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The dual nature of T_{DC} – bridging dendritic and T cells in immunity

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T_{DC} are hematopoietic cells with unique features that provide intriguing insights into the interplay between innate and adaptive immunity. They express a combination of conventional dendritic cell (DC) and T-cell markers and are found in secondary lymphoid organs (SLOs), lungs and liver of naïve mice, as well as in human blood. When analyzed *ex vivo*, T_{DC} can behave either as DCs or as T cells, depending on the provided stimuli. Notably, T_{DC} numbers and activation significantly increase in SLOs following viral infection, suggesting a potential role for T_{DC} in antiviral immune responses. In this review, we discuss the properties of these fascinating cells, which call for more investigation on their physiological role during immune responses to both pathogens and tumors.

Keywords: antigen presentation; dendritic cell; T cell; viral infection; Zbtb46

Effective immune responses rely on the successful interplay between innate and adaptive immunity. Central to this orchestration is the intricate communication between dendritic cells (DCs) and T cells [1,2]. DCs, hailed as the most proficient antigen-presenting cells (APCs), process and present antigens via peptides loaded onto major histocompatibility complex (MHC) molecules. The trajectory of a specific adaptive immune response hinges upon the maturation and activation status of DCs; immature DCs tend to induce tolerance, whereas mature DCs prompt robust and tailored immune reactions. One mechanism driving DC maturation involves the activation of pattern-recognition receptors (PRR) by specific microbial or danger-associated patterns, leading to the upregulation of costimulatory molecules and MHC molecules, as well as the secretion of pro-inflammatory cytokines such as IL-12, IL-6, and tumor necrosis factor-alpha (TNF-α). Upon maturation, DCs migrate to T-cell zones within secondary lymphoid organs (SLOs), where they present antigens loaded onto MHC molecules to T cells [1]. The recognition of MHC complexes by the T-cell receptor (TCR), along with co-stimulation provided by mature DCs, results in the initiation of the adaptive T-cell response. This response consists in T-cell activation, proliferation, and differentiation into effector cells capable of mounting specific immune responses tailored to the encountered antigen: on one side CD8+ cytotoxic T cells directly eliminate infected or tumor cells and on the other side CD4+ helper T cells provide a cytokine milieu that supports both cytotoxic and humoral responses [1].

Although DCs and T cells both originate from bone marrow (BM) progenitors, their developmental trajectories diverge early on and are considered to be as

Abbreviations
T_{DC}, T dendritic cell; DC, dendritic cell; SLO, secondary lymphoid organ; APC, antigen-presenting cell; MHC, major histocompatibility complex; PRR, pattern-recognition receptors; TNF-α, tumor necrosis factor-alpha; TCR, T-cell receptor; BM, bone marrow; DN, double negative; NK, natural killer; ILC, innate lymphoid cell; RORγt, Retinoic Acid-Related orphan receptor gamma; Treg, T regulatory; R-DC-like, $ROR\gamma t^{+}$ DC-like; LN, lymph node; MHC-II, MHC class II; LPS, lipopolysaccharide; rIFN-γ, recombinant Interferon-γ.

distinct as their respective functions. Commitment of T cells occurs within the thymus, where precursor cells undergo a complex series of steps resulting in the production of mature naïve $CD4^+CD8^-$ and $CD4^-CD8^+$ T cells [3–5]. This process unfolds through distinct stages, beginning with the most immature thymocytes being double negative (DN), lacking both CD4 and CD8 markers, and further categorized into four subsets (DN1–DN4) based on CD44 and CD25 expression. DN1–DN2 thymocytes maintain the potential to differentiate into various myeloid cell types, such as natural killer (NK) cells and thymic dendritic cells (DCs) [6]. Commitment to the T-cell lineage and subsequent recombination of the TCRβ locus, along with pre-TCR expression, occur at the DN3 stage ($CD25^+CD44^-$), a process regulated by Notch signaling and involving the downregulation of specific transcription factors crucial for myeloid development [6].

