HHS Public Access

Author manuscript

Nat Rev Immunol. Author manuscript; available in PMC 2020 December 22.

Published in final edited form as:

Nat Rev Immunol. 2019 February ; 19(2): 89–103. doi:10.1038/s41577-018-0088-1.

Dendritic cell subsets in T cell programming: location dictates function

S. C. Eisenbarth

Department of Laboratory Medicine, Immunobiology, Section of Allergy & Immunology, Yale University School of Medicine, New Haven, CT, USA.

Abstract

Dendritic cells (DCs) can be viewed as translators between innate and adaptive immunity. They integrate signals derived from tissue infection or damage and present processed antigen from these sites to naive T cells in secondary lymphoid organs while also providing multiple soluble and surface-bound signals that help to guide T cell differentiation. DC-mediated tailoring of the appropriate T cell programme ensures a proper cascade of immune responses that adequately targets the insult. Recent advances in our understanding of the different types of DC subsets along with the cellular organization and orchestration of DC and lymphocyte positioning in secondary lymphoid organs over time has led to a clearer understanding of how the nature of the T cell response is shaped. This Review discusses how geographical organization and ordered sequences of cellular interactions in lymph nodes and the spleen regulate immunity.

T cells differentiate into discrete functional subsets that reflect the nature of their activation. Specification of the appropriate T cell fate is crucial for a tailored and effective adaptive immune response. T cells kill infected cells (for example, $CD8^+$ cytotoxic T lymphocytes (CTLs)), help macrophages destroy intracellular bacteria (for example, $CD4^+$ T helper 1 (T_H1) cells), recruit eosinophils to attack parasites (for example, T_H2 cells) or neutrophils to clear fungi (for example, T_H17 cells) or help B cells generate antibodies (for example, T follicular helper (T_{FH}) cells). The inflammatory response associated with each T cell subset is distinct and tightly controlled. If the nature of an immune response is inappropriate, it can be ineffective or, worse, cause pathology.

Experimental evidence accumulated over two decades identified developmentally distinct dendritic cell (DC) subsets that specialize in priming different types of effector T cells and thus tailor the outcome of an immune response. However, the mechanistic underpinning of this specialization is only partially understood, and plasticity exists, allowing DCs to

stephanie.eisenbarth@yale.edu.

Competing interests

The author declares no competing interests.

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Reviewer information

Nature Reviews Immunology thanks J. Cyster, S. Mueller and the other, anonymous reviewer(s) for their contribution to the peer review of this work.

differentially respond to distinct stimuli. What then makes a particular DC subset preferentially activate $CD4^+$ versus $CD8^+$ T cells or particular T_H cell fates? Recent work using sophisticated tools to interrogate individual cellular interactions over space and time suggests that the physical segregation of DC subsets in the secondary lymphoid organs (SLOs) helps regulate the nature of the T cell response. Such insights are of importance for areas such as rational vaccine design[1], as exemplified by the observation that fibrotic disruption of the basic human lymph node (LN) architecture results in impaired antibody responses to the highly effective yellow fever vaccine[2]. Here, I discuss how the temporal microanatomy of immune cells within SLOs allows for effective and tailored T cell immunity.

Introduction to dendritic cell subsets

Initially, DCs were divided into lymphoid and myeloid groups, but this nomenclature does not accurately reflect the developmental origin of each DC subset (reviewed previously[3]). Later, subsets were proposed on the basis of function, but again, DC plasticity defies rigid functional categories. What has emerged is a new, simplified classification system based on ontogeny (reviewed previously[4]), which often correlates with function (FIG. 1). This divides DCs and related myeloid lineages into conventional (also known as classical) DCs (cDCs), plasmacytoid DCs (pDCs), monocyte-derived DCs (MoDCs) and Langerhans cells (LCs). All other macrophage DC progenitor (MDP)-derived cells labelled DCs, here loosely grouped with MoDCs and LCs under the heading non-conventional DCs, have a variety of names and subgroups, some of which are termed DC owing to the expression of CD11c but otherwise have little in common with cDCs. Although some non-conventional DCs can augment cDC-mediated T cell priming, in general, these cells have distinct functions restricted to tissues rather than SLOs. As the focus of this Review is DC-dependent T cell priming and differentiation, only cDCs will be discussed in detail.

Type 1 and 2 conventional dendritic cells.

Across all tissues, developmentally and functionally similar subsets of cDCs exist, which are divided by ontogeny[5]. These two DC lineages have recently been termed type 1 (cDC1) and type 2 (cDC2) cDCs, and there are largely analogous subsets in mice and humans[6-11]. Using multiple methods to eliminate or target cDC1s across a variety of tissues, it has been established that cDC1s are the primary subset that cross-presents antigen to $CD8^+$ T cells in mice; they also produce more IL-12 than cDC2s and uniquely express Toll-like receptor 3 (TLR3)[10,12-20]. Although numerous studies using transcriptomic, surface marker and functional analyses have concluded that $CD141^+$ DCs in humans correspond to cDC1s in mice[7,11,21-23], whether cross-presentation is restricted to this human DC subset is less clear[24,25]. In mice, cross-presentation is not subset restricted when antigen is delivered to a cell in vitro, in particular, when the antigen is soluble rather than cell associated[26]. By necessity, this is the primary method of testing cross-presentation of human DC subsets. As multiple cellular pathways exist for processing exogenous antigen for MHC class I (MHCI), different DC subsets might be able to cross-present soluble but not cell-associated antigens, and therefore the capability of different subsets for cross-presentation might depend on how they are tested[27]. In contrast to cDC1s, cDC2s and subsets within this group have been

largely associated with a variety of $CD4^+$ T_H cell responses[19,20,26,28-35]; however, cDC2s can be difficult to distinguish from MoDCs and other DCs induced during inflammation owing to an overlap in CD11b expression.

What engenders this division of labour between cDC1s and cDC2s is only partially understood (reviewed previously[36]). Different methods of antigen uptake[37] and lysosomal processing[38] or different levels of peptide-loaded MHCI versus MHC class II (MHCII)[29,39] have been proposed to account for the ability of a DC to stimulate either T_H cell or CTL responses. Yet, in vitro or even ex vivo, both cDC subsets (as well as non-cDCs) effectively activate $CD4^+$ and $CD8^+$ T cells[15,26,40,41]. Therefore, restricted processing of antigen on either MHCI or MHCII does not sufficiently explain this preferential activation of $CD4^+$ versus $CD8^+$ T cells observed in vivo[3,36]. Many aspects of cellular function are context-dependent; as will be discussed, intrinsic functional differences between DC subsets combined with their physical location within SLOs create immunologically distinct compartments that engender particular types of T cell induction.

Migratory versus LN-resident conventional dendritic cells.

A final level of sub-division of cDC1s and cDC2s into LN-resident and migratory derives from their steady state location and therefore how they access antigen. This classification by location and development results in four different subsets of cDCs across all LNs in the body. cDC1s and cDC2s seed both parenchymal tissue and SLOs and act as sentinels at both sites. LN-resident cDCs continually enter the LN from the blood and receive antigen via either lymphatic drainage or transfer from other cells and can use these acquired antigens for both $CD4^+$ and $CD8^+$ T cell priming[42-44]; despite their name, however, they are not stationary cells and can traverse different areas within the SLO. By contrast, migratory cDCs reside in parenchymal tissues and must migrate to LNs in order to interact with naive T cells.

The primary method of distinguishing LN-resident from migratory DCs is by measuring MHCII and CD11c expression levels. In a naive animal, there is a clear distinction between steady state migratory ($CD11c^{int}MHCII^{hi}$) and LN-resident ($CD11c^{hi}MHCII^{int}$) populations[45]. However, during inflammation-induced DC activation, these populations merge on the basis of these markers, making discrimination difficult[36,46]. The unique expression of $CD8\alpha\alpha$ on LN-resident cDC1s allows their discrimination from $CD103^+$ migratory cDC1s. But an equivalent segregating marker for LN-resident versus migratory cDC2s does not exist. Immunization with large labelled beads that cannot drain into the LN can distinguish migratory from LN-resident DCs[47], but this approach cannot always be applied.

The same cDC division between resident and migratory cells can be applied to splenic DCs, although the accepted nomenclature becomes problematic. DC migration within the spleen has long been appreciated[48-53]; however, it has been generally accepted that migratory DCs do not exist in the spleen, and instead all DCs are grouped together as resident[3,24]. This is perhaps due to the lack of lymphatics draining into the white pulp (WP), equivalent to LN afferent lymphatics. Indeed, all DCs migrate (including resident DCs), whether to LNs or within LNs, and the same is true for splenic DCs. In the periphery, migratory DCs are characterized by antigen scouting in tissue and, upon receiving activating signals,

immigration to LN compartments rich in naive T cells. Indeed, subsets of splenic DCs sample systemic antigen in the red pulp (RP), marginal zone (MZ) and bridging channels, mirroring the sentinel role of DCs in other parenchymal tissues. Exposure to the same innate immune stimuli that induce DC migration from peripheral tissues to draining LNs induces splenic DC migration to the WP T cell zone (TCZ)[26,48-53]. If one views the RP of the spleen as a tissue embedded with multiple SLOs (that is, WP), rather than as one large LN, then it becomes clear that the same division of function between resident and migratory DC subsets exists in the spleen and that these subsets perform parallel functions to DCs across the rest of the body. Indeed, CC-chemokine receptor 7 (CCR7) is important for this migratory step[26,54]. Further, a subpopulation of splenic cDC1s that reside in the MZ and migrate to the WP after immunization express the marker CD103, which is used in other tissues to identify migratory cDC1s[55]. Therefore, a universal classification system of migratory and resident DCs across all tissues can be used to help understand the relative role of each of these subsets, whether the antigen is injected into tissue, inhaled or carried via the bloodstream.

