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Chemokine-guided cell positioning in the lymph node orchestrates the generation of adaptive immune responses

Jeffrey Lian and Andrew D. Luster

Center for Immunology & Inflammatory Diseases, Division of Rheumatology, Allergy & Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

Abstract

The generation of adaptive immune responses occurs in the lymph node (LN) and requires that lymphocytes locate and interact with cognate antigen-bearing dendritic cells. This process requires the coordinated movement of both innate and adaptive immune cells, and is orchestrated by the chemokine family of chemotactic cytokines. Upon initiation of inflammation, the LN undergoes dramatic changes that include the marked induction of specific chemokines in distinct regions of the reactive LN. These chemokine rich domains establish LN niches that facilitate the differentiation of CD4⁺ T cells into effector cell subsets and the rapid activation of memory CD8⁺ T cells. This review will focus on recent advances highlighting the importance of LN chemokines for shaping adaptive immune responses by controlling immune cell migration, positioning, and interactions in the reactive LN.

Introduction

The generation of adaptive immune responses requires interactions between naïve lymphocytes and antigen-presenting cells (APCs). Naïve lymphocytes expressing the chemokine receptor CCR7 continuously migrate into lymph nodes (LN) from the blood via specialized high endothelial venules (HEV), while antigen-bearing dendritic cells (DC), also expressing CCR7, carry antigen from the periphery into the LN via afferent lymphatics [1]. CC chemokine ligand 19 (CCL19) and CCL21 guide both cell types into the deep paracortex of the LN, where colocalization of these cells facilitates T cell scanning and early initiation of immune responses [2]. Upon the initiation of inflammation and the onset of the immune response, LNs undergo marked changes to their structure coinciding with dramatic changes in LN chemokine expression. This review will focus on recent studies that have identified the guidance cues that position both innate and adaptive immune cells in this highly dynamic environment that allow for the cellular interactions necessary for antigen-specific highly differentiated immune responses (Figure 1).

Corresponding author: Andrew D. Luster (aluster@mgh.harvard.edu).

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Innate Immune Response

The initiation of adaptive immunity to antigens in the tissue depends on DC expression of CCR7 for successful navigation into afferent lymphatics and into draining LNs. Soluble CCL19 in the tissue helps guide DC polarization and chemotaxis towards lymphatic vessels [3], although CCL19 is not absolutely required for DC migration into the LN, since CCL21 alone was sufficient to mediate this migration [4]. Experiments tracking DC behavior in mouse ear explants showed that DCs migrate using immobilized CCL21, a process called haptotaxis, to navigate the interstitial space and enter lymphatic vessels. CCL21 was immobilized in the tissue bound to heparan sulfate containing proteoglycans, in a gradient directing DCs towards lymphatic vessels [5]. DCs continue their migration into afferent lymphatic vessels until arriving at the subcapsular sinus (SCS) of LN. Until recently, the mechanism by which DCs entered LNs was unclear. Ulvmar and colleagues showed that the atypical chemokine receptor ACKR4 (CCLR1) was expressed only on lymphatic endothelial cells comprising the ceiling and not the floor of the SCS [6*]. ACKR4 scavenging of CCL21 specifically by ceiling lymphatic endothelial cells is thus able to create a local gradient for CCL21-directed migration into the LN cortex.

Specialized macrophages reside just underneath the LN capsule in the SCS, acting as a filter for the LN and preventing the systemic spread of lymph-borne pathogens, while medullary macrophages play a crucial role in handling particulate antigens to promote B cell responses [7]. Experiments using a modified vaccinia virus infection demonstrated that several innate lymphoid effector cell populations (e.g., NK, NKT, $\gamma\delta$ and innate-like CD8⁺ T cells) are prepositioned and accumulate near SCS macrophages, making them able to quickly respond to IL-18 released by infected macrophages by producing IFN γ [8**]. In addition, NK cell recruitment to inflamed LNs depended on the production of IFN γ , CXCL9, and CXCL10 in the LN, as well as expression of CXCR3 on NK cells, and was abolished with depletion of SCS macrophages using clodronate-loaded liposomes [9,10]. Highlighting the dynamic nature of the LN response, Gaya and colleagues visualized draining LNs after skin ear infection with *Staphylococcus aureus* and found that the arrival of mature DCs during inflammation disrupted the organization of SCS macrophages [11]. The authors speculate that this may also present an opportunity for the rapid trafficking of lymph-borne immune cells through the SCS floor to facilitate antigen relay to follicular dendritic cells and B cells. However, this reorganization rendered the draining LN refractory to newly arriving pathogens by impairing the ability of the draining LN to retain and present antigen to a subsequent secondary infection, resulting in diminished B cell responses to a secondary infectious pathogen. These studies highlight the rapid generation of localized chemokine and cytokine environments during the dynamic LN response to inflammation.