On the other hand, conventional DCs (cDCs) arise from a common DC progenitor in the BM and subsequently migrate to peripheral lymphoid organs [7,8]. Various transcription factors including Zbtb46, PU.1, Ikaros, IRF8, RelB, and Batf3 have been implicated in DC development, yet none can exclusively define the DC lineage due to their pleiotropic roles and the high heterogeneity of DC subsets. For instance, Zbtb46 has been reported to be a specific marker for cDCs and their precursors, although its functional role in cDC differentiation is still to be fully understood [9–12], but it is also expressed by endothelial cells and innate lymphoid cell (ILC) 3 in the intestine [11,13,14]. Although no single master regulator of DC lineage commitment has been identified, interactions between FLT3 and its ligand (FLT3L) are crucial for DC development and homeostasis, as evidenced by the absence of DCs in peripheral lymphoid organs in FLT3L-deficient mice [15].

In this review, we will discuss the origin and function of T_{DC}, a novel hematopoietic cell type that has been recently described and characterized and that encompasses features and functional properties of both DC and T cells. This duality renders them the first innate polyclonal T cells to have been reported and suggests that they might play specific roles during the immune response.

Atypical DC or T cell populations with a mixed phenotype

Before going into the depth of T_{DC} description, we would like to discuss other examples of atypical cell populations that combine both myeloid and lymphoid cell features. One example is the family of murine Retinoic Acid-Related orphan receptor gamma $(ROR\gamma t)^+$ APC, which includes subsets of group 3 innate lymphoid cells (ILC), extrathymic autoimmune regulator-expressing cells and the $Rorc^+$ DC-like cells [16–19]. All members of the family express the *Rorc* gene, which encodes for two major transcription factor isoforms: RORγ (expressed by many different cell types) and RORγt (enriched in double-positive thymocytes, in group 3 ILCs, and in T helper 17 cells) [20–23]. $ROR\gamma t^+$ APC express Zbtb46, and other APC-related markers such as MHC-II and costimulatory ligands (although with a degree of variability within the different subsets), and induce microorganism-specific T regulatory (Treg) cells [16]. Interestingly, it was recently reported that Zbtb46, which is mostly restricted to the DC lineage [11,24], is also expressed by some intestinal ILC3 and regulates their proinflammatory functions [14]. A human counterpart of $ROR\gamma t^+$ APC was identified in the mucosae and in tonsils and named $ROR\gamma t^+$ DC-like (R-DC-like) cells; these cells express MHC-II and costimulatory molecules, can be expanded *in vitro* with FLT3, and can stimulate T cell activation [25].

In addition to $ROR\gamma t^+$ APC, another interesting and atypical cell type, named MyT, was discovered in mice in 2021 [26]. These cells were identified by transcriptomic and flow cytometry analysis as part of the effector CD4 T cells induced upon bacterial infection in the gut. MyT simultaneously express T cell and myeloid cell (among which MHC-II) markers, and are characterized by polyclonal TCRs [26]. This novel cell type has many features overlapping with T_{DC}, as it will be described later.

As demonstrated by the abovementioned examples, the coexistence of DC-specific and lymphocyte-specific transcription factors and surface markers in the same cell is possible, although our understanding of these "chimeric" cell types is still limited and requires further investigation.

T_{DC} identification

T_{DC} were identified in 2012, as described in Kuka et al. [27]. While investigating the interaction between cDCs and T cells through the costimulatory pair CD70-CD27 ([28,29], a subset of splenic cDCs expressing the CD27 receptor, which is also present on all T cells in naïve mice) was identified. Although the frequency of these cells was low, it was consistent across different experiments. A further examination of this subset performed by staining it with various marker combinations revealed that these cells expressed classical markers of both cDC and naïve T cells, thus resulting in the T_{DC} name. T_{DC} comprise about 7–10% of

