)2 differentiation and PKC-theta-mediated activation of NF-kappaB1. Immunity.2004;21:693–706. [PubMed: 15539155]

161. Cannons JL, Wu JZ, Gomez-Rodriguez J, et al. Biochemical and genetic evidence for a SAP-PKC-theta interaction contributing to IL-4 regulation. Journal of immunology (Baltimore, Md : 1950).2010;185:2819–2827.

162. Sims TN, Soos TJ, Xenias HS, et al. Opposing effects of PKCtheta and WASp on symmetry breaking and relocation of the immunological synapse. Cell.2007;129:773–785. [PubMed: 17512410]
163. Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. Annu Rev Immunol.2007;25:337–379. [PubMed: 17201683]
164. van Panhuys N TCR Signal Strength Alters T-DC Activation and Interaction Times and Directs the Outcome of Differentiation. Front Immunol.2016;7:6. [PubMed: 26834747]
165. Wu C, Nguyen KB, Pien GC, et al. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. Nat Immunol.2001;2:410–414. [PubMed: 11323694]
166. Marsland BJ, Soos TJ, Spath G, Littman DR, Kopf M. Protein kinase C theta is critical for the development of in vivo T helper (Th)2 cell but not Th1 cell responses. The Journal of experimental medicine.2004;200:181–189. [PubMed: 15263025]
167. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. Nature.2008;455:764–769. [PubMed: 18843362]
168. Wojciechowski W, Harris DP, Sprague F, et al. Cytokine-producing effector B cells regulate type 2 immunity to H. polygyrus. Immunity.2009;30:421–433. [PubMed: 19249230]
169. Lindell DM, Berlin AA, Schaller MA, Lukacs NW. B cell antigen presentation promotes Th2 responses and immunopathology during chronic allergic lung disease. PLoS One.2008;3:e3129. [PubMed: 18769622]
170. Langhorne J, Cross C, Seixas E, Li C, von der Weid T. A role for B cells in the development of T cell helper function in a malaria infection in mice. Proc Natl Acad Sci U S A.1998;95:1730–1734. [PubMed: 9465085]
171. Ronet C, Voigt H, Himmelrich H, et al. Leishmania major-specific B cells are necessary for Th2 cell development and susceptibility to L. major LV39 in BALB/c mice. Journal of immunology (Baltimore, Md : 1950).2008;180:4825–4835.
172. Linton PJ, Bautista B, Biederman E, et al. Costimulation via OX40L expressed by B cells is sufficient to determine the extent of primary CD4 cell expansion and Th2 cytokine secretion in vivo. The Journal of experimental medicine.2003;197:875–883. [PubMed: 12668647]
173. Perrigoue JG, Saenz SA, Siracusa MC, et al. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. Nat Immunol.2009;10:697–705. [PubMed: 19465906]
174. Sokol CL, Chu NQ, Yu S, et al. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. Nat Immunol.2009;10:713–720. [PubMed: 19465907]
175. Simpson TR, Quezada SA, Allison JP. Regulation of CD4 T cell activation and effector function by inducible costimulator (ICOS). Curr Opin Immunol.2010;22:326–332. [PubMed: 20116985]
176. Crotty S Follicular helper CD4 T cells (TFH). Annu Rev Immunol.2011;29:621–663. [PubMed: 21314428]
177. Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. Annu Rev Immunol.2011;29:665–705. [PubMed: 21219180]
178. Coquet JM, Schuijs MJ, Smyth MJ, et al. Interleukin-21-Producing CD4⁺ T Cells Promote Type 2 Immunity to House Dust Mites. Immunity.2015;43:318–330. [PubMed: 26287681]
179. Hondowicz BD, An D, Schenkel JM, et al. Interleukin-2-Dependent Allergen-Specific Tissue-Resident Memory Cells Drive Asthma. Immunity.2016;44:155–166. [PubMed: 26750312]
180. Dullaers M, Schuijs MJ, Willart M, et al. House dust mite–driven asthma and allergen-specific T cells depend on B cells when the amount of inhaled allergen is limiting. Journal of Allergy and Clinical Immunology.2017;140:76–88.e77. [PubMed: 27746238]
181. Iborra S, Martinez-Lopez M, Khouili SC, et al. Optimal Generation of Tissue-Resident but Not Circulating Memory T Cells during Viral Infection Requires Crosspriming by DNGR-1(+) Dendritic Cells. Immunity.2016;45:847–860. [PubMed: 27692611]
182. Kastenmuller W, Torabi-Parizi P, Subramanian N, Lammermann T, Germain RN. A spatially-organized multicellular innate immune response in lymph nodes limits systemic pathogen spread. Cell.2012;150:1235–1248. [PubMed: 22980983]