The LN is supported by a network of stromal cells, including fibroblastic reticular cells (FRCs), that provide a physical structure for the LN. Some antigens reaching the LN subcapsular sinus can enter a network of conduits lined by FRCs and travel throughout the LN before exiting through efferent lymphatics [12]. Recent studies have highlighted an important role for FRCs in directing lymphocyte localization under steady state and inflammatory conditions. *In vivo* depletion of FRCs using *Ccl19*-directed diphtheria toxin receptor (DTR) transgenic mice resulted in follicle disorganization and impaired B cell

viability due to a loss of B cell activating factor (BAFF) and CXCL13 in the B cell follicle microenvironment [13*]. Denton and colleagues depleted FRCs by an alternative method using fibroblast activation protein (FAP)-DTR transgenic mice, which similarly disrupted LN architecture and resulted in reduced homing of transferred CD8⁺ T cells to LNs and Peyer's Patches due to a loss in IL-7, CCL19, and CCL21 in T cell zones. FRC depletion prior to influenza infection resulted in impaired T and B cell responses due to disruptions in homeostatic lymphocyte maintenance, but depletion after infection had minimal effect, suggesting a reliance on other cues after the onset of inflammation [14*]. These studies establish the environment that naïve lymphocytes must successfully navigate for generating appropriate immune responses.

CD8⁺ effector T cells

Naïve T cells arriving in the LN enter through HEVs and localize deep in the center of the LN underneath B cell follicles in T cell zones. Priming of T cells was thought to occur in the LN paracortex adjacent to HEVs near the T cell zone [15,16]. To address the location of DC presentation to naïve CD8⁺ T cells in the LN, Hickman and colleagues used two-photon intravital microscopy to visualize the response of adoptively transferred antigen-specific T cells after infection with fluorescent vaccinia virus (large, double-stranded DNA virus) and vesicular stomatitis virus (small, negative-stranded RNA virus). Surprisingly, naïve antigen-specific CD8⁺ T cells relocated to the peripheral interfollicular region near the subcapsular sinus, forming stable and lasting contacts with infected DCs, becoming activated effector cells and trafficking to infected tissue [17]. Subsequent experiments highlighted a chemokine-based mechanism for CD8⁺ T cell activation in this region, demonstrating that infected DCs release CCR5 ligands promoting preferential interaction and stable contacts of naïve CD8⁺ T cells with these specialized cross-priming APCs over the nearby SCS macrophages [18]. These studies established the principle that after initial priming events, terminal effector T cell activation during the primary immune response takes place in the outer regions of the LN.

CD4⁺ effector T cells

Until recently, intranodal positioning of T-helper type 1 (Th1) cells during activation remained unclear. Newly activated T cells upregulate CXCR3, which has been associated with IFN- γ producing Th1 responses. To investigate the importance of CXCR3 ligands in Th1 activation, Groom et al generated a transgenic reporter mouse (REX3) in which CXCL9- and CXCL10-expressing cells are RFP- and BFP-positive, respectively (CXCL11 is not expressed in C57BL/6 mice). Immunization of these mice with model antigen and Th1 adjuvant led to an appearance of CXCL9- and CXCL10-expressing cells outside the T cell zone in the cortical ridge, interfollicular and medullary regions of the LN. Antigen-specific T cells migrated out of the deep T cell zone to these regions, requiring CXCR3, which led to optimal differentiation into IFN γ and TNF α -producing Th1 cells [19**]. Experiments using whole LN imaging showed that vaccination with UV-inactivated influenza (PR8) led to LN-resident DC localization to medullary and interfollicular regions where they captured viral antigens. Analysis in REX3 mice revealed that these DC expressed CXCL10 and promoted CXCR3⁺ T cell-DC clustering [20**].

While relatively less is known regarding intranodal Th2 localization, this process is guided by a different set of cues. Infection with *H. polygyrus* resulted in DC localization in the perifollicular region of the LN. Mature DCs and priming CD4⁺ T cells upregulated CXCR5, positioning these cells around B cell follicles in a CXCL13-dependent manner leading to differentiation of IL-4 producing Th2 cells [21**]. Blockade of lymphotoxin- β or depletion of B cells secreting lymphotoxin- β led to decreased levels of CXCL13 and impaired Th2 differentiation.

