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Dendritic cells steering antigen and leukocyte traffic in lymph nodes

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Dendritic cells (DCs) play a central role in initiating and shaping the adaptive immune response, thanks to their ability to uptake antigens and present them to T cells. Once in the lymph node (LN), DCs can spread the antigen to other DCs, expanding the pool of cells capable of activating specific T-cell clones. Additionally, DCs can modulate the dynamics of other immune cells, by increasing naïve T-cell dwell time, thereby facilitating the scanning for cognate antigens, and by selectively recruiting other leukocytes. Here we discuss the role of DCs in orchestrating antigen and leukocyte trafficking within the LN, together with the implications of this trafficking on T-cell activation and commitment to effector function.

Keywords: antigen presentation; antigen trafficking; Dendritic cells; leukocyte trafficking

Dendritic cells: an overview

Dendritic cells (DCs) play a key role in the immune system, exhibiting unique functions in sensing, capturing, and presenting antigens to other immune cells. These cells are thus fundamental in initiating and regulating immune responses against pathogens, tumors, and foreign entities [1,2]. Positioned strategically throughout the periphery, DCs act as vigilant sentinels in tissues, detecting invading pathogens. Moreover, they reside within lymphoid organs, establishing a network that facilitates antigen presentation to T cells. Given their brief lifespan, they undergo continuous replenishment to keep an effective immune surveillance [3].

Dendritic cells form a highly heterogeneous population distinguished by specific surface markers, functional attributes, and ontogeny. The current classification of DCs based on ontogeny identifies conventional dendritic cells (cDCs), monocyte-derived dendritic cells (MoDCs), plasmacytoid dendritic cells (pDCs), and the recently described dendritic cells type 3 (DC3) [4–6].

cDCs are characterized by the expression of the transcription factor *Zbtb46* and arise from the bone marrow from the conventional DC progenitor (CDP) [7], formerly known as the 'common DC progenitor', since it was initially believed to be the precursor of plasmacytoid DCs as well [8]. These cells then differentiate into pre-dendritic cells (pre-DCs), which exit the bone marrow to seed the periphery and are characterized by the expression of the CD11c marker [9]. Subsequently, pre-DCs undergo further differentiation into two distinct subtypes: cDC type 1 (cDC1) and cDC type 2

Abbreviations

BMDC, bone marrow-derived dendritic cell; cDC, conventional dendritic cell; CFSE, carboxyfluorescein succinimidyl ester; DC, dendritic cell; DC3, dendritic cell type 3; FDC, follicular dendritic cell; FRC, fibroblastic reticular cell; GPCR, G protein-coupled receptor; HEV, high endothelium venules; IFN-I, type I interferon; IFR, interfollicular region; LN, lymph node; MHC, major histocompatibility complex; migDC, migratory dendritic cell; MoDC, monocyte-derived dendritic cell; NK, natural killer; pDC, plasmacytoid dendritic cell; PRR, pathogen recognition receptors; resDC, resident dendritic cell; SCS, subcapsular sinus; TCR, T-cell receptor.

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(cDC2). cDC1s are identified by the expression of XCR1 and the transcription factors BATF3 and IRF8 [10,11]. In contrast, cDC2s represent a more abundant and heterogeneous population, distinguished by the surface markers SIRPα and high levels of CD11b, and express various transcription factors including IRF4, KLF4, ESAM, RORγT, and T-bet [12–14].

In line with their distinct classification, these two cDC subtypes exhibit functional differences in antigen presentation and T-cell activation. cDC1s excel in cross-presenting antigens, showing a remarkable ability to stimulate the $CD8^+$ T-cell response [15,16]; moreover, the XCR1 marker facilitates the clustering of cDC1 with CTLs, which secrete its ligand: XCL1. [17]. These characteristics make cDC1s the privileged initiators of cellular immune responses. On the other hand, cDC2s play a major role in the context of $CD4^+$ T-cell activation and humoral response, thanks to their efficiency in MHC-II antigen processing pathway [18], their privileged role in T_{FH} induction [19], and their anatomical localization (discussed below). However, it is important to mention that this distinction is more plastic than initially proposed. For example, it was shown that interaction with $CD4^+$ T cells is fundamental for cDC1 to efficiently prime the $CD8^+$ mediated cytotoxic response [20,21]. Additionally, in defined inflammatory conditions, cDC2 can develop the ability to present the antigen directly to $CD8^+$ T cells [22,23].