183. Kastenmuller W, Brandes M, Wang Z, et al. Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8+ T cell responses in the lymph node. Immunity.2013;38:502–513. [PubMed: 23352234]

184. Sung JH, Zhang H, Moseman EA, et al. Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. Cell.2012;150:1249–1263. [PubMed: 22980984]

185. Rao DA, Gurish MF, Marshall JL, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature.2017;542:110–114. [PubMed: 28150777]

186. Qin L, Waseem TC, Sahoo A, et al. Insights Into the Molecular Mechanisms of T Follicular Helper-Mediated Immunity and Pathology. Front Immunol.2018;9:1884. [PubMed: 30158933]

187. Woodruff MC, Heesters BA, Herndon CN, et al. Trans-nodal migration of resident dendritic cells into medullary interfollicular regions initiates immunity to influenza vaccine. The Journal of experimental medicine.2014;211:1611–1621. [PubMed: 25049334]

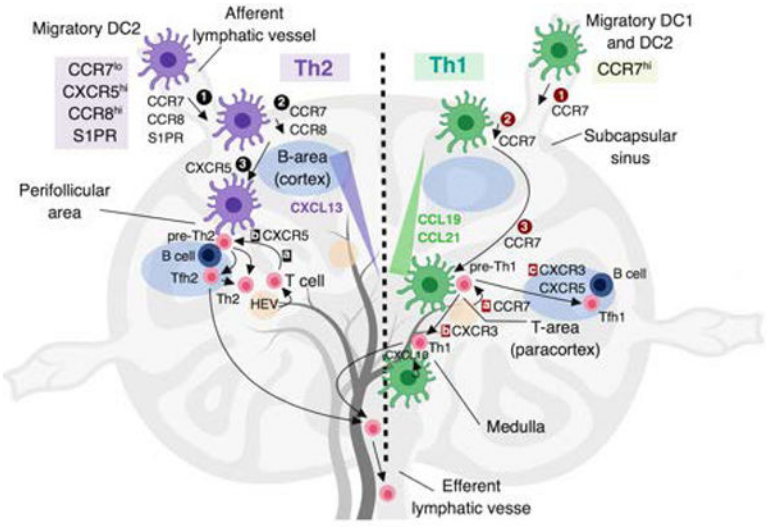

Figure 1. Compartmentalization of Th1 and Th2 cell responses in the LN.

The LN supports the differentiation of naïve CD4$^+$ T cells into multiple effector populations that exhibit distinct functional properties. We propose that the priming and full commitment of naïve CD4$^+$ T cells to type 1 IFN**γ**-producing (Th1) and type 2 IL-4-producing (Th2 and Tfh2) subsets requires sequential encounters with different populations of APCs. Furthermore, we propose that development of Th1 and Tfh2/Th2 effector T cells requires compartmentalization of these T cell-APC encounters within different LN niches. This compartmentalization is achieved, at least in part, through distinct patterns of chemokine receptor expression by the Ag-presenting migratory cDC1 and cDC2 subsets. For the induction of Th2 cell immune responses (left), Ag-containing migratory cDC2 cells arrive the LN subcapsular sinus via the lymph through afferent lymphatic vessels by a mechanism that requires the cooperative function of CCR7, S1PR and CCR8 **(**1). cDC2 cells exit the subcapsular sinus and enter the LN parenchyma by the cooperative function of CCR7 and CCR8 **(**2**)**. Once in the LN parenchyma, the high expression of CXCR5 and low expression of CCR7 on cDC2 cells allows their preferential localization adjacent to the B cell follicles, within the perifollicular region of the LN **(**3**)**, where the cDC2 cells can interact with naïve CD4$^+$ T cells that entered the LN through HEVs that are located in the paracortex and at the T-B border in the perifollicular region of the LN **(a)**. Following this initial activation by the Ag-bearing cDC2 cells in the perifollicular region, the T cells upregulate CXCR5 (pre-Th2 cells) **(b)**, which prevents the activated T cells from entering the T cell zone and allows the T cells to undergo secondary interactions with Ag-presenting B cells. Those T cells that interact transiently with B cells can fully commit to the Th2 cell lineage while those T cells that undergo sustained interactions with B cells will differentiate into Tfh2 cells. Prolonged interactions with B cells allows for long-term maintenance of Tfh2 cells that retain the capacity to differentiate into effector Th2 cells following later encounters with Ag-presenting DCs. By contrast, Th1 cell immune responses (right) are supported by Ag-presenting migratory cDC1 cells or cDC2 cells that arrive in the subcapsular sinus of the LN **(**1**)**, enter the LN parenchyma **(**2**)** and localize in the T cell area **(**3**)** via CCR7-mediated signaling. CD4$^+$ T cells expressing CCR7 interact with cDCs in the T cell zone **(a)**. This initial transient interaction induces the T cells to upregulate CXCR3 (pre-Th1 cells), which