Memory CD8⁺ T cells

The hallmark of adaptive immunity is the generation of immune memory. Central memory CD8⁺ T cells (T_{cm}) survey the lymphatics and ensure rapid immune responses in the draining LN to secondary infection. *In vitro*-generated antigen-specific T_{cm} rapidly localized within hours to the SCS, medullary, and interfollicular regions in response to LCMV infection, while naïve T cells (T_n) remained mostly in the T cell zone at this early timepoint. T_{cm} express high levels of CXCR3 while CD8⁺ T_n do not, allowing for the preferential migration and localization of T_{cm} within CXCL10 expressing regions of the LN. In these peripheral LN niches, T_{cm} interacted with infected cells, namely macrophages in the SCS, and DCs in the medullary and interfollicular regions, and rapidly produced IFN γ . Notably, IFN γ released by T_{cm} induced CXCL9 production by macrophages and stromal cells, potentially acting as a feed-forward mechanism to direct other immune cells to these infected areas [22]. Similar experiments demonstrated that *in vivo*-generated T_{cm} are prepositioned towards peripheral regions of the lymph node compared to T_n in response to viral challenge and confirmed the requirement of CXCR3 sensing of CXCL9 and CXCL10 for localization to the SCS, medullary, and interfollicular areas. Interestingly, CXCL10 staining colocalized with medullary macrophages, while CXCL9 was found on SCS myeloid cells and CD11c⁺ cells. The feed-forward CXCL9 production in response to rapid IFN γ production directed T_{cm} more specifically to the outer most areas of these regions, even closer to the SCS, a distinction from the behavior of T_n, which remained in the deep paracortex [23**].

Taken together, these studies demonstrate the nonredundant roles for CXCL9 and CXCL10 in guiding T cells to areas conducive to Th1 effector cell differentiation, and positioning central memory T cells for rapid response to secondary infection.

B cells and T follicular helper cells (T_{fh})

B cells localize in organized follicles outside the T cell zones closer to the SCS and separated by interfollicular regions. Chemokine sensing is critical for newly arriving B cell entry into LNs, requiring CXCR4 and CCR7 in a partially redundant manner [24]. Interestingly, in contrast to LNs, CXCR5 rather than CXCR4 is required for B cell entry into Peyer's Patches, while CXCR4 is required for sensing CXCL12 for egress from Peyer's Patches to draining lymphatics [25]. Indeed, CXCR5 is an important chemokine receptor for directing B cell migration, and is expressed on mature B cells. B cell migration throughout the follicle is guided, at least in part, by CXCR5 and CXCL13 interactions [26].

Germinal centers (GC) are structures that form during the immune response to T-cell dependent antigens. They can be separated into a light zone oriented toward follicular dendritic cells and the area of antigen entry, and a dark zone extending toward the T cell zone [27]. GCs are the predominant site of B cell affinity maturation, class switch recombination, plasma cell and memory B cell differentiation [28]. These processes are facilitated by interactions with T follicular helper cells (Tfh), which highly express CXCR5 [29,30] as a result of their specific differentiation program [31,32,33]. CXCR5 induction combined with CCR7 downregulation guides Tfh migration towards the T-B border of the follicle, while recently activated B cells upregulate CCR7 and also migrate towards the T-B border of the follicle [34]. Tfh-B cell interactions provide initial T cell help to B cells, and result in the retention of Tfh in the B cell follicle and participation in the GC reaction [35,36]. Experiments visualizing GC dynamics *in vivo* using photoactivatable mice showed that differential CXCR5 and CXCR4 expression regulated B cell localization between light and dark zones for proper affinity maturation and clonal expansion, respectively [37]. Tfh cells largely remain in the light zone of the GC and maintain high expression of CXCR5, although expression of the chemokine receptor is not solely restricted to GC Tfh cells and is also expressed on extrafollicular Tfh [38].