MoDCs are a hybrid population originating from circulating monocytes under inflammatory conditions [24,25]. MoDCs are known to be highly proficient in antigen transport, although their capacity in antigen processing and antigen presentation is subject of debate [26–28]. This is in part because the correct identification of these cells has been particularly challenging due to their shared markers with cDCs and monocytes/macrophages. Recently, some works identified two new markers, CD26 and CD88, which could help discriminating MoDCs from cDCs, facilitating a more accurate detection and study of this population [29,30].

pDCs are a specialized cell subset known for their ability to produce large amounts of type I interferons (IFN-I) in response to viral infections; on the other hand, their role in antigen presentation is not well-defined [31]. Beyond that, it was shown that they collaborate in the cytotoxic-mediated response optimizing cDC1 maturation and cross-presentation [32,33].

In this *In a Nutshell* Review, we will discuss the role of dendritic cells in directing antigen and leukocyte trafficking within the lymph node. We will focus our attention on mouse cDCs, since studies carried out on these processes have been mostly conducted on this DC type in murine models.

cDC localization in the lymph node

Lymph nodes (LNs) are small bean-shaped lymphoid organs distributed throughout the body; they have the fundamental role of concentrating at the same site antigens from the periphery and different types of immune cells, serving as a privileged platform for antigen presentation and the initiation of the adaptive response. LNs are encapsulated organs with a hilum, entry site of the vascularization of the organ, and origin of the efferent lymphatic vessel. The lymph reaches the organ directly through the capsule, entering the lymphatic sinus that develops underneath: the subcapsular sinus (SCS) [34]. This region is patrolled by subcapsular sinus macrophages, leukocytes specialized in the capture of antigens carried in a soluble form by the lymph [35]. From the capsule, several trabeculae arise and deepen within the LN, surrounded by trabecular sinuses. The entire architecture of the LN is supported by a network of Fibroblastic Reticular Cells (FRCs), specialized fibroblasts that form conduits facilitating the transport of the lymph deep into the lymph node parenchyma.

The LN parenchyma can be divided into three distinct regions: the cortex, the paracortex, and the medulla. The cortex (or B-cell zone) is the outermost region of the LN parenchyma, in which B cells are organized in follicles and are in close contact with Follicular Dendritic Cells (FDCs). These cells, despite their name, have a mesenchymal origin and do not have evident phagocytic capacity; nevertheless, they are particularly proficient in capturing and displaying on their surface immune complexes [36]. The paracortex (or T-cell zone) is mainly composed of T cells occupying an area underneath the cortex; additionally, T cells surround the follicles creating zones called interfollicular regions (IFRs). In the paracortex, blood vessels from the hilum give rise to specialized structures called High Endothelium Venules (HEVs). These vessels, thanks to their conformation and surface expression of adhesion molecules, facilitate leukocyte extravasation from the bloodstream to the LN parenchyma. Finally, the medulla is the closest area to the hilum and contains the branching vessels from arteries and veins that irrigate the LN. These vessels along all their path are surrounded by different sheaths of pericytic FRCs and recirculating lymphocytes that together form structures named cords [37,38].

Activated dendritic cells mostly exert their function in the paracortex, IFRs, and at the T-B border, i.e. the interface between T-cell and B-cell zone. Upon encounter with activation stimuli, cDCs start to express CCR7, a G protein-coupled receptor (GPCR)

that binds CCL19 and CCL21, cytokines secreted by LN stromal cells, creating a chemokine gradient from the periphery to the lymphoid organ. cDC1s and cDC2s can localize in distinct areas of the LN [39]. cDC1s, which express higher levels of CCR7, continue to follow the gradient of CCL21, secreted by the FRCs. This promotes their migration toward the T-cell zone, going through the same migration path followed by the T cells themselves [19,39,40]. cDC2s, instead, probably due to their heterogeneity, can follow different routes. In specific contexts, they upregulate CXCR5 that binds the chemokine CXCL13 secreted by the FDCs. This allows their migration toward the T-B border and the IFRs (Fig. 1A). cDC2 localization at those sites is crucial for Th2 induction upon *Heligmosomoides polygyrus* infection and for T_{FH} differentiation in response to an inhaled antigen [19,41]. Additionally, in an allergy model, it has been shown that penetration of some skin-derived cDC2 ($CD301b^+$, described below in more detail) in the LN parenchyma relies on CCR8-mediated sensing of CCL8, a chemokine secreted by interfollicular macrophages [42]. Also in this case, cDC2 localization was crucial for Th2 response. In addition to LN positioning, exposure to specific cytokines in a time-dependent manner strongly influences DC function and T-cell activation. For instance, it was recently observed that temporal differences in IFN-I sensing are crucial in shifting the response toward either Th1 or T_{FH} fates [43,44].