Orchestration of an LN immune response

B cells require cognate $CD4^+$ T cell help to proliferate, class switch and affinity mature (a pathway termed ‘linked recognition’); CTLs require $CD4^+$ T cell help to generate effective memory; $CD4^+$ T cells are licensed by activated DCs carrying their cognate antigen. This simplified list highlights that, at a minimum, four different types of leukocytes must all interact with each other and a single antigenic target, in sequence, to generate a productive adaptive immune response. Even if all the cellular players are in the same general vicinity (for example, the LN TCZ), chance interactions seem unlikely to promote such a choreographed and ordered development. Instead, the spatiotemporal organization within SLOs is crucial for not only initiating immunity but also tailoring the type of response generated (BOX 1). Indeed, there is no uniform dispersal of DCs across the LN, nor is there an isolated concentration of DCs at high endothelial venules (HEVs), the sites of T cell entry. Instead, each cDC subset homes to a specific region of the LN after immunization and acts as a nidus for particular types of immune reactions. This asymmetric DC positioning within the LN corresponds to $CD4^+$ and $CD8^+$ T cell segregation and has implications for the nature of the T cell response (FIG. 2). Given the rarity of an antigen-carrying DC encountering a naive T cell that recognizes its cargo, this DC subset–T cell lineage colocalization likely promotes efficient adaptive immune responses and creates potentially unique cellular niches for T cell specification. B cells similarly undergo intricate LN organization and reorganization over the course of an immune response, the discussion of which is beyond the scope of this Review (reviewed previously[56]).

How antigen gets to LNs.

The antigenic context (route of immunization, nature of immunogen and dose of antigen) impacts which DC subsets are exposed to antigen and where in the LN the DC–T cell interactions occur, as well as the kinetics of the interaction. Antigen can reach LNs via two mechanisms: free draining via lymph or carried by cells, primarily migratory DCs.

The first mechanism requires a high antigen dose at the tissue site or injection of antigen into a restricted tissue compartment such as the footpad or ear pinnae (BOX 2), and the antigen needs to be <200 nm in diameter[57]. Once such draining antigen reaches the LN, it travels into the subcapsular sinus (SCS) and marginal sinus, where it can be sampled by macrophages and DCs. Low-molecular-mass molecules (<70 kDa) such as chemokines, certain antigens and cytokines can continue further, into the vicinity of HEVs in the cortex, via lymph conduits[58-60]. These conduits are not freely permeable, and therefore antigen does not percolate through an LN. Instead, interdigitating LN-resident DCs can sample conduit lymph for antigen[58,60]. A similar conduit system has been proposed to exist to deliver antigen to LN follicles[59,61]. Recently, nonhomogeneous antigen dispersal in the LN was shown to influence the amount of antigen each resident DC subset can present based on differential localization[62]. When antigen is injected into the footpad, and therefore a substantial amount of cell-free antigen drains via lymph into the LN, it enters the lymphoid sinus, where it can be acquired by LN-resident cDC2s[62]. By contrast, when antigen is subcutaneously injected at other sites, little free antigen reaches the LN until migratory DCs carry it there[47,63].

For the second mechanism, activated antigen-laden migratory DCs, following lymphatic-derived CCR7 ligands and the signalling lipid sphingosine-1-phosphate (S1P), can enter the LN parenchyma and either present the antigen or transfer it to LN-resident DCs or B cells[42,44]. After 1–3 days of antigen presentation following DC activation, DCs then die[64,65]. They do not migrate to other LNs[66] and must be replaced by new waves of DCs. Depending on the tissue, timing and the type of immunization, the relative number of migratory cDC1s versus cDC2s versus non-conventional DCs carrying antigen to an LN varies. For example, a relative paucity of cDC1s in the skin results in a migratory cDC2-dominated response in the LN after subcutaneous immunization and, days later, antigen-carrying LCs[47,65,67]. By contrast, an almost equal number of migratory cDC1s versus cDC2s transit antigen to mediastinal LNs from the lung during the first day of a response[47,68]. After intramuscular immunization, both migratory cDC1s and cDC2s can carry antigen to local LNs; however, the number of migrating DCs reaching draining LNs from this site is an order of magnitude less than from the skin or lung[47,69]. Therefore, the size and dose of antigen along with the route of exposure dictate which DCs present antigen and, as will be discussed, where in the LN antigen is presented.

Spatiotemporal organization of dendritic cells.

Migratory DCs finish their CCR7-dependent transit from tissues at the SCS of the LN, where they cross the SCS floor in a CCR7-independent manner[66]. From here, migratory cDC1s and cDC2s enter the interfollicular zone (IFZ)[66,70] and then home to the different LN regions (BOX 3). An early study that could not discriminate between migratory versus LN-resident cDC2s observed segregation of cDC1s and cDC2s, with cDC1s located in the deep TCZ and cDC2s in the T cell–B cell border[63]. Other imaging studies of skin-draining LNs localized migratory dermal DCs to the peripheral TCZ and/or outer paracortex but could not distinguish cDC1s from cDC2s[71-73]. Using a Langerin (also known as CD207) reporter, which marks both dermal cDC1s and LCs[74], it was shown that these two cell populations migrate into the deep TCZ after application of tracers to the skin[75]. By contrast, the

Langerin-negative dermal migratory DCs localized to the outer paracortex; on the basis of recent work, this latter population likely consists of migratory cDC2s[31,47]. Two studies using photoconvertible fluorescent proteins that change upon exposure to light determined the kinetics and homing of DC migration from the skin. They found that migratory cDC1s arrive within 1 day after immunization but require another 24 hours to reach the deep TCZ, where they intermingle with LN-resident cDC1s[65,76]. The pattern that emerges from these varied studies is a central TCZ populated by migratory and LN-resident cDC1s along with LCs and a peripheral ring of cDC2s at the T cell–B cell border (FIG. 2c).

Intravital imaging of LNs following adoptive transfer of either bone marrow-derived or splenic DCs tracked them to the T cell–B cell border, proximal to HEVs, and therefore this site was proposed to act as a locale for DC scanning by incoming naive T cells from the circulation[71,73,77,78]. However, these types of transferred DCs do not faithfully phenocopy native DCs and likely migrate to different sub-anatomic LN regions than endogenous subsets of migratory DCs[79]. These studies were also not designed to identify the sub-anatomic location of the four cDC subsets; this is a difficult task given the number of markers required. One approach to solve this problem, called histo-cytometry, is a hybrid of flow cytometry and microscopy that uses multiplex staining of LN sections with intensity measurements to both locate and quantify cDC1 and cDC2 subsets[80]. However, it relies on differing levels of MHC and CD11c expression to distinguish migratory versus LN-resident DCs and therefore is best applied to steady state rather than post-immunization LNs[36]. In steady state skin-draining LNs, this method indeed showed that migratory cDC1s, LN-resident cDC1s and LCs were in the deep TCZ, whereas LN-resident cDC2s were adjacent to lymphatic sinuses, and migratory $CD11b^+$ cDC2s homed to the IFZ in the T cell–B cell border[31]. Using immunization with labelled beads that cannot freely drain into lymphatics to discriminate migratory from LN-resident DCs, and six-colour immunofluorescence to distinguish subsets after immunization, a similar pattern was found in lung-draining LNs; migratory cDC2s localized to the T cell–B cell border, whereas migratory cDC1s were in the deep TCZ[47]. Therefore, these patterns likely hold across a variety of tissues at both steady state and after immunization. Recent work suggests they are also mirrored in human LNs; an elegant survey of multiple SLOs from humans found cDC2s in the T cell–B cell border, whereas cDC1s were located in the TCZ[81].

Most studies suggest that both LN-resident cDC1s and cDC2s can acquire antigen through lymph sampling, especially following immunization by footpad or intra-auricular injection[31,82]. However, whether this occurs at cortical conduits in the outer TCZ or lymphatic sinuses located in the cortico-medullary junction adjacent to peripheral follicles is debated. As the size of antigen that is accessible at each site can differ and the proximity to particular lymphocyte subsets is different, where LN-resident cDC2s reside could matter for the nature of the induced response. For example, it was shown that LN-resident $CD11b^+$ DCs sampled small antigens from LN conduits after footpad injection, presumably in the outer LN T cell–B cell border[60]. By contrast, another study identified LN-resident $CD11b^+$ cDC2s at the lymphatic sinus on the medullary side of the LN, where they could capture large, particulate antigen from the lymph[31]. Whether these represent two different LN-resident cDC2 subsets or the same cDC2 subset migrating to different locations during different types of inflammation is not clear. Two studies using intravital imaging of LNs

following footpad immunization with inactivated virus indeed documented rapid *trans*-nodal repositioning of both LN-resident cDC1s and cDC2s into the peripheral lymphoid sinus[83,84]. By using photoconversion of skin-derived DCs to distinguish migratory versus LN-resident cDC1s following skin painting, some of LN-resident cDC1s were identified in the lymphatic sinus[76]. However, in this study and many others, most LN-resident cDC1s, in contrast to LN-resident cDC2s, were found to form a network with migratory cDC1s in the deep TCZ[31,75,76]. Again, the overall pattern that emerges is a central region of both migratory and resident cDC1s flanked by peripheral migratory and resident cDC2s (FIG. 2).