T regulatory cells (Treg)

The role of Tregs in regulating adaptive immune responses and chemokine production in the LN is an area of interest. *In vivo* ablation of Tregs prior to HSV infection resulted in more severe disease and increased viral load in the vaginal tissue [39]. Surprisingly, levels of inflammatory chemokines and cytokines were increased in the draining LN, which correlated with enhanced CD4⁺ T cell priming. However, inflammatory cytokine levels and NK, pDC, DC, and CD4⁺ effector T cell numbers were decreased in the vaginal tissue, suggesting that, in the absence of Tregs, immune cells necessary to control viral replication in the tissue were retained in the lymph node due to abnormally high levels of inflammatory chemokines. In addition, Tregs were shown to play a critical role in regulating CCL3, 4, and 5 levels for restricting initial priming of antigen-specific cells to only high-avidity CD8⁺ T cells rather than low-avidity clones, for the establishment of protective memory [40]. FoxP3⁺ Treg also play an important role in supporting B cell responses. CXCR5-expressing FoxP3-expressing regulatory Tfh are controlled by the receptor PD-1 [41] and limit Tfh and GC B cell numbers after immunization for optimal antigen-specific responses [42]. Additional work is required to fully elucidate the contribution of Treg to regulating chemokine production in the LN for the establishment of specialized niches for the generation of effective immune responses.

Conclusions and Future Directions

Mounting an adaptive immune response requires the dynamic orchestration of innate and adaptive immune cell positioning in the LN for engagement of lymphocytes with cognate antigen-loaded and appropriately activated APCs. Studies using modern imaging techniques have significantly improved the understanding of immune cell migratory behavior and cellular interactions in the LN that lead to lymphocyte activation and differentiation as well as memory responses. To date, this has been studied for the differentiation of CD4⁺ T cells

into Th1-type and Th2-type helper subsets and for the amnestic response of central memory CD8⁺ T cells (Figure 1). It remains to be determined if chemokine-driven specialized LN niches facilitate the differentiation of CD4⁺ T cells into Tregs, Tfh and other helper subsets, such as Th17, Th9, Th22. Further, in addition to understanding more completely how Tregs influence the quality of adaptive immune responses in the LN, the role of other immune cells, such as innate lymphocytes (e.g., NK, NKT, innate lymphoid cells), also remains to be more fully explored. A more complete understanding of the processes that regulate immune cell migration, positioning, and interactions in the reactive LN will help inform vaccine design for fine-tuning cell activation and differentiation to obtain the desired level and type of effector and memory responses.

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Figure 1. Chemokine-mediated cellular positioning in the reactive lymph node creates specialized niches to facilitate T cell differentiation and activation

A) Antigen from the site of infection arrives via the afferent lymphatics in soluble form or carried by dendritic cells (DC). Atypical chemokine receptor 4 (ACKR4) expressed on ceiling lymphatic endothelial cells scavenges CCL21 and creates a localized gradient for DC entry into the draining lymph node (LN) via the floor of the subcapsular sinus (SCS).

B) Specialized macrophages are positioned within the subcapsular sinus (SCS Mac) and take up antigen, secreting inflammatory cytokines, such as IL-18 and type I interferons upon activation. Innate lymphocytes expressing CXCR3 are positioned near the SCS, responding to IL-18 and producing IFN γ upon activation. Type 1 and II IFNs induce the production of CXCL9 and CXCL10 by local stromal cells and DCs, attracting and priming CXCR3-expressing CD4⁺ T helper-type 1 (Th1) cells. CD8⁺ central memory T cells (Tcm) are prepositioned in the outermost region of the T cell zone or are preferentially recruited to these regions by their constitutive expression of CXCR3.

C) Naïve T cells (Tn) enter the LN via high endothelial cells (HEV) in the deep T cell zone. In response to a Th1-type inducing stimulus, such as influenza virus, Tn are initially primed by antigen-bearing DCs, become activated, and upregulate CXCR3 expression. CXCL9 and CXCL10 gradients direct priming CD4⁺ T cells and Tcm out of the T cell zone and towards peripheral regions of the LN. T cells localize to the interfollicular and medullary regions and interact with infected and activated antigen presenting cells (APCs), resulting in optimal Th1 differentiation and IFN γ production.

D) Newly activated naïve T cells respond to a Th2-type inducing stimulus, such as a parasite, by upregulating CXCR5 and migrating out of the T cell zone via a CXCL13 gradient into the perifollicular areas near B cell follicles. There they interact with DCs, which have also upregulated CXCR5 in response to a Th2-inducing stimulus to generate a Th2 response.

E) Naïve B cells also enter the LN via HEV in chemokine-mediated process that is dependent on CXCR4 and CCR7 in a partially redundant manner. B cells are then recruited into the B cell follicle in response to CXCL13. Following B cell activation, B cells upregulate CCR7, which guide their movement to the border of the follicle where they are in position to interact with T follicular helper cells (Tfh). Tfh cells express high levels of CXCR5 and are specialized to provide B cell help for class switching.

Figure was modified from [43].