In the spleen and mucosal-associated lymphoid organs, DC positioning has been shown to rely on different pathways, as the sensing of 7α,25-hydoxycholesterol [45], CCL9 [46], 5-hydroxyindoleacetic acid [47], and MIP-3α [48]. Interestingly, perturbations of these pathways deeply influence the immune response elicited, supporting the notion that anatomical and microanatomical DC positioning is an important variable in immune regulation in secondary lymphoid organs.

Migratory DCs contribute to lymph node functional heterogeneity

Even though LNs share a common spatial organization and overall cellular composition, distinct LNs can steer the immune response toward different outcomes. Functional heterogeneity between LNs (comprehensively reviewed in [49]) depends on several factors, including lymph composition as well as identity and state of migratory DCs reaching the LN from the periphery. In a seminal paper from 1996, Everson *et al.* [50] first observed that DCs display different stimulatory capacities toward $CD4^+$ T cells depending on the tissue of origin. Precisely, coculture experiments with splenic or Peyer's patches-derived DCs and T cells demonstrated that the origin of DCs played a critical role in driving the production of Th1 cytokines (IL-2, IFN-gamma) or what at the time was considered a Th2-related cytokines (IL-6). This article paved the way for the study of how the tissue of origin of DCs influences the immune environment among different lymphoid structures and even between LNs draining the same district. In this sense, work of Esterházy *et al.* [51] showed that gut-draining LNs, despite their anatomical contiguity and continuity, almost exclusively drain a specific tract of the intestine, with no or small lymph leaking between them. These LNs present different DC subpopulations which confer them diverse characteristics, with the duodenal, jejunal, and ileal LNs expressing a more tolerogenic environment compared to the inflammatory-prone colonic LN. In a specular way, Brown *et al.* [52] showed that DCs migrating from different organs of the gastrointestinal tract to the same LNs, maintain a signature that reflects the tissue of origin. Thus, the crosstalk between DCs of different origins contributes to define the delicate balance between immunity and tolerance typical of the gut. More recently, in a setting of skin allergy model, it was shown that the same antigen-adjuvant combination administered at different sites determines different clustering of DCs in the LN which led to a different magnitude of Th2 response. Interestingly however, this difference was not appreciated in a context of Th1 immunization, relating this phenomenon to the different expression profile of costimulatory molecules between specific skin-derived DCs in the two compartments [53]. In conclusion, the environment in the periphery can lead to distinct functional adaptations and localization patterns of DCs which, in turn, influence LN immune function.

Role of migratory and resident dendritic cells in antigen presentation

Hitherto, we described all dendritic cells as cells that, after activation in the periphery, acquire the capacity to migrate in the LN. However, both cDC1 and cDC2 can also directly localize in the LN before the encounter of the antigen. For this reason, besides the previous classification in cDC1 and cDC2, we can also distinguish resident and migratory dendritic cells based on their relative localization to the LN before activation. Migratory dendritic cells (migDCs) are the prototype of the DCs described above: they patrol peripheral non-lymphoid tissues and upon antigen encounter move to the draining LN. Resident Dendritic Cells

(resDCs) instead, directly seed the LN before activation. They reach the lymphoid organ through the bloodstream and extravasate across the medullary cords and the medullary HEVs, as described by a recent paper by Ugur *et al.* [54] (Fig. 1A).

In a steady-state condition, resDCs and migDCs can be distinguished by flow cytometry, thanks to their differential expression of the CD11c and the MHC-II markers. resDCs show high levels of CD11c (CD11c^{hi}) and intermediate expression of MHC-II (MHC-II^{int}), while migDCs, since they are already activated when they reach the LN, are MHC-II^{hi} and CD11c^{int}. This distinction, however, is not entirely clear-cut, since once a resDC undergoes activation, it also upregulates MHC-II, making its discrimination from a migDC more complex. To better discriminate between migDCs and resDCs, both chemical and genetic approaches have been employed. It was shown that carboxyfluorescein