The nature of the immunization can alter the anatomic organization of DC subsets in LNs. Eight days after influenza infection, mature DCs in the LN were shown to primarily express CCR7, whereas at the same time point after infection with the parasite *Heligmosomoides polygyrus*, most DCs in the LN expressed CXC-chemokine receptor 5 (CXCR5), which binds CXC-chemokine ligand 13 (CXCL13)[85]. Accordingly, the pattern of CD11c staining in LNs is remarkably different, with a strong DC concentration in the deep TCZ after viral infection and a T cell–B cell border staining after parasite infection[85]. Consequently, loss of CXCR5 on DCs or CXCL13 neutralization during parasite infection results in DC migration into the TCZ instead. Although the DC subset expressing CXCR5 was not identified in this study, work using other types of immunization demonstrating preferential expression of CXCR5 and placement adjacent to B cell follicles argues that these are migratory cDC2s[47,79,86]. A similar change in chemotactic regulation of DC subset positioning has been described in the spleen. cDC2 migration to the T cell–B cell border in the WP requires sensing of oxysterol chemoattractants through the G protein-coupled receptor Epstein–Barr virus induced gene 2 (EBI2; also known as GPR183), but type I interferon production disrupts EBI2 expression and accordingly relocalizes cDC2s across the TCZ[87].

Spatiotemporal organization of T cells.

Multiple cell types cooperate to slow the egress of, pre-prime, prime and differentiate naive T cells. This is supported by in vivo imaging studies demonstrating that induction of effector function in naive $CD4^+$ T cells requires repeated antigenic stimulation; the nature of these sequential interactions differs in length and potentially in quality[88-90]. Some interactions may require a specific DC subset, whereas promiscuous stages could be achieved by a variety of cell types, potentially even in the absence of cognate antigen. However, the anatomical context of each of these stages is often lost by in vivo imaging. Evidence from a combination of other imaging modalities suggests that particular T cell activation steps occur in distinct LN regions, as will be discussed.

Naive and memory T cells that travel to the LN via the blood enter through HEVs into the paracortex (FIG. 2a). T cells arriving via lymphatics circle around to the medullary side and enter the LN via medullary sinuses[66]. Brief, serial interactions with DCs in inflamed LNs then induce early T cell activation over the first 8 hours, often in a non-antigen-specific manner. For example, surface binding of CC-chemokine ligand 21 (CCL21) via its heparin-binding domain to DCs helps pre-prime naive human and mouse $CCR7^+$ T cells, even in the presence of sub-optimal T cell receptor (TCR) stimulation[91-95]. Expression of CCR7 quickly decreases, while the expression of other chemokine receptors such as CXCR5, CXCR3 and

CCR5 increases[96-98]. CD69 upregulation is necessary to dampen responsiveness to S1P egress signals emanating from cortical sinuses[99] and can be induced independently of cognate antigen recognition[100]. These changes in responsiveness to chemoattractants help retain T cells in the LN for the second phase of activation, which is characterized by DC–T cell interactions, each lasting more than an hour, and induces fulminant T cell activation including cytokine production[78,90]. The final phase, characterized by T cell proliferation, occurs >24 hours after immunization and requires less interaction with DCs. Evidence suggests that multiple DC subsets can cooperate to complete phases one and two[101,102]. Using footpad antigen injection, it was demonstrated that LN-resident DCs can induce T cell proliferation but not effector differentiation within 3 hours of immunization; skin migratory DCs arrived 24 hours later and induced functional effector T cells[102]. Therefore, both resident and migratory DCs participated in $CD4^+$ T cell activation but at different time points after immunization.

During phase one of activation, naive $CD4^+$ and $CD8^+$ T cells upregulate distinct chemoattractant receptors, such as EBI2, which induce their segregation to different poles of the TCZ (FIG. 2b). Some studies of endogenous T cells suggest that segregation of the two T cell lineages already exists in steady state LNs[85], something that was also observed in the TCZ of the spleen[26]. Using adoptively transferred $CD4^+$ and $CD8^+$ TCR transgenic T cells, such a distinction was not seen; instead both lineages appear scattered across the TCZ[76,96,103,104]. Regardless, within 24 hours after immunization, a consistent pattern of segregation is observed for both TCR transgenic and endogenous T cell lineages; $CD4^+$ T cells migrate towards the T cell–B cell border, and $CD8^+$ T cells concentrate within the deep TCZ[63,71,72,76,103]. Margination of $CD4^+$ T cells to the T cell–B cell border requires upregulation of CXCR5 and EBI2 and stromal CXCL13 production and is promoted by lymphotoxin-producing B cells[85]. Migratory cDC2s also preferentially express CXCR5 and EBI2 and localize to the T cell–B cell border. LN-resident cDC2s also localize to the T cell–B cell border but on the medullary side of the LN[31,80]. Not coincidentally, cDC2s rather than cDC1s are most often found to be required for $CD4^+$ T cell priming[19,20,28-31]. By contrast, migratory and LN-resident cDC1s are located in the deep TCZ, where they colocalize with and are necessary for activation of $CD8^+$ T cells[76,103]. Two recent papers showed that $CD4^+$ and $CD8^+$ T cell activation is indeed spatially and temporally separated in LNs early during viral infection, but 1–2 days after immunization, both T cell subsets come together with a LN-resident cDC1 subset[72,103], which may optimize $CD4^+$ T cell help for CTL activation, as will be discussed in the next section.

Although, in many studies, a general segregation of $CD4^+$ and $CD8^+$ T cells is observed, differentiation of particular $CD4^+$ T cell fates might occur outside of the T cell–B cell border. Loss of DC localization to the T cell–B cell border impairs T_H2 cell and T_{FH} cell induction during parasite infection or vaccination with inert antigen and adjuvant but does not impair T_H1 cell induction in response to influenza[31,80,85], suggesting that cDC2s might not be the relevant DCs for T_H1 cell induction. In fact, T_H1 cell differentiation appears to be one exception to the cDC2–$CD4^+$ T cell priming rule. Consistent with marked IL-12 production, cDC1s are often required for T_H1 cell differentiation[13,47,50,105]. Migratory and LN-resident cDC1s express more CCR7 and less CXCR5 and EBI2 and thereby localize to the deep TCZ. This is the same site where $CD8^+$ T cell priming occurs. IL-6-producing and

IL-23-producing cDC2s or LCs favour T_H17 cell differentiation[13,106-109]; however, less is known about the geography of this interaction.

Niches for T cell fate specification

Intranodal positioning of DCs supports niche-restricted T cell differentiation by establishing specialized microenvironments. These microenvironments, in addition to the particular DC subset, include accessory cells such as stromal cells, granulocytes, B cells, pDCs, natural killer (NK) cells, NKT cells and regulatory T (T_{reg}) cells, which can differentially favour particular T cell fates and are geographically concentrated during particular types of responses. Owing to either the intrinsic nature of the DC subset and/or accessory cells in the niche, sites are created that differ with regard to the abundance of particular cytokines, co-stimulatory signals and strength of antigenic stimulation.

$CD4^+$ T cell fate specification.

Antigen dose is a well-known variable regulating T_H cell differentiation, with the highest doses favouring T_{FH} cell and the lowest favouring T_H2 cell differentiation[110,111]. T_{FH} cells are a subset of $CD4^+$ T cells that promote long-lived, high-affinity antibody production by B cells[112]. T_{FH} cells are characterized by the expression of the transcription factor BCL-6 and surface expression of programmed cell death 1 (PD-1), inducible T cell co-stimulator (ICOS) and CXCR5. The current paradigm of T_{FH} cell induction is that DCs and B cells must cooperate to first induce (DC phase) and then solidify (B cell phase) T_{FH} cell fate. Multiple studies have demonstrated that both mouse and human cDC2s induce the first pre-T_{FH} cell phase and are required for humoral immune responses[31,47,86,113-115]. This is consistent with preferential antigen presentation on MHCII by cDC2s[29,39] and stronger antigenic stimulation favouring T_{FH} cell differentiation[110,111]. However, the geography of this interaction is likely also relevant. Tracking of T_{FH} cell development demonstrated that the pre-T_{FH} cell phase occurs in the T cell–B cell border and/or the IFZ[116,117]. Early CXCR5 expression on T cells allows them to stray from the deep TCZ and localize adjacent to $CXCL13^+$ follicles[85,116,118]. EBI2 is also crucial for this localization[86]. What signals and cells induce these early changes in chemokine receptors is not clear. Concomitantly, antigen-specific $CXCR5^+$ B cells upregulate CCR7 and EBI2 and stray from the follicle centre into the T cell–B cell border, where IL-4, potentially produced by NKT cells, acts as an early survival signal[119-121]. Therefore, homing of cDC2s to the T cell–B cell border provides antigen for T cells and also possibly B cells and allows the formation of a potent niche for T_{FH} cell development[120]. Yet cDC2s are not sufficient to enforce T_{FH} cell differentiation. Cytokines that promote T_{FH} cell differentiation, such as IL-6, IL-12 and IL-21, are not preferentially expressed by cDC2s[47,50,112,114]. Instead, recently stimulated $CD4^+$ T cells in the T cell–B cell border can receive conditioning signals from adjacent B cells and stromal cells[122]. This is in line with a study using mixed bone marrow chimaeras demonstrating that sustained upregulation of CXCR5 and BCL6 on nascent T_{FH} cells required IL-6 production by non-haematopoietic cells[116]. Additionally, B cells express both CD40 and OX40 (also known as TNFRSF4), which provide important co-stimulatory signals to T cells[123,124]. As IL-2 can downregulate CXCR5 expression on T cells and inhibit T_{FH} cell differentiation, it has been proposed that IL-2 sinks promote T_{FH} cell responses[86,112,118]. T_{reg} cells, B cells

and cDC2s can express CD25, the IL-2 receptor α-chain, which can sequester IL-2. Altogether, cDC2s with accessory cells in the T cell–B cell border create a powerful niche for the pre-T_{FH} cell development. Pre-T_{FH} cells then enter the follicle, where they encounter cognate B cells that induce the second phase of T_{FH} cell commitment[117].