all cDC found in naïve spleens and lymph nodes (LNs), and can be identified using either CD11c or the cDC lineage marker Zbtb46 [11,24,30]. In addition to CD11c and Zbtb46, T_{DC} express other murine cDC markers such as MHC class II (MHC-II), CIITA, PU.1, CD74, IRF-8, FLT3, and intermediate levels of CD11b and FcγR. Interestingly, whereas MHC-II can be expressed by human activated T cells, murine T cells never express these molecules since their transcriptional activator CIITA is actively repressed in activated murine T cells [31]. With regard to T-cell markers, T_{DC} express polyclonal αβ TCRs as well as genes of the TCR complex, Thy1, IL-7R, CD28, CD27, and either CD4 or CD8 [27]. Other lineage markers or genes related to T cell activation are not expressed, with the exception of a group of cytotoxic and effector genes such as *Prf1*, *Gzma*, *Gzmb*, *Eomes*, and *Ifng* [27,30].

The hypothesis of facing an artifact caused by imperfect exclusion of T-DC doublets, which frequently form when DC and T cells interact through an immune synapse, particularly during an active immune response, was considered [32]. However, it seemed unlikely that T_{DC} were T-DC cell doublets because these cells were detected in organs of naïve mice, where no active immune response was occurring. Additionally, a double-round doublet exclusion strategy was used and media supplemented with chelating agents to prevent doublet stability used [27,30,33]. Definitive proof that T_{DC} are not T-DC doublets was obtained by sorting 100% pure T_{DC} and subsequently examining them via confocal microscopy: this approach represents an alternative to modern imaging flow cytometry (which was not available at the time) and revealed that all sorted cells displayed surface expression of CD11c, MHC-II and TCRβ [27,33]. The expression of these three markers was uniform and not confined to specific membrane areas, thereby ruling out other potential artifacts such as trogocytosis and cross-dressing [34–37]. Furthermore, it was observed that T_{DC} express DC and T cell markers not only on their surface but also at the mRNA level, which is typically not seen in trogocytosis [37,38]. Indeed, the gene expression profile of sorted T_{DC} was explored by either microarray or bulk RNA sequencing, and both assays revealed that their transcriptional profile is distinct with respect to T cell and cDC, although some genes were shared with either cell type. Interestingly, the unique gene signature expressed by T_{DC} closely resembles that of MyT, a recently identified $CD4^+$ T cell population that exhibits MHC-II molecules and other myeloid markers [26]. MyT were identified as a specific cluster within a single-cell RNAseq dataset of murine effector $CD4^+$ T cells in the gut of Salmonella-infected mice, and different experiments were performed to exclude that they were T-DC doublets [26]. The fact that the transcriptional signature of T_{DC} and MyT is very similar strongly suggests that T_{DC} are a specific cell type and not an artifact due to T-DC doublets.

T_{DC} ontogeny

The identification of T_{DC}, which express lineage-specific markers of both DCs (PU.1, Zbtb46, IRF8) and T cells (TCRβ), suggests that the lymphoid and myeloid differentiation pathways might not be mutually exclusive, as previously thought. Indeed, DC originates from a Zbtb46-expressing common DC precursor, whereas conventional αβ-T cells originate from a lymphoid precursor but necessitate a thymus for their maturation [4,5,7,39]. However, as mentioned above, commitment to the T-cell lineage and subsequent recombination of the TCRβ locus, along with pre-TCR expression, does not occur immediately but only at the DN3 stage. Before that stage, thymocytes maintain the potential to differentiate into various myeloid cell types, such as NK cells and thymic DCs [6,40]. This indicates that the differentiation fate of DCs and T cells is not defined until the latest stages of the thymic development. Interestingly, human thymocytes exhibiting low IRF-8 expression levels, retain the capability to differentiate into either T cells (where IRF-8 levels are then shut down) or DCs (where IRF-8 levels increase even more) [41,42]. In addition, Zbtb46 which is normally expressed by the DC lineage, has been recently shown to be expressed also by a subset of innate lymphoid cells, which originate from the same early precursor as T lymphocytes in the BM [14]. Therefore, transcription factors that are thought to be specific for DC commitment might indeed play pleiotropic functions and be involved also in the ontogeny and differentiation of lymphoid cells.