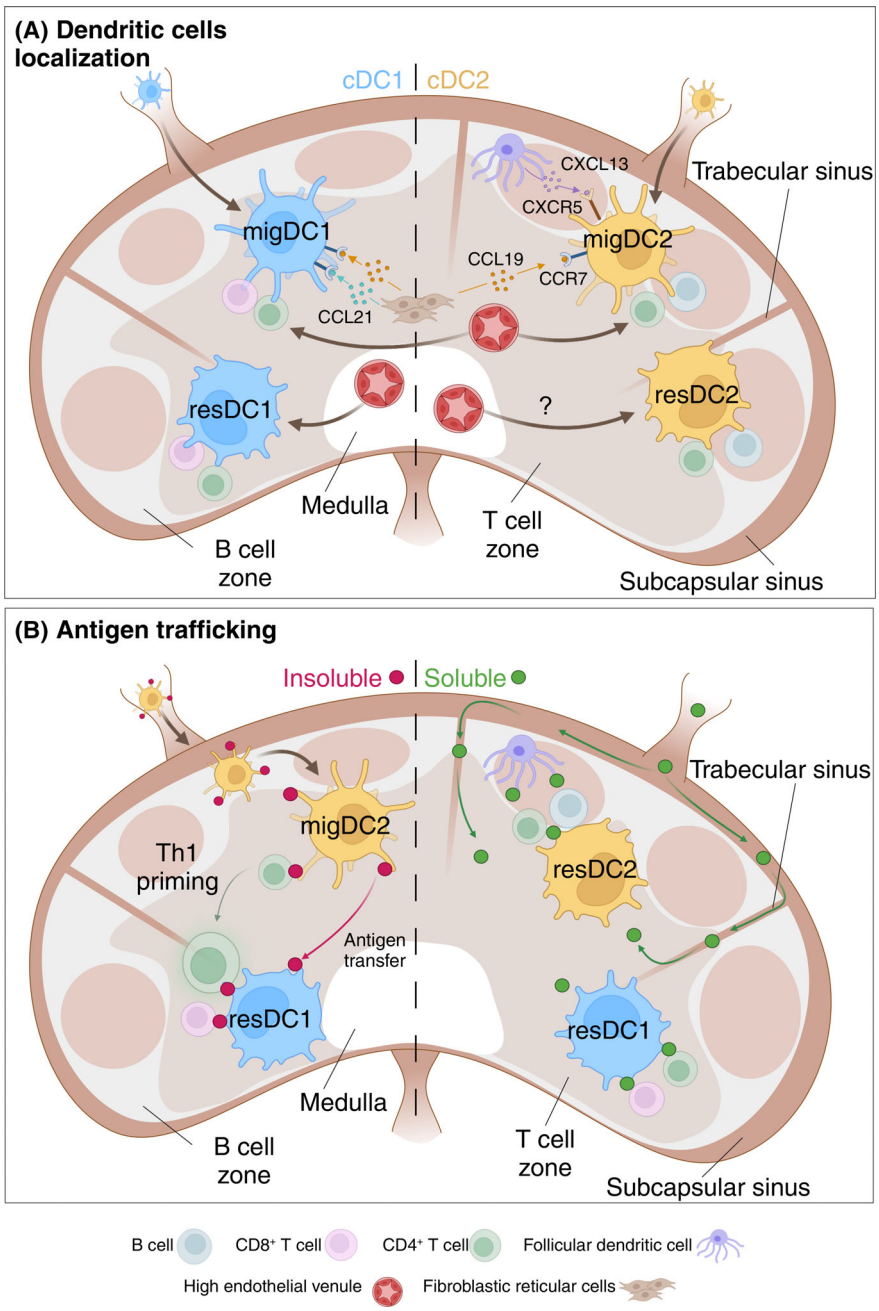

Fig. 1. Dendritic cell and antigen trafficking within lymph node. (A) Dendritic cell localization in the lymph node (LN). Upon activation, migratory dendritic cells (migDCs) upregulate CCR7 expression and migrate toward the LN, guided by the CCL19/21 chemokines secreted by LN stromal cells. Within the LN, conventional dendritic cells type 1 (cDC1s) primarily localize to the T-cell zone, thanks to their high expression levels of CCR7. In contrast, conventional dendritic cells type 2 (cDC2s) can express CXCR5 and localize to the T-B-cell border and interfollicular regions (IFRs), responding to the combined gradients of CXCL13 secreted by follicular dendritic cells (FDCs) and CCL19 secreted by fibroblastic reticular cells (FRCs). Resident dendritic cells type 1 (resDC1s) seed the LN during steady-state conditions via the medullary high endothelial venules (HEVs), following the bloodstream. Upon maturation, they migrate to the T-cell zone in a CCR7-dependent manner. Similarly, resDC2s originate from the medullary HEVs, although their precise migration paths and development require further elucidation (the hypothesized path is represented in the figure). (B) Antigen trafficking within the LN. In the context of a non-lymph-born antigen, migDCs directly transport the antigen to the LN and then present it to the naive T cells. In this scenario, migDCs can transfer the antigen to resDCs that, in turn, reinforce the immune response. In this figure, a migDC2 transfers the antigen to a resDC1, which in turn cross-presents it to a naive $CD8^+$ T cell supported by a previously activated $CD4^+$ T cell. Soluble antigens are transported to the LN by the lymphatic vessels and disseminate throughout the organ via the subcapsular sinus (SCS). These antigens are subsequently captured by resident dendritic cells (resDCs), which then preferentially present them to $CD4^+$ (resDC2) and $CD8^+$ T cells (resDC1).

succinimidyl ester (CFSE), thanks to its fast cell penetration and reactivity to cytosolic and cell membrane amines, is a suitable solution to label DCs in the periphery, thus allowing to distinguish them once in the LN [55,56]. Alternatively, mouse models expressing photoconvertible reporters were exploited to precisely discriminate the two populations [57,58]. In this approach, peripheral tissues are beamed with a laser, inducing a photoconversion so that migDCs, once in the lymph node, can easily be recognized from their resident counterpart.