T_H1 cells and T_H2 cells also segregate within LNs and the spleen, with T_H1 cells often in the deep TCZ and T_H2 cells adjacent to B cell follicles in the IFZ and T cell–B cell border[125]. Differential expression of CCR7 appears to be a dominant force guiding this segregation, with high levels on T_H1 cells and low levels on T_H2 cells, mirroring the chemokine receptor pattern on cDC1s and cDC2s, respectively[47]. Accordingly, the geography of these two $CD4^+$ T cell subsets coincides with the DC subset shown to regulate each fate. In most studies, including those using human-derived DCs, cDC2s promote T_H2 cell differentiation[32-35,67,68,126-129] and cDC1s promote T_H1 cell differentiation[13,105,130]. Although both T_{FH} cell and T_H2 cell differentiation are fostered in the IFZ by cDC2s, whether this fate choice is stochastic or deterministic is currently unclear. A particular subset of cDC2s that is characterized by the expression of CD301b and is dependent on the transcription factor krueppel-like factor 4 (KLF4) has been shown to specifically induce T_H2 cell but not T_{FH} cell or T_H17 cell responses[32-34,129]. Therefore, a division of labour and even localization between subsets within the cDC2 population could potentially account for the induction of T_H2 cell versus T_{FH} cell differentiation, but this remains to be determined.

Why these subsets and/or sites selectively promote T_H1 cell versus T_H2 cell differentiation is only partially understood. Lower antigen doses promote T_H2 cell over T_H1 cell development[110]. This paradigm is inconsistent with the finding that cDC2s, which preferentially express peptides on MHCII[29,39], are required for T_H2 cell rather than T_H1 cell differentiation[32-35,68,126,127,129]. This argues for another level of regulation involving accessory cells in microenvironments. In some studies, basophils were shown to augment T_H2 cell differentiation by providing IL-4, and the recruitment of basophils to LNs by activated DCs and direct basophil–DC interactions have been demonstrated[67,131]. However, other studies questioned whether basophils actually enter LNs and their relevance to T_H2 cell induction[132,133], and so the role of T_H2 accessory cells requires further investigation. Innate lymphoid cell (ILC) populations have been identified in the T cell areas of LNs and the spleen and have been proposed to have both antigen-presenting functions as well as cytokine-dependent effects on T cell activation[134]. However, the exact function of group 2 ILCs (ILC2s) in the LN and whether they directly regulate DC function need clarification[135]. One report has suggested that ILC2s in the lung influence DC migration and thereby affect T_H2 cell induction to allergens, but a direct interaction in the LN has not yet been identified[136].

T_H1 cell differentiation in some studies has been demonstrated in the IFZ and medullary regions of the TCZ[96]. The latter site, adjacent to the central TCZ, can be populated by cDC1s, which intrinsically produce more IL-12 upon stimulation than cDC2s and therefore favour T_H1 cell differentiation[13,47,105]. DC-derived CXCL10 attracts $CXCR3^+$ T cells to the outer TCZ, prolongs DC contact time and favours T_H1 cell differentiation[96,137]. Other CXCR3 ligands such as CXCL9 also organize favourable microenvironments for T_H1 cell differentiation through recruitment of IFNγ-producing NK cells[97]. Indeed, these innate cells

are present in the TCZ (parafollicular and medullary regions), and during infection they colocalize with DC–T cell clusters[138], where they can influence both DC activity and survival[139-141] as well as T cell polarization. NK cell activation of human and murine DCs, in particular cDC1s, induces IL-12 production, which can reciprocally enhance NK cell activity[139-141] and also promote T_H1 cell differentiation. Similarly, activated NKT cells promote cDC1 cross-priming of CTL responses[142]. Another innate lymphocyte population, γδ T cells, has also been shown to influence DC activation by producing TNF and can thereby affect the nature of the T cell response generated[143]. Therefore, NK, NKT and γδ T cells create a milieu rich in IL-12, IFNγ and TNF in the deep TCZ and, along with cell–cell contact with cDC1s, can promote T_H1 cell and CTL activation.

CD8$^+$ T cell fate specification.

Although most studies conclude that murine cDC1s are the relevant antigen-presenting cells for $CD8^+$ T cell priming, studies differ on whether migratory or LN-resident cDC1s are the dominant populations facilitating this process[41,42,72,103,144]. This difference is likely to be due to the type of immunization used in the study and whether antigen and/or virus can directly disseminate to the LN. A study using photoconvertible tracers to distinguish migratory versus LN-resident cDC1s found that $CD8^+$ T cells interacted more with migratory rather than LN-resident cDC1s and that this occurred in the deep TCZ over the course of 3 days[76]. Resident cDC1s did not participate significantly in this response[76,145]; however, migratory and LN-resident cDC1s are in close proximity in the TCZ and can share antigen, and so other studies demonstrate a crucial role for resident cDC1s in $CD8^+$ T cell priming[42,44]. When DCs are directly infected, any subset can prime $CD8^+$ T cells, and, accordingly, priming occurs outside the deep TCZ, including the IFZ[72,103,146,147].

In addition to antigen presentation, cDC1s have recently been shown to act as an important platform for $CD8^+$ T cell help. Activated $CD4^+$ T cells promote CTL activation and are particularly important for the generation of $CD8^+$ T cell memory (reviewed previously[148]). Recent work demonstrated that cDC1s expressing the chemokine XC receptor 1 (XCR1) act as a bridge between previously activated $CD4^+$ T cells and naive $CD8^+$ T cells[72,103]. It was shown that during viral infection of the skin, migratory DCs carry antigen to draining LNs to prime $CD4^+$ T cells during the first 1–2 days. On the basis of the location in the TCZ and the nature of the infection, it can be surmised that these $CD4^+$ T cells were T_H1 cell differentiated and further that other T_H cells might be poor partners for $CD8^+$ T cell activation given their peripheral location in the IFZ. Subsequently, primed T_H cells licensed LN-resident cDC1s to induce CTL priming over days 2 and 3 (REFS[72,103]). Loss of this cDC1 platform for $CD4^+$ to $CD8^+$ T cell collaboration impaired the generation of memory CTLs[103]. Although the exact location of cDC1s in these two studies was not identified, we can postulate that this three-way DC–T cell interaction happens in the deep TCZ on the basis of the T cell localization and previous DC work[31,75,76]. Separate studies demonstrated that $CD4^+$ T cell recognition of cognate antigen on DCs induces local production of CCL3 and CCL4 and thereby attracts naive $CCR5^+CD8^+$ T cells for antigen scanning of the $CD4^+$ T cell–DC pair[72,73,98,103,147]. CCR5 ligands also draw pDCs into foci of cDC1–$CD8^+$ T cell interactions, which promotes cDC1 function via pDC-derived type I interferon

production[149]. Therefore, the spatial organization of multiple cell types around cDC1s is important for the nature and magnitude of $CD8^+$ T cell responses.

Viral infection of the LN or spleen itself induces unique chemokine gradients that alter the microanatomy of these SLOs for $CD8^+$ T cell priming[72,103,147] and promote recognition of virally infected cells. SCS macrophage infection results in adjacent DC antigen presentation to CTLs outside of the TCZ by CCR5-dependent chemokine production[147]. Under this condition and in contrast to studies of other immunizations, $CD4^+$ T cell activation occurred in the deep TCZ[103], and $CD4^+$ and $CD8^+$ T cell activation was synchronous[72]. If the same virus instead infected peripheral tissue but not LNs, then the temporally and spatially segregated $CD4^+$ and $CD8^+$ T cell activation pattern was restored[72,150].

Orchestration of a splenic immune response

An analogous organization to LNs exists in the spleen to selectively promote tailored T cell activation to systemic antigens. But given the differences in architecture, T cell activation in the spleen is discussed separately using landmarks appropriate to the spleen. As was summarized for the LN, DC–T cell lineage niches also exist in the spleen (FIG. 3); however, less is known about $CD4^+$ T cell differentiation niches in the spleen than in the LN.

How antigen gets to the spleen.

The spleen is the largest SLO and serves as a filter for ageing red blood cells as well as foreign antigens or pathogens that have gained access to the bloodstream. It is also the site where immune responses to these antigens are initiated. In this way, the spleen is the draining SLO for the circulatory system, analogous to LNs that drain particular regions of the body via lymphatics.

Antigens from the blood are released by terminal arterioles into the MZ (mouse) or perifollicular region (human) and surrounding RP and are encountered by multiple macrophage populations and, in mice, innate MZ B cells and a subset of DCs[55,151,152]. Much less is understood about how this encounter happens in the human spleen. Similar to LNs, intravenous antigen does not freely percolate through the naive lymphocyte-rich WP. Although conduits can carry antigens smaller than 60 kDa into the WP[153], cellular transport is a major mechanism of antigen delivery. MZ B cells carry antigen into the follicles[154], and DCs from the RP carry antigen to the periarteriolar lymphatic sheaths (PALSs). Is DC migration within the spleen required for T cell priming, as it is for many forms of antigen in peripheral tissues? Confusion on this point has arisen because DCs reside in the TCZ at steady state (like LN-resident DCs), and the unique architecture of the spleen juxtaposes antigen-exposed tissue (the MZ and/or RP) directly with the lymphoid compartment (WP). Using selective inhibition of cDC2 migration, impaired T cell responses to blood-borne antigens were found, suggesting that intrasplenic DC migration helps to set a threshold for T cell activation to innocuous or self-antigens in the blood, just as happens in the periphery[26].

Spatiotemporal white pulp organization mirrors LNs.