promotes subsequent migration of the T cells to the medullary region of the LN **(b),** where the T cells can interact with Ag-presenting CXCL10-expressing cDC2 cells. This secondary sustained interaction between the pre-Th1 cells and the DCs is required for optimal and maximal effector Th1 cell differentiation. Alternatively, upregulation of CXCR3 and CXCR5 by pre-Th1 cells may facilitate migration of pre-Th1 cells to perifollicular area, where both CXCL9 and CXCL13 are expressed, and eventually to the CXCL13-expressing B cell follicle, where pre-Th1 cells interact with Ag-presenting B cells and differentiate into fully functional Tfh1 cells **(c)**.

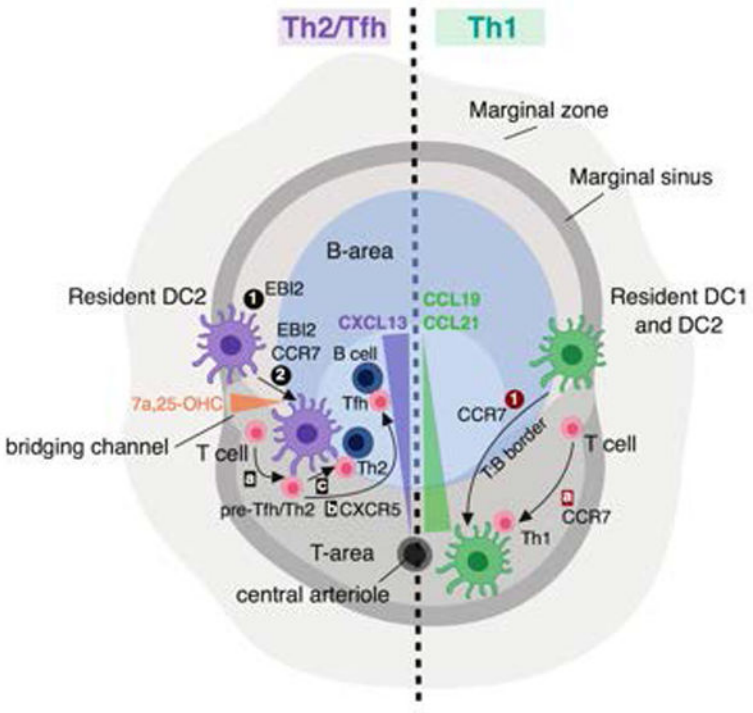

Figure 2. Compartmentalization of Th1, Th2 and Tfh cell responses in the spleen.

In response to blood-borne Ags, white pulp regions in the spleen foster the activation and differentiation of naïve $CD4^+$ T cells into Th subsets, including Th1, Th2 and Tfh cells. As in the LN, we propose that the development of these T cell subsets requires the compartmentalization of T cell-APC encounters within different niches in the splenic white pulp. The splenic DC subsets include the IFN-producing pDCs and the resident cDC1 and cDC2 cells. Resident cDC2 express EBI2 and preferentially localize in the marginal zone bridging channels (left) where the EBI2 ligand 7α,25-OHC is highly expressed and where the resident cDC2 cells can rapidly access blood-borne Ags. **(**1**)**. Following exposure to Ags that promote Th2 and Tfh cell immune responses (left), resident cDC2 cells upregulate expression of CCR7, while simultaneously maintaining EBI2 expression. Dual expression of CCR7 and EBI2 allows for repositioning of the Ag-engaged cDC2 cells to the T-B border **(**2**)**. At the T-B border, resident cDC2 cells interact with naïve $CD4^+$ T cells that entered the splenic white pulp via the marginal bridging channels **(a)**. Following this initial activation by the Ag-bearing resident cDC2 cells at the T-B border, the T cells can differentiate into either pre-Tfh cells or pre-Th2 cells. Pre-Tfh cells that up-regulate CXCR5 to high levels can, in turn, migrate into the B cell follicles (**b**) to undergo sustained interactions with B cells, which ultimately will lead to the full differentiation of Tfh cells. pre-Th2 cells that only undergo transient interactions with B cells at the T-B border will commit to the Th2 cell lineage (**c**). By contrast, Th1 cell immune responses (right) are supported by Ag-presenting resident cDC1 cells or cDC2 cells that move from the marginal bridging channels to the T cell area normally by CCR7-mediated signaling. This process is particularly enhanced under inflammatory conditions that promote further upregulation of CCR7 expression by the DCs or enhance CCL19/CCL21 ligand production by stromal cells within the T-area **(**1**)**. $CD4^+$ T

cells expressing CCR7 interact with resident cDCs in the T cell zone **(a)** where the activated T cells differentiate into Th1 cells.