From a functional perspective, resDCs are specialized in uptaking and presenting soluble small antigens (< 70 KDa, < 200 nm) carried by the lymph stream, while migDCs directly acquire the antigen in the periphery bringing it to the LN [59–62]. This suggests that, depending on their physical characteristics, antigens not only follow different paths to reach the LN (cell-mediated or cell-independent transport) but also that distinct cell types intervene in their presentation, giving different contributions in the T-cell priming (Fig. 1B). In a seminal article Itano *et al.* [63] showed that subcutaneously administered soluble antigen quickly reaches the lymph node through the lymph stream even 30 min after injection and that 4 h later the antigen is presented by resDCs. migDCs, instead, upon antigen capture, need 18 h to reach the draining LN and subsequently interact with T cells [63,64]. In the same work, it was observed that resDCs are sufficient to activate $CD4^+$ naïve T cells, which start expressing CD69 and secreting IL-2 already 6 h after antigen administration. On the other hand, the contribution of migDCs is fundamental for the generation of a long-lasting immune response, acting as a reinforcement for T-cell priming and survival. This indicates that resDCs and migDCs are not working independently from each other. Still, their contribution and coordination in the T-cell licensing are necessary to mount an efficient immune response.

Of note, resDCs and migDCs do not only show differences in their CD11c and MHC-II expression but other markers can be used to discriminate their status depending on their belonging to the cDC1 or the cDC2 subtypes. resDC1 expresses univocally the CD8αα homodimer, while its migratory counterpart is characterized by the expression of the Integrin α E (CD103). For the cDC2 population instead, given its higher heterogeneity, the discrimination between migratory and resident DCs is more complicated. It was shown in mice, that most of the skin-draining DCs are $CD301b^+$ [65] but a common marker of migratory or resident cDC2 is still lacking. Based on these markers, Sokol *et al.* [42] showed that subcutaneous Th2 immunization promotes specific migration of the $CD301b^+$ DCs, while the Th1 immunization induces a more $CD103^+$ DCs skewed trafficking. This finding can be partially explained by the different expression profiles of Pathogen Recognition Receptors (PRRs) between cDC1 and cDC2 [13,66,67], which can lead to different likelihood of activation (and migration) depending on the administered adjuvant. In conclusion, these articles report that the type of DCs that will bring the antigen is dependent on the contextual information of the periphery. Nevertheless, it must be taken into account that even if different types of immune responses are elicited (Th1, Th2, Th17), the majority of DCs that migrate will still belong to the cDC2 type [42,68]. This could lead to the hypothesis that even very small variations in the relative number of migDC subtypes can contribute to substantial differences in the type of immune response.

