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T cell and dendritic cell interactions in lymphoid organs: more than just being in the right place at the right time

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Summary

The initiation of T lymphocyte responses within secondary lymphoid organs involves interactions with different subsets of dendritic cells (DCs). Recent studies have revealed the complexity of microanatomical organization within lymphoid organs. Exactly how T cells and DCs locate each other and the type of cellular interactions required for optimal priming of effector and memory T cell responses are beginning to be unravelled. Here we review advances in our understanding of how T cell priming is choreographed during infections, highlight the importance of cell positioning in this process and discuss how a spectrum of cellular interactions shapes T cell activation and differentiation.

Keywords:
T lymphocytes, dendritic cells, secondary lymphoid organs, antigen presentation, cell migration, intravital imaging.

INTRODUCTION

The adaptive immune system relies on encounters of T cells with antigen presenting cells (APCs) such as dendritic cells (DCs) for the initiation of immune responses to infection. Although as few as 85 DCs are predicted to be sufficient to induce a T cell response (1), the rarity of cognate T cells specific for pathogen antigens poses a challenge for these encounters. Secondary lymphoid organs (SLOs) including the lymph nodes (LNs) and the spleen provide an anatomical solution for this challenge as meeting points for naïve T cells and APCs. DCs generally do not migrate from one SLO to another while naïve T cells continuously circulate between multiple SLOs. T cells home to SLOs from blood, spend 12-24

hours scanning multiple APCs looking for their cognate antigens before exiting. Although T cells can theoretically scan hundreds of DC while transiting through LNs (2), and DCs are estimated to interact with as many as 5000 T cells per hour (3), initial cognate interactions between T cells and DCs within SLOs still rely heavily on chance encounters. Given the predicted scanning rate of naïve T cells, there will still be hundreds or thousands of DCs within a single LN that a T cell will not be able to scan during its time in the LN. However, during immune responses additional mechanisms that regulate the migration of T cells and APCs and the availability of antigen are employed by the immune system to increase the probability of such random encounters and ensure timely generation of T cell responses. This is particularly important in the context of infections with pathogens including viruses and bacteria that can rapidly invade and spread in hosts if not controlled by fast and specific T and B cell responses.

Bringing cognate T cells together with DCs is only the beginning and one aspect of successful T cell responses. T cells also need several contact-dependent and -independent signals for their proper differentiation. In addition to cognate TCR peptide-MHC (pMHC) interactions, costimulatory signals through CD28 binding CD80 and CD86 on DCs (signal 2) and DC-derived cytokines such as IL12 (signal 3) are also required for optimal T cell differentiation (4, 5). Moreover, cytokines such as IL-12, IL-4 and IL-6 are also involved in the polarization of T cell responses according to the type of infection ensuring the flexibility of the adaptive immune system (6). These factors make the local microenvironment around activated T cells extremely important for the development of effective T cell responses. Several recent papers have described the orderly and precise positioning of T cells and APCs in SLOs during infections for the successful induction of T cell responses. Here we discuss these recent advances in our understanding of the choreography between DCs and T cells that occur in SLOs during immune responses.

DENDRITIC CELLS AS CENTRAL REGULATORS OF ADAPTIVE IMMUNE RESPONSES

Conventional T cells need to recognize pMHC molecules on APCs with their unique TCR molecules to get activated. Peptides derived from endogenous proteins are generally presented on MHC-I molecules for the activation of $CD8^+$ T cells, while exogenous peptides

are taken up, processed and presented on MHC-II molecules by APCs (such as DCs and B cells) for the activation of $CD4^+$ T cells. However, certain DCs can also present peptides derived from exogenous antigens on MHC-I molecules via a mechanism called cross-presentation (7). Cells that can prime naïve T cells are usually referred to as professional APCs and may include conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), monocyte-derived dendritic cells, Langerhans cells, macrophages and B cells (8-10). Several studies have shown the central role of cDCs for the priming of T cells and in this review we focus on cDCs as the key initiators of many immune responses. It is important to note that antigen presentation by non-professional APCs, including stromal cells in SLOs and infected cells in tissues, can contribute to the magnitude of effector T cell responses (11), but considerably less is known about the contribution of such APCs during the priming phase of T cell activation.