Surveillance of different areas of the spleen is divided between the cDC subsets. All splenic cDC1s express XCR1, and using XCR1 reporter mice or immunofluorescence staining,

cDC1s were found to be localized to two regions of the spleen[17,26,40,155]. CD8αα $^{+}$DEC-205^{+} cDC1s reside in the PALSs at steady state in both mouse and human spleens, equivalent to LN-resident cDC1s[156-158]. The other cDC1s reside in the MZ and RP, some of which express CD103 and Langerin (depending on the mouse strain) and are equivalent to migratory cDC1s in other sites[51,55]. Similar to LN-resident cDC1s, WP-resident cDC1s must access antigen from conduits or immigrating cells.

The bridging channel is the sole site of DC immunoreceptor 2 (DCIR2)$^{+}$ cDC2s (using the 33D1 antibody for DCIR2 detection)[26,157,159]. The chemoattractant receptor EBI2 helps to localize cDC2s to the bridging channel and is required for the maintenance of this DC subset through interactions with B cells[160,161]. S1P also acts to localize cDC2s to the bridging channel through S1P receptor 1 (S1PR1)[162]. In vivo labelling has shown that approximately half of the cDC2s in the bridging channel are exposed to antigens in the MZ and half are not, indicating that some cDC2s are within the WP and therefore should not have access to large blood-derived antigens[26]. Whether these two cDC2 populations parallel LN-resident versus migratory cDC2s at other sites and are functionally and developmentally different is unknown.

Following systemic immunization in mice and possibly also in humans, this cellular organization is highly dynamic[163]: antigen-laden murine MZ B cells shuttle into and out of the WP[154], the marginal zone metallophilic macrophage-defined border dissolves (analogous to the SCS macrophage disruption that happens in LNs)[164], activated B cells upregulate CCR7 and therefore migrate towards the T cell–B cell border[165], and multiple types of DCs migrate to specific regions in or around the WP. pDCs form a ring around the MZ[53], cDC1s evacuate the RP and MZ to enter the WP[26,50-52,55], and cDC2s in the bridging channel migrate into the WP[26,87,161]. After immunization with an array of pathogen-associated molecular patterns, the two cDC subsets were found to migrate into the WP, where they segregated into non-overlapping regions in the PALSs[26]. The outer bridging channel cDC2s migrated into the peripheral PALS region, adjacent to B cell follicles[26,159]. Migratory cDC1s in the RP and/or MZ migrated to the WP-resident cDC1s in the central TCZ[26,51]. This mirrors the pattern described for cDC subsets in LNs. The molecular regulation of cDC1 and cDC2 segregation in the WP after immunization has recently been elucidated; increased expression of EBI2 on cDC2s allows these to respond to oxysterols, which are primarily produced by stromal cells in the bridging channel and T cell–B cell border[87]. However, the enzyme 3 β-hydroxysteroid dehydrogenase type 7 (HSD3B7), which is produced by cDC1s, inactivates these ligands. Therefore, as migratory cDC1s concentrate within the central TCZ, possibly owing to XCR1 ligand production by CTLs[17], the chemoattractant for cDC2s is erased in these areas[87]. The requirement for CCR7 in DC migration within the spleen has been questioned[162], but this has been difficult to answer because the basic immune structure of the WP, including a defined TCZ, is severely disrupted in CCR7-deficient mice[26,166]. Several studies have used mixed bone marrow chimaeras to restrict CCR7 deficiency to DCs and therefore partially restore splenic architecture and have found that intrasplenic DC migration indeed requires CCR7 (REFS[26,161]). Downregulation of CCR7 on splenic DCs by parasite infection also demonstrated a loss of PALS homing[54].

Analysis of PALSs revealed that the steady state location of $CD4^+$ and $CD8^+$ T cells coincided with the post-immunization location of cDC2s and cDC1s, respectively[26]. Given that most splenic cDC2s express CD4 and cDC1s express CD8, it should be noted that this DC–T cell co-segregation places $CD4^+$ DCs with $CD4^+$ T cells and $CD8^+$ DCs with $CD8^+$ T cells. More importantly, this colocalization is consistent with the geographical DC organization in LNs and the preferential priming of CTLs by cDC1s and $CD4^+$ T cells by cDC2s in the spleen[26,86,114,155,160,161,167]. Loss of cDC2 maintenance, development or migration within the spleen impairs $CD4^+$ T cell activation and T cell-dependent antibody responses to red blood cells as well as protein antigens[26,113,159,160]. Loss of cDC1s conversely impairs CTL priming in the spleen to protein antigens, pathogens and tumours[16,26] as well as memory CTL reactivation and appropriate localization within the spleen during recall responses[168]. Localization of naive $CD8^+$ T cells to the PALSs requires CCR7, and loss of T cell CCR7 impairs their activation during systemic *Listeria* infection[169]. Forced expression of CCR7 drives naive $CD8^+$ T cells to the PALSs, favours memory cell over effector cell differentiation[169] and blocks activated $CD8^+$ T cell egress to the RP, thereby inhibiting their effector function[170]. CXCR3-deficient CTLs also mislocalize to PALSs instead of the MZ and become memory rather than effector CTLs[171,172]. Therefore, the intricate coordination of lymphocyte movement between splenic compartments helps to determine $CD8^+$ T cell fate and, ultimately, function in fighting systemic insults.

Concluding remarks

The intrinsic differences in DC subsets, the type of DCs in the affected tissue and the geography of the induced response in the SLO all create a recipe for a particular type of T cell response (BOX 4). The T cell niches described above that promote differentiation of a particular T cell fate are chemoattractant driven. However, when and how different chemoattractant receptors are upregulated on a recently activated naive T cell to form these niches and engender a nascent fate are not clear. Is it driven by the strength of TCR–peptide:MHC interaction or stochastic T cell-intrinsic properties or regulated by another signal? Are particular DC subsets hard-wired to express a restricted set of chemokine receptors, allowing for differentiation of only a particular T cell fate? Defining this early stage of T cell–DC interaction will provide insight into how adaptive immunity is tailored to deal with a particular insult.

Acknowledgements

The author thanks all the members of her lab, A. Williams, S. Cassel and many wonderful colleagues at Yale for helpful discussion over the years and critical review of this manuscript. This work was supported by R01 AI108829, CTSA UL1 TR001863 and P01HL13281901.

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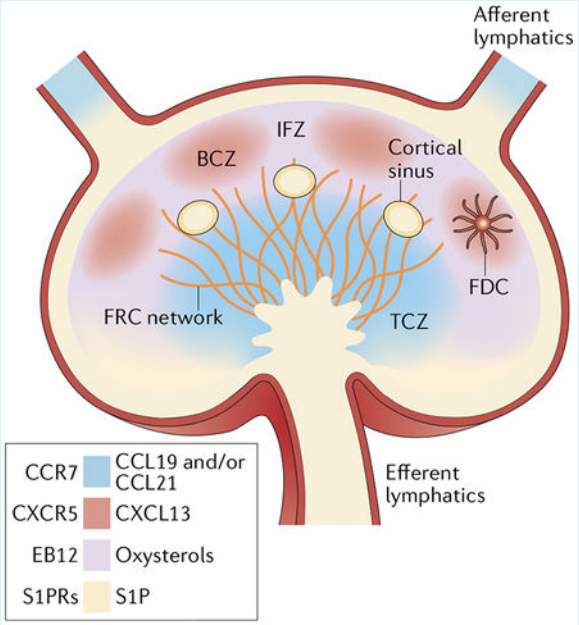
Box 1 |

Chemoattractant gradients that establish LN cellular architecture

The expression of a single chemokine receptor is insufficient to predict where in the lymph node (LN) a particular cell will be stationed. Instead, competitive, potentially opposing gradients of chemoattractants emanating from niches within the LN guide cells to precise subdomains within the LN. Depicted in the figure is a layout of a typical LN with gradients (concentration indicated by degree of shading) of select chemoattractants relevant for leukocyte positioning. Some of the relevant receptors for the depicted ligands are indicated.

Stromal cell network

This is the scaffold of an LN on which cellular organization and directional migration occurs. This includes non-haematopoietic follicular dendritic cells (FDCs) in B cell zones (BCZs) and fibroblastic reticular cells (FRCs) in the paracortex (reviewed previously[173]). FRCs ensheath the lymphatic conduit system that carries lymph from the subcapsular sinus (SCS). The FRC networks also act as ‘roads’ for leukocytes to travel to their sub-anatomic LN region. Recent work using single-cell RNA sequencing identified nine non-endothelial stromal cell niches containing transcriptionally divergent FRCs that pattern different LN regions[174]. These FRC subsets are distinct even within different regions of the T cell zone (TCZ), potentially accounting for partitioning of $CD4^+$ and $CD8^+$ T cells in the TCZ and perhaps helping form niches that favour particular T cell effector fates. T cells follow CC-chemokine ligand 21 (CCL21)-producing FRCs to populate the TCZ, while B cells initially follow FRCs but then transition to CXC-chemokine ligand 13 (CXCL13)-producing FDCs to populate BCZs[173,175]. Migratory dendritic cells (DCs) use the C-type lectin receptor C-type lectin domain-containing 2 (CLEC2) to bind to podoplanin on lymphatic endothelial cells and immigrate to LNs. Once in the SCS, they bind to podoplanin on FRC networks to migrate into the paracortex[176]. This establishes the basic cellular architecture of an LN.



T cell zone

The chemokine receptor CC-chemokine receptor 7 (CCR7) on leukocytes and its two ligands, CCL19 and CCL21, which are produced by stromal cells, organize leukocytes in secondary lymphoid organs. Immobilized gradients of CCL21 on FRCs guide DCs to the lymphatics and lymphocytes through the high endothelial venules; then, both CCL19 and CCL21 act to further guide DCs and T cells to the parafollicular region. Loss of CCR7 impairs immigration of lymphocytes and DCs[45,177] and disrupts TCZ organization[166].