Increasing the chances: antigen spreading

Another fundamental feature of DCs is their capability to share the antigen with other DCs, other than process and present it to lymphocytes through MHC. In

groundbreaking work, Allan *et al.* [69] observed that, in a skin infection model, resDC1s have a privileged role in activating the CTL response, but at the same time the blockade of the migration of skin migDCs drastically reduced CTL activation and expansion. This corroborated the hypothesis that migDCs can also transfer the antigen to the resDCs, which, in turn, could present them to T cells. In a more recent study, Gurevich *et al.* [70] pulsed bone marrow-derived DCs with ovalbumin and mixed them with unpulsed bone marrow-derived DCs at a ratio of 1 : 200. After several hours, these DCs were cocultured with ovalbumin-specific $CD8^+$ T cells and it was observed that these cells clustered with both the pulsed and unpulsed DCs. Conversely, no clustering was seen with MHC-I lacking DCs, suggesting that the antigen was transferred from the original rare antigen-donor DC to be presented on recipient DCs. In the same work, exploiting an elegant *in vivo* setting of replication-defective virus infection, it was also observed that antigen transfer occurred within the lymph node in an LFA-1-dependent manner delegating resDCs to present the viral antigen in place of the viral downregulated-MHC-I migDCs [70]. migDCs might then need to reach the LN to transfer the antigen to their resident counterpart in order to propagate the immune activation. Today, this accepted model is known as antigen spreading or antigen dissemination, and it represents a strategy to expand the number of antigen-bearing dendritic cells, therefore increasing the possibility of an encounter between the antigen and the cognate T-cell clone [71].

Several mechanisms were theorized and studied to explain how the antigen spreading occurs: (a) antigen transfer by exosomes [72], (b) MHC-II cross-dressing [73], (c) direct soluble antigen transfer [74], and (d) antigen uptake from apoptotic DCs that reached the LN [75]. More recent findings, however, are underlying the role of a fifth path, consisting of direct antigen transfer during a DC–DC immunological synapse. A recent work, exploiting a ZsGreen expressing tumor, showed that tumor antigens are transferred from migDCs to resDCs in the context of an immunological synapse formation without free exosomes transfer [76]. In a similar model of lung infection with 'BrightFlu' (Influenza A Virus genetically tagged with ZsGreen), it was also shown that migDCs not only transfer the antigen to the resDCs but also the contextual information (e.g. PAMPs/DAMPs) found at the infection site [77]. This means that antigen spreading not only allows resDCs to present peptides of the same antigen that was collected in the periphery but also that the immune response elicited is coherent with the context of immunization that was found by migDCs in the peripheral tissue (Fig. 1B).

Dendritic cells dictate leukocyte trafficking in the lymph node

Immune cells communicate with each other to mount the most appropriate response toward a given stimulus. DCs are not an exception since they can influence the effector T-cell response. This modulation could be accomplished in at least two different ways: directly, through signals exchanged in the context of DC-T-cell interactions, and indirectly, through signals arising from other leucocytes recruited by DCs at the priming site. Increasing evidences indicate that DCs can drive the dynamics of cells belonging to both innate and adaptive immunity coherently with the peripheral perturbation, with possible consequences on the induction of T-cell effector functions. Here, we summarize the most important findings on the role of DCs in recruiting and regulating specific leukocyte migration in the LN compartment, listing the type of immune cells recruited.

NK cell

It is established that NK cells play a fundamental role in supporting the Th1 response, thanks to the secretion of IFN-γ [78,79]. NK cells reach the LN through HEVs and can make stable contacts with DCs localizing at the T-B border and in the medulla [80–82]. In *Leishmania major* infection, it was shown that NK cells are recruited from the bloodstream and localize in the T-cell zone, starting to secrete IFN-γ [81]. In 2004, Martín-Fontecha *et al.* [83], showed that in immunization setups with certain Th1-skewing adjuvants (R848, Ribi, and LPS, but not CpG and CFA) migDCs reach the dLN and recruit NK cells in a CXCR3-dependent manner. Here, stimulated by DCs-secreted IL-12, NK cells start to produce IFN-γ (Fig. 2A). In addition to this, more recently it was shown that in Th1 immunization settings, activated $CD4^+$ T cells upregulate CXCR3 which allow them to reach CCL10 and CCL9-secreting DCs localized more across the outer LN [84]. Together with previous observation, this finding suggests that this chemokine gradient determines the co-clustering of DCs, $CXCR3^+$ T cells, and NK cells at the lymph node border to reinforce the naïve T-cell differentiation toward the Th1 fate.