Splenic T_{DC} express FLT3 receptor, and can respond and expand *in vivo* in response to FLT3L, just like cDC [27]. In addition, their frequencies are dramatically impaired in the spleens of FLT3L-deficient animals which suggests that FLT3-dependent signals are needed for their early development ([27] and Fig. 1). Nevertheless, bone marrow cell cultures supplemented with granulocyte–monocyte-colony-stimulating factor or FLT3L failed to generate T_{DC} (unpublished data). This would suggest that BM is not sufficient and that T_{DC} need thymus for full development and acquisition of T cell markers, as confirmed by the absence of T_{DC} in the spleens of athymic mice ([27] and Fig. 1). In the thymus, T_{DC} can be identified

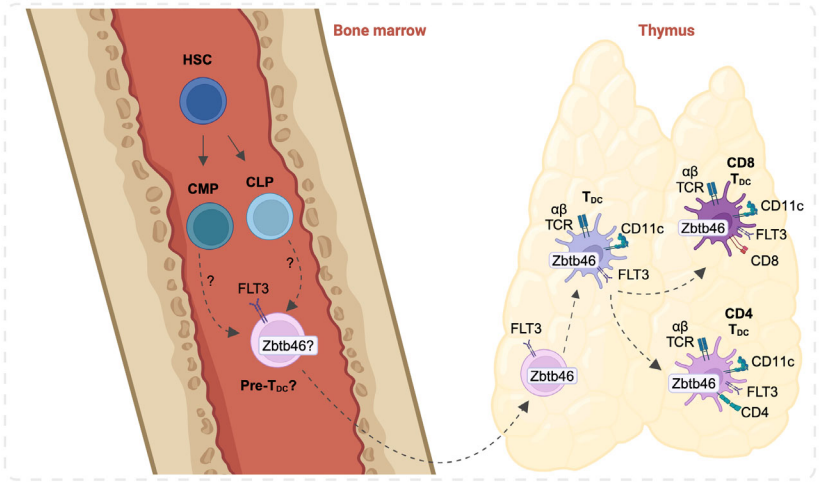

Fig. 1. Proposed model for T_{DC} ontogeny. Hypothetical mechanism was proposed based on the published data, according to which T_{DC} arise in the bone marrow (BM) from a precursor expressing FLT3. No formal data on whether the T_{DC} precursor is originated from the common lymphoid (CLP) or myeloid precursor (CMP) is available. T_{DC} precursors leave the BM and reach the thymus, where they rearrange polyclonal TCRs and undergo thymic positive selection to become either $CD4^+$ or $CD8^+$ T_{DC}. Although the stage where Zbtb46 is expressed in T_{DC} is not known, we hypothesize that it happens early during development in the BM (like in DCs) since T_{DC} precursors in the thymus already express Zbtb46.

within both the CD3-intermediate and CD3-high thymocyte subsets, indicating that they are found throughout all T cell developmental stages. Investigations into whether T_{DC} undergo antigen-specific selection in the thymus involved the study of mice expressing αβ-TCR transgenes (Tg) on either positively selecting or non-selecting MHC haplotypes. T_{DC} were present in mice with positively selecting haplotypes but not in those with non-selecting MHC, strongly indicating that T_{DC} require the same positive selection signals in the thymus as conventional T cells [27]. Further evidence supporting the positive selection of T_{DC} was derived from studies of ThPOK expression, a transcription factor crucial for CD4 helper lineage specification [43,44]. Consistent with conventional T cells, ThPOK-deficient mice lacked only $CD4^+$ T_{DC}, whereas the frequency of $CD8^+$ T_{DC} was increased [27]. Taken together, these findings underscore the requirement for T_{DC} of both DC and T-cell developmental cues (Fig. 1).