B cell zone

FDCs in follicles produce the chemokine CXCL13, the ligand for CXC-chemokine receptor 5 (CXCR5). This guides $CXCR5^+$ B cells entering from HEVs in the paracortex to the follicles in the cortex. The receptor Epstein–Barr virus induced gene 2 (EBI2) on B cells also guides these cells to areas with oxysterol ligands in the outer follicular region. During germinal centre reactions, other chemokines such as CXCL12, which binds to the receptor CXCR4, help to establish zones within the follicle.

T cell–B cell border

Increased CXCR5 expression upon T cell receptor stimulation draws T follicular helper (T_{FH}) cells out of the deep TCZ, but simultaneous expression of CCR7 keeps them out of the central BCZ[85,104,116,178]. $CXCR5^+$ B cells, migrating towards the follicles, encounter antigen on DCs in the paracortex and upregulate CCR7 (REFS[120,165]). Therefore, competing gradients of CXCR5 and CCR7 ligands retain early activated T and B cells with migratory $CXCR5^+$ type 2 conventional DCs (cDC2s) in the T cell–B cell border[47]. The EBI2 oxysterol ligands are similarly expressed at the T cell–B cell border, and EBI2 is preferentially expressed by $CD4^+$ T cells (in particular, T_{FH} cells) rather than $CD8^+$ T cells. It is also expressed by cDC2s and B cells, thereby bringing the triad of developing T_{FH} cells, cDC2s and B cells together at the T cell–B cell border.

IFZ, interfollicular zone; S1P, sphingosine-1-phosphate; S1PR, sphingosine-1-phosphate receptor.

Box 2 |

Site matters: immunization routes and antigen acquisition

In some studies, the requirement for dendritic cell (DC) migration in T cell priming has been questioned because lymph node (LN)-resident DCs seem to be sufficient[31,84]. Yet, the elimination of migratory DCs impairs T cell-dependent immune responses across numerous sites in the body (recently reviewed[179]). These discrepancies might be due in part to the immunization site and methods used and, therefore, whether antigen can directly access LNs.

Studies in mice using immunizations at two sites that do not mimic vaccination in humans, the footpad and ear pinnae, often conclude that LN-resident rather than migratory DCs are required for T cell priming; the immune response also follows a more rapid tempo[31,84,102]. Depending on the size of the formulation, hydrostatic pressure can force antigen delivery to skin-draining LNs within minutes[47,57,99,180]. Even adjuvant formulations that partially work through a depot effect, if injected into the ear or footpad, allow delivery of antigen to LNs hours after administration[181]. By contrast, natural tissue infection and skin and mucosa immunization result in antigen delivery to the LN over the course of days and most often demonstrate a requirement for migratory DCs in direct presentation or antigen delivery[42,47,72,144]. Therefore, these two forms of subcutaneous immunization (auricular and footpad) differ from other subcutaneous injections or mucosal immunization. For these reasons, the use of ear removal minutes after intra-auricular injection in multiple studies is not an accurate way of gauging the relative role of migratory versus LN-resident DCs because it bypasses normal antigen trafficking and does not represent the amount of antigen delivered directly to lymphatics via other sites of immunization[180].

Delivering antigen into lymphatics indeed demonstrates that LN-resident DCs are highly functional antigen-presenting cells when sufficiently antigen-exposed, but during other forms of immunization, these pathways might play a minor role. Instead, these immunization methods might mimic a condition in which abundant antigen is available, such as infection within the LN itself. This discrepancy is important to highlight as it can explain why studies using different methods of immunization reach dramatically different conclusions about the role of migratory versus LN-resident DCs.

Box 3 |

How is dendritic cell subset organization achieved?

Dendritic cells (DCs) have been called professional antigen-presenting cells because of their potent ability to prime naive T cells. Much of this ability stems from their unique migratory pattern. Unlike most innate sentinel cells, activated DCs leave the affected area to migrate to draining lymph nodes (LNs). As naive T cells can access only secondary lymphoid organs (SLOs), they rely on this sentinel function of DCs. The migratory pattern of DCs from peripheral tissues to and within SLOs has been well studied for decades, but a coherent set of rules for how these migratory journeys impact the most important roles for DCs, T cell priming and differentiation, is only now becoming clear.

After activation, type 1 conventional DCs (cDC1s) and cDC2s in lung-draining LNs express distinct migratory transcriptional signatures[47]. Many of these subset-specific chemokine receptors and adhesion molecules correspond to the differential positioning of migratory cDC1s in the deep T cell zone (TCZ) and migratory cDC2s at the T cell–B cell border. DCs deficient in the chemokine receptor CC-chemokine receptor 7 (CCR7) or DCs with pathogen-mediated CCR7 downregulation demonstrate that DC homing to the TCZ requires CCR7 signalling[26,54,66,182]. Plt (paucity of LN T cells) mice, which have impaired production of the chemokines CC-chemokine ligand 19 (CCL19) and CCL21, have impaired but not ablated DC migration to draining LNs[183]. Because lymphatic-derived CCL21 is still produced, DCs home to LNs but become trapped in the interfollicular zone of LNs or the bridging channel of the spleen[184]. This is consistent with the TCZ of both LNs and the spleen being areas high in CCL19 and/or CCL21 (REFS[54,94]). The subcapsular area of LNs is devoid of CCR7 ligands[179], thereby creating an effective gradient. Higher expression of CCR7 on migratory cDC1s than cDC2s is consistent with the preferential localization of cDC1s in the deep TCZ[47]. The chemokine receptor XCR1 is also selectively expressed by cDC1s, and $CD8^+$ T cells secrete its ligand, lymphotactin, during early activation, thereby potentially helping to establish this niche[17,149].

Similar to T and B cells at the T cell–B cell border, the ratio of CXC-chemokine receptor 5 (CXCR5) to CCR7 expression is much higher in cDC2s than cDC1s, and, accordingly, migratory cDC2s are positioned at the T cell–B cell border after immigration into the LN[47]. Differential expression of the chemotactic receptors Epstein–Barr virus induced gene 2 (EBI2) and sphingosine-1-phosphate (S1P) receptor 1 (S1PR1) and S1PR3 has also been observed on cDC subsets. EBI2 was recently shown to guide splenic cDC2s to oxysterols at the T cell–B cell border, analogous to findings in the LN[86,87]. cDC1s can actually express enzymes that degrade oxysterols and therefore create a central T cell area relatively devoid of EBI2 ligands[87]. The reciprocal expression of S1PR1 and S1PR3 could also potentially contribute to distinct anatomic microdomain organization of cDC subsets. Both receptors recognize S1P, which regulates the egress of lymphocytes from LNs[99]. However, it is unclear why DCs express S1PRs once in LNs, as DCs do not egress[66]. Both receptors are suggested to be important for DC migration to LNs[185-187] and localization within the spleen[162,188]. Some of this work was done using S1PR blockade through the immunomodulatory drug fingolimod (FTY720), which can have

on-target, but DC-extrinsic effects on SLO organization, and therefore data generated using this approach must be interpreted carefully. However, because S1PR3 is neither desensitized as readily as S1PR1 nor downregulated by CD69 like S1PR1 (REFS[154,187]), the selective expression of S1PR3 by cDC2s could result in sustained responsiveness to S1P gradients. S1P is concentrated in cortical sinusoids in the paracortex[99]. This area largely overlaps with the T cell–B cell border[99] and the region with the highest concentration of migratory cDC2s[47].

Box 4 |

Orchestration of an immune response

On the basis of the literature discussed in this Review, the following model may explain the ultimate fate of an antigen-specific naive T cell activated in a secondary lymphoid organ (SLO). At high doses of antigen, resident dendritic cells (DCs) in the lymph node (LN) or spleen present antigen captured from the draining lymph or blood and present to naive T cells. The time frame for this interaction is approximately 6–8 hours after immunization but remains to be precisely determined. For low antigen doses, this first interaction is delayed until cellular transport of antigen reaches the SLO; it is not clear whether migratory DCs are sufficient for early stimulation or whether antigen must be transferred to another DC. Regardless, this initial exposure to cognate antigen induces proliferation, and the multiplied antigen-specific T cell population moves along fibroblastic reticular cells into different areas of the T cell zone (TCZ) and T cell–B cell border. Here, the second encounter happens approximately 18 hours after immunization, primarily with migratory DCs carrying antigen from the tissue. This second stimulation occurs within discrete niches and dictates the differentiation fate of the proliferated T cells on the basis of both the nature of the DCs and associated niche cells. Type 1 conventional DCs (cDC1s) in concert with γδ T cells, natural killer T cells, natural killer cells and other innate lymphoid cells (ILCs) activate cytotoxic T lymphocytes and T helper 1 (T_H1) cells primarily in the deep TCZ. Transcription factor krueppel-like factor 4 (KLF4)-dependent cDC2s in concert with ILCs, γδ T cells, basophils or other innate cells drive T_H2 cell responses in the interfollicular zone. By contrast, the NOTCH2-dependent cDC2 population induces T_H17 cell responses possibly in an outer region of the T cell–B cell border. Finally, cDC2s throughout the T cell–B cell border, but secluded from these other effector niches, induce T follicular helper (T_{FH}) cell differentiation. Once priming and differentiation are established, effector T cells (except T_{FH} cells) depart the LN via efferent lymphatics or the red pulp of the spleen to re-enter circulation and ultimately home to the affected tissue. There, a wide variety of cells are capable of eliciting T cell effector function though antigen presentation and co-stimulation.

Secondary lymphoid organs

(SLOs). Structures that are organized to facilitate antigen concentration, resulting in T and B cell activation or tolerance. In contrast to the primary lymphoid structures, thymus and bone marrow, SLOs include lymph nodes, spleen and mucosa-associated lymphoid tissues.