Basophils

Despite the role of DCs in driving the T-cell fate decision, there is no clear evidence supporting their ability to secrete IL-4, the most potent cytokine for

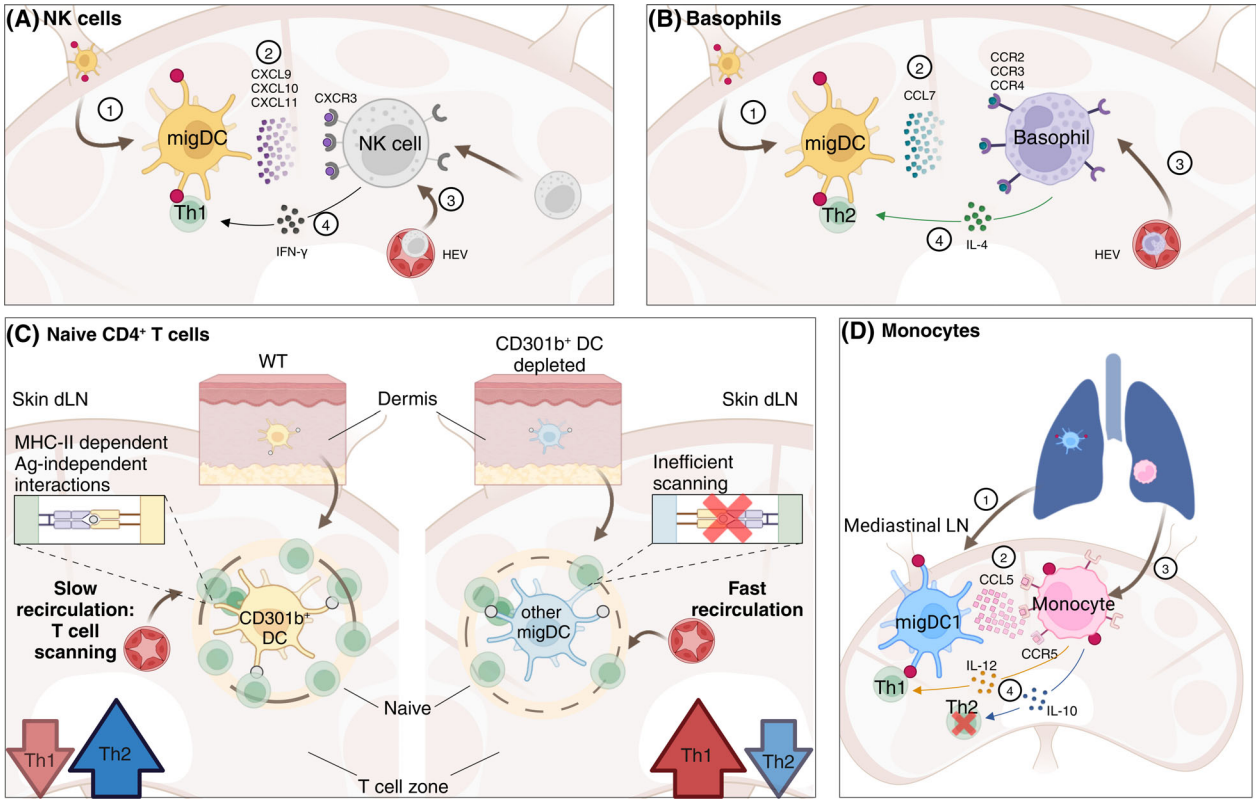

Fig. 2. Dendritic cells orchestrate leukocyte trafficking in the lymph node to elicit context-appropriate immune responses. (A) Upon stimulation with a Th1 immunogen, migratory dendritic cells (migDCs) migrate to the lymph node (LN) and recruit natural killer (NK) cells from both the LN and the bloodstream in a CXCR3-dependent manner. NK cells subsequently produce IFN-γ, promoting Th1 differentiation. (B) In an allergic context, migDCs migrate to the LN and secrete the basophil chemoattractant CCL7. Once basophils reach the LN, they secrete IL-4, reinforcing the Th2 immune response. (C) Following subcutaneous administration of an antigen, CD301b$^+$ migDC2s reach the draining LN (dLN) and localize near high endothelial venules (HEVs), forming close MHC-II-dependent antigen-independent interactions with circulating naive T cells and prolonging their retention within the LN. Moreover, CD301b$^+$ DCs skew the immune response toward the Th2 fate, dampening the Th1 response. In the absence of this DC type, T cells exhibit reduced dwell time and faster recirculation together with a more Th1-oriented response, probably due to the reduced number of migDCs and the reduced capability of other DCs to perform the same TCR scanning. (D) After intranasal administration of an immunogen, migDC1s migrate to the mediastinal LN (mLN), where they induce monocyte chemotaxis through the CCL5-CCR5 axis. Monocytes in turn contribute to Th1 fate determination through two proposed mechanisms: IL-12 secretion to promote Th1 differentiation and IL-10 production to suppress the Th2 response.