T_{DC} localization and potential functions

T_{DC} can be identified as those cells expressing concomitantly the TCR and either CD11c or the cDC lineage marker Zbtb46 [11,30]. Indeed, positivity for CD11c can be used for the identification of T_{DC} in steady-state conditions but not in inflammation or other pathological settings, since CD11c is upregulated on some T-cell populations upon activation [45–48]. For this reason, T_{DC} localization and frequency in organs other than naïve SLOs was difficult to study until Zbtb46 was used as a substitute marker for T_{DC} by taking advantage of the Zbtb46-GFP fluorescent reporter [11]. Indeed, Zbtb46 has been reported to be expressed only by classical DC and their precursors, by a fraction of endothelial cells, and by ILC3 in the intestine, but not by activated T cells [11,13,14,24,30]. $Zbtb46^+$ T_{DC} were found to be present in the lungs of naïve mice at the same frequencies as in the spleens of the same animals, and were found to be even more abundant in the livers, indicating that they might play some specific role in this latter organ [30]. In the SLOs, T_{DC} seem to localize specifically in interfollicular areas near B cell follicles or subcapsular sinuses, reminiscent of previously described innate αβ CD8 T cells known for early IFN-γ production after infection [49].

One of the most important questions arising upon T_{DC} identification is relative to their physiological relevance. Their potential functions can be interrogated *ex vivo* by stimulating in different ways either total splenocytes or high-purity T_{DC} obtained upon a two-round sorting strategy [27,33] (Fig. 2). To assess the innate immune response of T_{DC}, total splenocytes from WT mice were stimulated with lipopolysaccharide (LPS) or other TLR agonists [27]. $CD11c^+$MHC-II^+TCR-β^+ cells (T_{DC}) displayed a notable increase in costimulatory molecules similar to DCs, whereas