Cross-presentation

Processing of extracellular antigens for MHC class I (MHCI) for presentation to $CD8^+$ T cells; this is primarily accomplished by type 1 conventional dendritic cells.

DC activation

A process that is alternatively called dendritic cell (DC) maturation, which can cause confusion with the developmental maturation programme that also occurs in tissues after immigration from the bone marrow as pre-specified DC subset precursors. Therefore, in this Review, the term DC activation is used to summarize the variety of changes the DC undergoes after innate stimuli are detected that induce T cell stimulatory signals, antigen processing and presentation and changes in chemokine receptor expression.

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High endothelial venules

(HEVs). Unique post-capillary venules in secondary lymphoid organs, except the spleen, where lymphocytes exit the bloodstream into the lymph node paracortex.

Antigen dose

This can be thought of as the weighted average of the ratio of antigen-bearing dendritic cells (DCs) to cognate T cells, the amount of cognate peptide–MHC per DC, the duration of DC–T cell interaction and the affinity of the T cell receptor for that antigen.

Chemokines

Small basic chemotactic proteins that guide migration of motile cells by binding to G protein-coupled receptors.

Sphingosine-1-phosphate

(S1P). A lipid that acts as a chemoattractant and is most concentrated in blood and lymph; multiple S1P receptors direct dendritic cell and lymphocyte migration.

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Interfollicular zone

(IFZ). The area of the lymph node immediately beneath the subcapsular sinus (SCS) (approximately 0–30 μm from the SCS) and in between B cell follicles. The medial section of the IFZ overlaps with the outer paracortex and is part of the T cell–B cell border.

Paracortex

The region of the lymph node medial to the B cell follicles in the cortex but distal to the medulla and efferent lymphatics. This is the area where T cells are typically distributed and therefore is often referred to as the T cell zone. High endothelial venules are also present in this region. The paracortex can be divided into an inner (90 μm from the subcapsular sinus (SCS)) and outer (30 μm from the SCS) region.

Follicular dendritic cells

(FDCs). Non-haematopoietic cells in the follicle of secondary lymphoid organs that capture and present antigen and immune complexes to B cells.

Fibroblastic reticular cells

(FRCs). Non-haematopoietic stromal cells in the T cell zone of secondary lymphoid organs that produce chemokines and express surface molecules necessary for lymphocyte and dendritic cell migration.

Epstein–Barr virus induced gene 2

(EBI2). A receptor that responds to the oxysterol ligands 7α,25-hydroxycholesterol and 7α,27-hydroxycho-lesterol and regulates B cell, T cell and dendritic cell (DC) positioning within secondary lymphoid organs. Signalling through EBI2 is particularly important for localizing lymphocytes and type 2 conventional DCs to the T cell–B cell border.

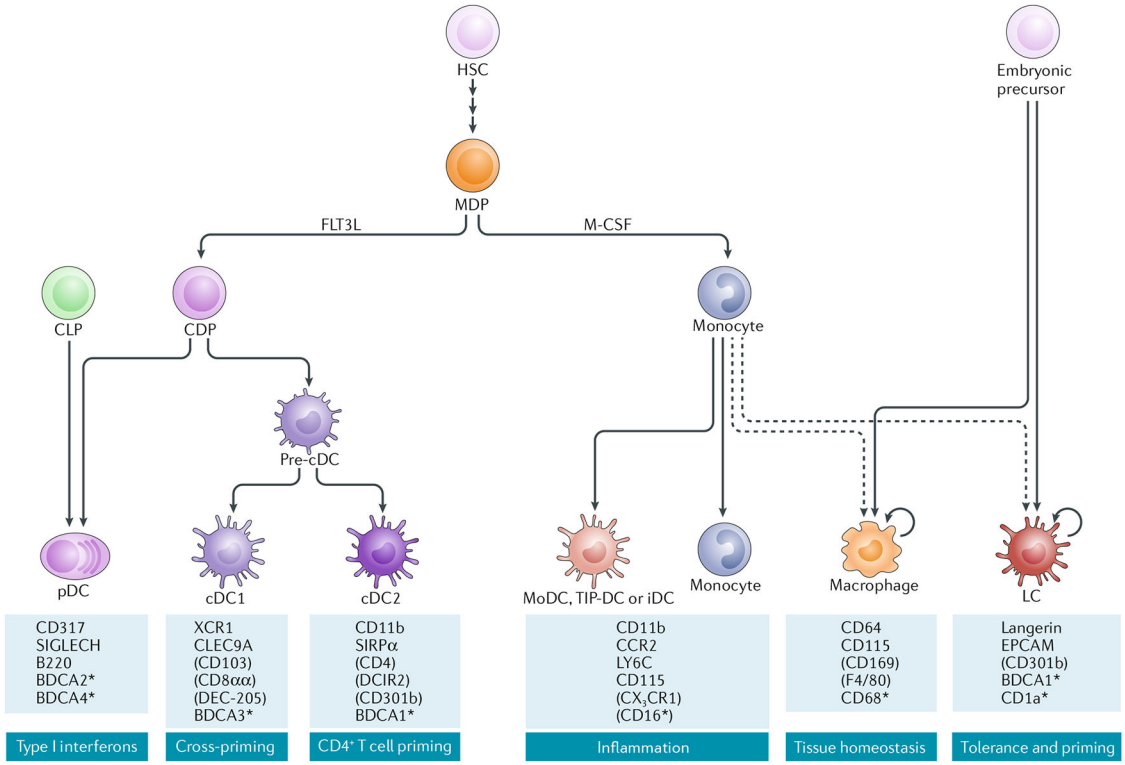

Fig. 1 |. Functionally specialized conventional and non-conventional dendritic cell subsets and related lineages.

The recent discovery of sets of particular transcription factors that define lineage ontogeny now allows for a clear distinction between monocytes and dendritic cells (DCs) as well as between different subsets of conventional DCs (cDCs) and non-cDCs[4,10]. cDCs derive from a common DC precursor (CDP), require the transcription factor FMS-like tyrosine kinase 3 ligand (FLT3L) for development and express, but do not require, the transcription factor ZBTB46 (REFS[189,190]). The genetic signature of cDCs from different tissues is similar but differs from that of plasmacytoid DCs (pDCs), monocytes and macrophages[191]. As professional antigen-presenting cells, their primary function is to prime naive T cells. The BATF3-dependent and IRF8-dependent type 1 cDC (cDC1) subset expresses the chemokine XC receptor 1 (XCR1)[12,17,23] (in both mice and humans) and includes DCs that may express different surface markers such as CD8αα, DEC-205 or CD103. Shared expression of the surface receptor Langerin by both Langerhans cells (LCs) and dermal cDC1s initially caused confusion about the relative role of LCs in T cell priming, but newer models delineate the specific function of each population[74]. The IRF4-dependent cDC2 subset can be identified by expression of surface markers CD11b, DC immunoreceptor 2 (DCIR2) (by staining with the antibody 33D1), CD301b (MGL2), CD4 or signal regulatory protein-α (SIRPα), depending on the tissue investigated[26,28,35,161,192]. This subset includes the $CD4^{+}CD11b^{+}ESAM^{+}$ DCs, which are dependent on the expression of NOTCH2 and the DNA-binding protein RBPJ, but also the double-negative ($CD4^{-}CD8^{-}$) DCs in the spleen, which are $ESAM^{low}$ but $CD11b^{+}$ (REFS[107,193]). Recent studies from other mouse tissues as well as human blood and lymph nodes (LNs) have similarly found two subsets of cDC2s[129,194-196] (this division has not been depicted). Although some pDCs share a common developmental

precursor with cDCs, they require distinct differentiation factors, express a unique pattern of surface markers and are functionally distinct from cDCs[197,198]. This includes being poor antigen-presenting cells for naive T cells (except under rare circumstances or potentially a unique subset) but robust producers of type I interferons[4,196]. One of the original DCs, LCs, has been re-classified with macrophages on the basis of ontogeny but performs many functions overlapping with cDCs[74]. Both tissue macrophages and LCs derive from embryonic precursors and self-renew in situ (circular arrow indicates self-renewal in tissues). However, under inflammatory conditions, bone marrow-derived monocytes can differentiate to help repopulate both of these cells types. Tissue-resident macrophages maintain tissue homeostasis and a variety of other functions (reviewed previously[5]). Macrophage populations also exist in LNs, but their roles are distinct from DCs; they are poor activators of naive T cells, even when presenting cognate antigen[83,147,199], but are potent at clearing apoptotic cells and promoting B cell activation[164,199]. Monocyte-derived DCs (MoDCs) include cells with a variety of functions, including TNF/iNOS-producing (TIP)-DCs as well as a difficult-to-define, heterogeneous group of cells collectively termed inflammatory DCs (iDCs)[5]. Like classical monocytes, MoDCs are dependent on the cytokine macrophage colony-stimulating factor (M-CSF), do not express the transcription factor ZBTB46 and depend on the chemokine receptor CC-chemokine receptor 2 (CCR2) for recruitment into inflammatory sites. They typically perform functions in the tissues such as antigen presentation to effector T cells, pathogen clearance and cytokine production. iDCs are induced during states of inflammation and have a mixed ontogeny and function that remain to be fully characterized[4,36,189]. Some iDCs have, however, been shown to migrate to LNs and act more like cDCs[68], but these iDCs may in fact arrive in LNs through the blood via a CCR7-independent mechanism[11,200]. Select markers for each population are shown, with those in parentheses indicating heterogeneous or tissue-restricted expression. All populations express CD11c except monocytes. Below each population, the dominant (but not sole) function of each population is indicated. BDCA, blood DC antigen; CLEC9A, C-type lectin domain-containing 9A ; CLP, common lymphoid progenitor ; CX_3CR1, CX_3C-chemokine receptor 1; EPCAM, epithelial cell adhesion molecule; HSC, haematopoietic stem cell; MDP, macrophage DC progenitor ; SIGLECH, sialic acid-binding Ig-like lectin H. *Human-specific marker.