promoting Th2 differentiation [85,86]. For this reason, it was speculated that other cells, such as basophils, could secrete IL-4 in the context of the LN. In 2010, Tang *et al.* [87], observed that migDCs stimulated with papain, once in the lymph node secrete CCL7, a strong basophil chemoattractant, tightly regulated by ROS, TLR4 and TRIF signaling pathways (all present in an allergy setting). For this reason, it was hypothesized that recruited basophils could provide the lacking IL-4 to drive the immune response toward the Th2 fate (Fig. 2B). Nevertheless, in more recent works, even if the capacity of DCs to recruit basophils was not disproved, the role of these granulocytes in supporting Th2 response has been strongly resized [88,89]. It is important to mention, however, that these different conclusions were reached based on different experimental designs and considering different sites of immunization: a skin immunization for the basophil-dependent reaction and lung exposure with house dust mites for the basophil-independent Th2 differentiation.

T helper cells

As discussed above, the encounter of T cells with the cognate antigen presented by DCs is a rare event. In a recently published article, Tatsumi *et al.* [90] showed that a subset of skin migDCs are capable of regulating T-cell dynamics in the LN, increasing their chance to recognize the cognate peptide. In particular, they observed that once reached the LN, CD301b$^+$ DCs

localize around the HEVs in an S1PR-dependent mechanism, engaging multiple interactions with extravasating naïve T cells in an MHC-II-dependent, Ag-independent mechanism. This scanning strategy prolongs the $CD4^+$ T-cell dwell time in the lymphoid organ, increasing the likelihood of an interaction with the cognate T-cell clone. Moreover, they observed that this specific migDC2 population seems to be required both during the first immunization and upon re-challenge in a physiological context of rare antigen-specific T cells. Lastly, it was also reported that $CD301b^+$ DCs–naïve $CD4^+$ T interactions at early time point (before 24 h) are critical in driving T-cell differentiation toward the Th2 path while suppressing the Th1 and Th17, confirming other findings that highlight the role of $CD301b^+$ DCs in the Th2 differentiation [91] (Fig. 2C).

Monocytes

DCs also possess the ability to influence the migration of monocytes within the inflammatory context, as shown by Rawat *et al.* [30]. In their study, migDCs, once in the lymph node, secrete CCL5. This chemokine interacts with the GPCR CCR5 expressed by monocytes, which lack expression of CCR7. Consequently, this induces monocyte migration toward the lymph node in an inflammatory condition, thereby enhancing their capacity to stimulate the T-cell response. In this sense, this and previous studies also suggest that inflammatory monocytes can drive Th1 response directly stimulating the Th1 fate-producing IL-12 [92] and/or suppressing the Th2 fate-secreting IL-10 [30,93], even if these regulatory mechanisms are still not completely elucidated (Fig. 2D). These data, taken together, show the important role of DCs in modulating LN trafficking in different contexts to efficiently tune the immune response.

Conclusions

In this brief Review, we summarized less known abilities of DCs such as mediating antigen trafficking and dissemination, and guiding leukocyte trafficking within the LN coherently with the peripheral insult. The studies discussed contribute to paving the way for broadening the knowledge of the mechanisms underlying efficient and targeted immune activation, with potential applications in various translational approaches in the vaccinology field.

Nonetheless, much remains to be uncovered. For instance, while it is established that vaccines incorporating adjuvants bound to antigens are more effective in eliciting immune responses [94], the precise mechanisms governing antigen transfer together with its contextual information remain elusive. Furthermore, there is ongoing debate regarding the contributions of different DC subtypes in recruiting other leukocytes to initiate or amplify specific immune responses, with a special point on the resDCs which seem not to be involved in this fine-tuning. Further research is required to clarify these aspects, leading to a deeper understanding of the functioning of the immune system with main implications on human health.

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Author contributions

ED and GP wrote the manuscript with inputs from AKM and TB. ED and TB prepared the figures.

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