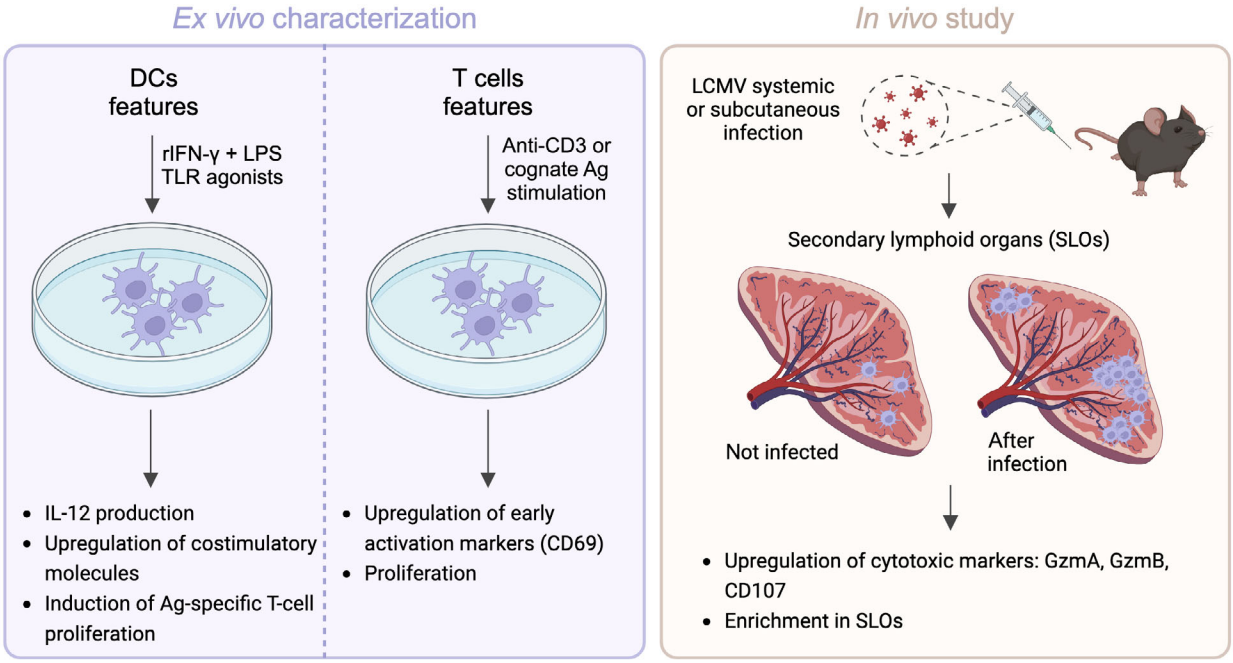

Fig. 2. T_{DC} functional properties *ex vivo* and *in vivo*. *Ex vivo* characterization (left panel): T_{DC} potential functions can be interrogated *ex vivo* by stimulating in different ways either total splenocytes or high-purity T_{DC} obtained upon a two-round sorting strategy. To assess DC features, total splenocytes from WT mice were stimulated with lipopolysaccharide (LPS) or other TLR agonists. T_{DC} displayed a notable increase in costimulatory molecules and IL-12 production. Sorted T_{DC} can induce antigen-specific T-cell proliferation comparable to DCs, suggesting that T_{DC} can process and present antigens on MHC-II. On the other hand, following stimulation of the TCR through anti-CD3 or cognate Ag stimulation, T_{DC} exhibit elevated levels of the activation marker CD69 and proliferate. *In vivo* study (right panel): LCMV infection leads to a notable increase in the frequency of T_{DC} in the SLOs of infected mice analyzed 2 and 7 days post-infection. Furthermore, significantly increased levels of the cytotoxic markers GzmA and GzmB, as well as the degranulation marker CD107 were observed 2 days after LCMV infection.

conventional T cells showed no such response (Fig. 2) [27]. Furthermore, T_{DC} stimulated with TLR agonists produce IL-12, a cytokine critical for T_H1 polarization and typically released by mature cDCs. Notably, IL-12 production by T_{DC} is enhanced when splenocytes are primed with recombinant Interferon-γ (rIFN-γ) followed by LPS stimulation [27]. An important feature of DCs is their ability to process and present antigens on MHC-II [1,50]. Interestingly, sorted T_{DC} can induce antigen-specific T-cell proliferation comparably to DCs, suggesting that T_{DC} can process and present antigens on MHC-II, and demonstrating functional properties characteristic of innate cells (Fig. 2) [27].

Additionally, when triggered through typical T-cell stimuli *ex vivo*, T_{DC} behave like conventional T cells (Fig. 2). Following stimulation of the TCR through an anti-CD3 approach, T_{DC} exhibit elevated levels of the activation marker CD69 and proliferate [27]. In addition, T_{DC} isolated from MHC-II-restricted TCR-transgenic mice and stimulated with cognate antigen proliferate in the absence of additional APCs, whereas conventional T cell activation is suboptimal without co-stimulation [27,51,52]. Thus, T_{DC} possess a functional TCR and are capable of antigen presentation independently of other APCs.

Unfortunately, while undergoing expansion in culture, T_{DC} exhibit a reduction in cell surface CD11c expression (although a fate mapping experiment for CD11c clearly showed that those same cells initially did express CD11c) [27]. Moreover, as mentioned above, CD11c is upregulated on some T-cell populations at the peak of the response [45–48]. This represents a potential caveat in addressing T_{DC} expansion *in vivo* and calls for the use of alternative markers for this purpose. Indeed, analysis of WT mice 2 months post-LCMV infection, when both T and T_{DC} numbers are stabilized, revealed gp33 tetramer-positive T_{DC} indicative of antigen-experienced cells. Notably, a relatively high proportion of T_{DC} were gp33-tetramer-positive, suggesting the persistence of memory T_{DC} *in vivo* akin to conventional memory T cells [27]. However, during LCMV infection, the gating strategy based on CD11c and MHC-II double-positive cells is suboptimal for identifying T_{DC} at day 7 post-infection