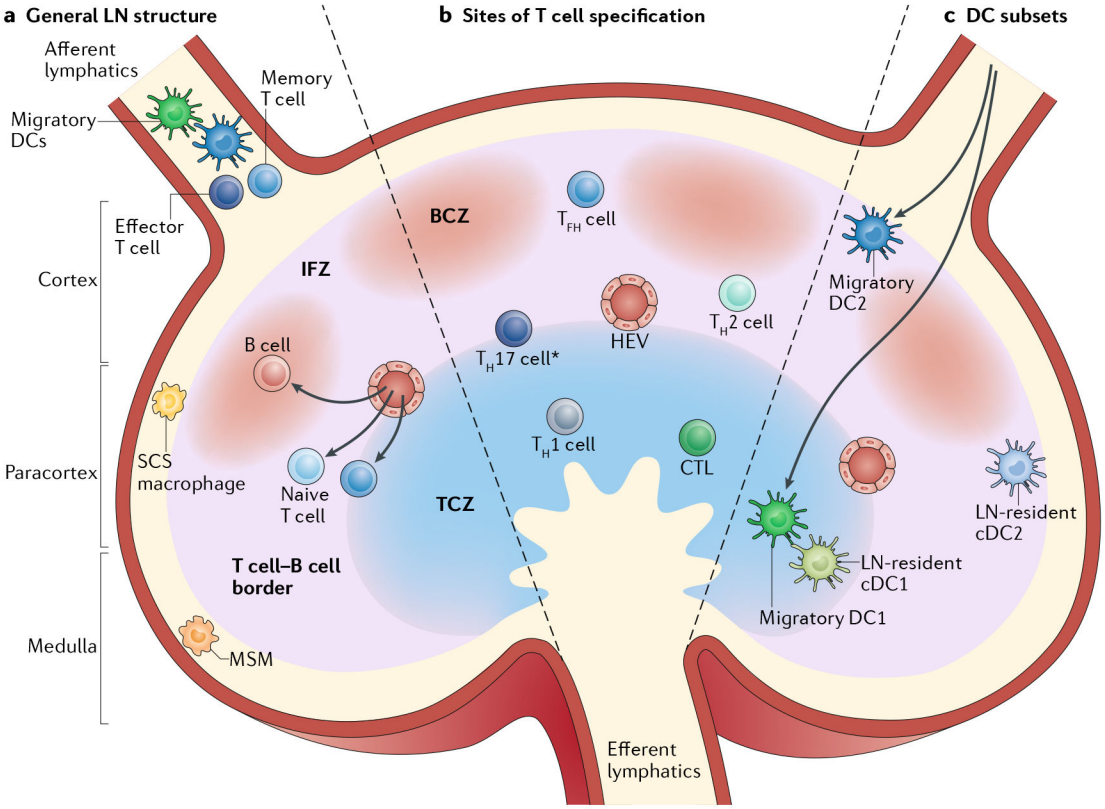

Fig. 2 | Immunologic microanatomy of an LN.

Shown is a lymph node (LN) with entry sites of naive, effector and memory T cells and migratory dendritic cells (DCs). **a** | The basic cellular structure of the LN divides it into T cell zones (TCZs) and B cell zones (BCZs). BCZs are also called follicles and may contain germinal centres depending on activation state. The basic anatomical structure of the LN divides it into the cortex (nearest the capsule and afferent lymphatics), paracortex (in between) and medulla (nearest the exiting efferent lymphatics). The area between the TCZ and BCZ, which contains fibroblastic reticular cell networks and many high endothelial venules (HEVs), has been called the cortical ridge[59]. The cortex is commonly thought of as the BCZ, whereas the paracortex is considered the TCZ; however, this over-simplifies the more intricate structure of the immune cellular compartments. The area between the B cell follicles through which immigrating cells from the lymph pass is called the interfollicular zone (IFZ) and can be considered part of the larger T cell–B cell border. No unique anatomical structure or cell is typically used to identify the IFZ, making a clear demarcation of this region difficult in some LNs. A cellular continuum exists between the outer paracortex and the IFZ and B cell regions, thereby forming a large and readily identifiable T cell–B cell border[47]. The border can be distinguished from the follicles and deep TCZ by the overlap in T and B cell staining. Only spare and scattered T cells can be identified in the follicles, and few B cells can be identified in the TCZ. **b** | Regions of effector T cell differentiation in an uninfected LN after immunization are depicted. The regions where $CD4^+$ T helper 1 (T_H1), T_H2 and T_H17 cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) accumulate are shown. Depending on the nature of the immunogen, T_H1 cell induction has

also been observed in the IFZ. **c** | The geographical organization of migratory and LN-resident conventional DC (cDC) subsets, including type 1 cDCs (cDC1s) and type 2 cDCs (cDC2s), in LNs after immunization is depicted. At high doses of antigen (LN infection, footpad or auricular injection or high concentration of soluble antigen), all DC subsets have access to antigen. By contrast, at low antigen dose (subcutaneous or mucosal immunization or infection), primarily migratory DCs would deliver antigen to LNs; this antigen might then be shared with LN-resident DCs. MSM, medullary sinus macrophage; SCS, subcapsular sinus; T_{FH} cell, T follicular helper cell. *The sub-anatomic LN region for T_H17 cell differentiation is based on indirect evidence.

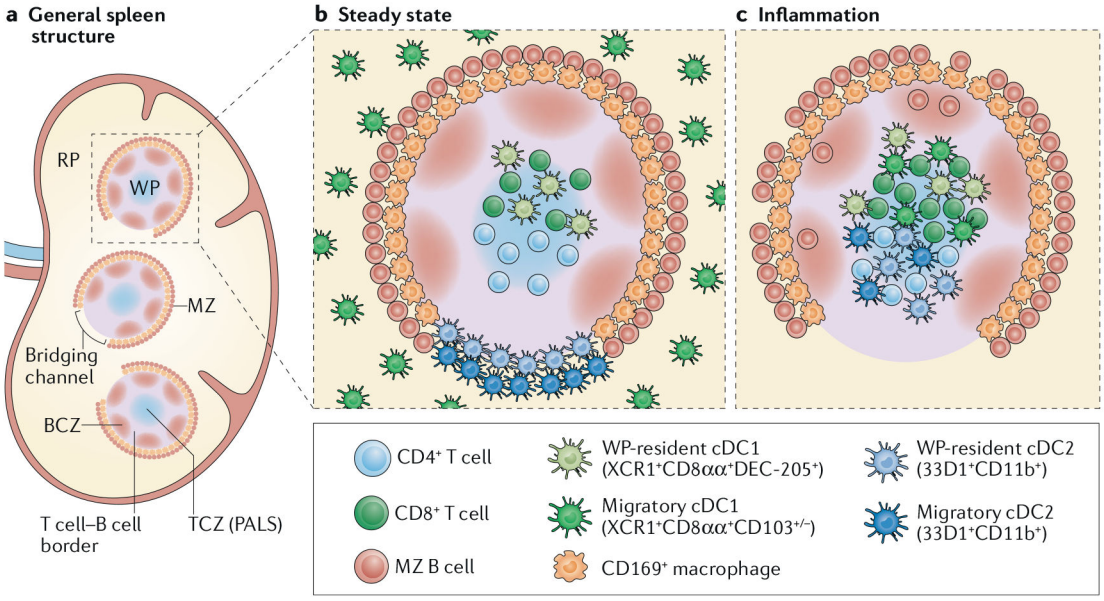

Fig. 3 |. Immunologic microanatomy of the spleen.

The spleen is divided by function and structure into red pulp (RP) and white pulp (WP); between these two regions is the marginal zone (MZ) (in mice), which is functionally equivalent to the less-well-characterized perifollicular zone in humans (not shown)[201,202]. These regions correspond to the subcapsular sinus of the lymph node (LN); in both LN and spleen, these are the regions of antigen delivery from the periphery and antigen sampling by innate immune cells such as macrophages. The RP contains multiple innate cell populations that help clear aged red cells and debris from the circulation as well as fight systemic infections but also houses particular effector lymphocyte populations. The MZ contains a conduit between the RP and WP called the bridging channel, which is thought to be a site of activated lymphocyte exit from the WP and an area where (in mice) MZ B cells produce T cell-independent antibodies[1,203,204]. The bridging channel lacks $CD169^+$ marginal zone metallophilic macrophages, which helps to define the border between the WP and RP and is the site where type 2 conventional dendritic cells (cDC2s) reside at steady state. Most naive lymphocytes are located in the WP and reside in distinct zones analogous to those found in LNs. Similar to the fibroblastic reticular cell and follicular dendritic cell structural framework in the LN, stromal cells establish the cellular organization of the WP[173,205]. B cells reside in WP follicles just beneath the MZ, surrounding a central T cell zone (TCZ) (also called the periarteriolar lymphoid sheath (PALS)). Similar to LNs, the WP is where adaptive immune responses are generated to blood-borne antigens. Part **a** shows the general spleen structure. Part **b** shows the geographical organization of dendritic cell (DC) and T cell subsets at steady state. cDC1 and cDC2 subsets are depicted in green and blue, respectively. WP-resident and migratory cDC2s are proposed but cannot currently be distinguished (whether the markers CD4 and ESAM are differentially expressed on the basis of location remains to be determined). Part **c** shows the geographical organization of DC and T cell subsets during inflammation. BCZ, B cell zone; XCR1; chemokine XC receptor 1.