(the peak of the immune response), since the frequency of $CD11c^+MHC\text{-}II^+$ cells significantly increases primarily because the majority of $TCR\beta^+$ cells start expressing CD11c [30]. Therefore, Zbtb46-GFP fluorescent reporter mice were instrumental in giving a hint on the role of T_{DC} *in vivo* early during viral infection (Fig. 2). Importantly, the Zbtb46 expression profile remains unchanged upon LCMV infection, making the Zbtb46-GFP reporter an ideal model for investigating whether T_{DC} expand in LCMV-infected mice. LCMV infection leads to a notable increase in the frequency of both T_{DC} and conventional T cells in the spleens of infected mice analyzed 7 days post-infection [30]. Similarly, mice infected subcutaneously in the footpad show a significant increase in $CD8^+$ T cells and $CD8^+$ T_{DC}. Confocal imaging of LN sections from infected mice shows numerous cells positive for both GFP and TCRβ, confirming an enrichment of T_{DC} in these organs following infection [30]. Interestingly, T_{DC} begin to expand earlier than conventional T cells following infection, which is in line with the observation that they represent a T-cell population with innate characteristics. Two days after systemic LCMV infection, the frequency of $CD8^+$ T_{DC} in the spleens of infected mice is higher compared with uninfected controls, whereas the frequency of conventional T cells and DCs does not increase at this time point. Furthermore, significantly increased levels of the cytotoxic markers GzmA and GzmB, as well as the degranulation marker CD107, in splenic T_{DC} 2 days after LCMV infection was observed [30]. Conversely, TCRβ levels were slightly downregulated consistent with TCR engagement during this activation process. Overall, these data suggest that T_{DC} may be activated by antigen and perform cytotoxic functions very early after infection, although further in-depth analysis is needed to corroborate these findings.

Conclusions and perspectives

T_{DC}, unconventional polyclonal T cells with both innate and adaptive properties, exhibit versatile responses *in vitro* but their physiological role remains to be clarified due to technical challenges stemming from their rarity and lack of specific markers for the generation of T_{DC}-deficient mice. However, knowledge gathered by combining data on their localization *in situ*, on their *in vivo* dynamics after infection, and on their unique transcriptional profile can allow some speculations on their possible functions. For example, their localization in interfollicular areas near B cell follicles or subcapsular sinuses of SLOs is reminiscent of other innate αβ CD8 T cells which were reported to be the earliest IFN-γ producers after infection [49]. Similarly, T_{DC} downregulated their TCR and upregulated cytotoxic molecules (GzmA, GzmB, CD107) already 2 days upon LCMV infection, suggesting that they might be responding earlier than conventional T cells [30]. On another note, T_{DC} transcriptional profile was found to overlap with another intriguing T cell subset expressing myeloid markers (MyT) and identified in the intestine of Salmonella-infected animals [26]. Although we did not find an enrichment for T_{DC} in the intestine of naïve animals, it is plausible that there might be pathogen-specific and organ-specific cues that could lead to expansion of T_{DC} in different organs and contexts. For example, with regard to unconventional T cells (UTC), it has been shown that there is a certain degree of heterogeneity of tissue-derived UTCs localizing preferentially to specific LNs and thus imprinting different immune responses [53].

In summary, T_{DC} combine features and functions of T cells and DCs, and it is tempting to speculate that such duality might render T_{DC} particularly responsive to infectious organisms, because they can bridge innate and adaptive traits. Infectious diseases are not the only context where T_{DC} might have a role. Several reports show that tumor-specific T cells engineered to produce IL-12 are very effective in eliciting regression of melanomas [54–56]. One could envision that tumor-specific T_{DC} might be effective in this setting because they produce IL-12 on stimulation, similar to innate APCs. Thus, T_{DC}, present in mice and humans, comprise a new and intriguing cell subset poised at the intersection of innate and adaptive immunity.

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Author contributions

Conceptualization: MK; Writing: MN and MK; Visualization: MN; Funding Acquisition: MK.

Data accessibility

Data sharing is not applicable as no new data were created or analyzed in this review.

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