Developmental studies have shown that cDCs can be divided into two broad categories known as cDC1 and cDC2 (8-10). cDC1s depend on the transcription factors Batf3, IRF8 and ID2 for their development and uniformly express the chemokine receptor XCR1 as well as Clec9a (DNGR1, CD370). cDC1s can cross present extracellular antigens for presentation on MHC-I and are specialized for the priming of $CD8^+$ T cells. cDC2s depend on IRF4 for their development and are characterized by expression of CD172a (SIRPα) and DCIR2 (in spleen). Several studies have shown that $CD4^+$ T cells are preferentially primed by cDC2s and they play key roles in Th2, Th17 and Tfh responses. Although, there appears to be specialization among cDCs for priming $CD8^+$ and $CD4^+$ T cells, this distinction does not refer to an innate inability of either cDC subset to stimulate different T cell subsets. *In vitro* studies have shown that the cDC subsets are capable of priming both T cell subsets (8-10) while only cDC1s are specialized for cross-presentation (12). As we discuss below, recent studies suggest that *in vivo* roles of cDC subsets for the preferential priming of different T cell subsets are influenced at least in part by their localization within SLOs and access to antigen.

Both cDC1s and cDC2s in LNs can further be classified into resident and migratory subsets (8-10). Resident cDCs develop from precursor (pre)-DCs that home to LNs and do not leave the LN. Conversely, migratory cDCs develop from pre-DCs that home to non-lymphoid tissues and these tissue cDCs can migrate to LNs via the afferent lymphatics. Under

homeostatic conditions resident and migratory cDCs are usually distinguished by the relatively higher expression of MHC-II on the more mature migratory cDCs. Under inflammatory conditions, however, this distinction becomes difficult as resident DCs also upregulate MHC-II expression as part of their activation process. The majority of cDC1s in peripheral tissues express the integrin CD103, thereby allowing distinction of migratory cDC1s from resident ones that express higher levels of CD8α coreceptor (8-10). Markers that distinguish resident and migratory cDC2s are less well defined, although many cDC2s in the dermis of the skin and the submucosa of tissues such as the vagina express CD301b (Mgl2) (13).

DC migration from non-lymphoid tissues depends on chemokine receptors, in particular CCR7 that binds the chemokines CCL21 and CCL19 produced by lymphatic endothelial cells (LECs) in tissues and both LECs and fibroblastic reticular cells (FRCs) in LNs. CCR7-dependent chemokines facilitate DC travel to LNs through the afferent lymphatics and allow DCs to cross the LN SCS and migrate into the T cell zones (14, 15). Recently, CCR8 on DCs was revealed to be required for CD301b$^+$ DC migration during allergic responses (16), implicating LN SCS macrophages for the provision of CCL8 to recruit DCs into the LN parenchyma during Th2 responses. Once migratory DCs arrive at the SCS of the LN, they migrate towards the parenchyma using gradients of CCR7 ligands that are established by LECs that line the ceiling of the SCS and express the scavenging receptor ACKR4 (CCRL1) (15, 17). In the absence of ACKR4, CCL21 gradients are abrogated in the LN SCS and in the skin, impairing DC trafficking and entry into T cell zones (17, 18).

Under steady state conditions, migratory cDC1s spend 4-6 days in the LN before dying, while migratory cDC2s spend 2-3 days (19, 20). Infection and inflammation generally increase the migration rate of DCs from tissues to LNs and induces a distinct program of activation in DCs (21) that may improve the capacity for antigen transport to the LNs, presentation and T cell activation. However, turnover rates of migratory DCs in LNs can also increase after inflammation as each DC spends less time in the LN (19). Migratory DCs that take up antigens in non-lymphoid tissues mature as they migrate to LNs and are therefore less efficient at taking up antigens in LNs, but more efficient at presentation (22).

DCs presenting cognate peptides that arrive in LNs from the tissues can prime T cell responses. Alternately, pathogens or antigens that drain from peripheral tissues via the lymphatics can be taken up by LN-resident DCs, or may infect LN SCS and medullary macrophages and DCs, for presentation to T cells. DCs have been shown to be a central and critical component of naïve T cell activation, in part using mouse models where subsets of DCs expressing the diphtheria toxin receptor (DTR) can be depleted. These models have downsides, including reduced LN cellularity due to genetically expressed DTR (23). Yet, they have revealed the complexity of T cell priming, which may involve multiple subsets of APCs in distinct spatial locations within LNs or spleen and is likely to vary considerably depending on the antigen type and dose and route of infection or inoculation.

MICROANATOMY OF SECONDARY LYMPHOID ORGANS

Microanatomy of Lymph Nodes

LNs have a highly organized structure to optimize the efficiency of adaptive immune responses. LNs act as meeting points for several immune cells and therefore each LN contains several distinct cell types, which occupy dedicated niches within the LN. Furthermore, continuous entry and exit of lymphocytes, migration of DCs from tissues, homing of pre-DCs from the blood and turnover via cell death and proliferation makes LNs highly dynamic structures. Nevertheless, LNs generally maintain their microanatomical structures under homeostatic conditions and can rapidly reorganize positioning of immune cells during infection. Broadly, LNs are composed of multiple B cell follicles, a central paracortex area that contains T cells, a medulla that contains large lymphatic sinuses and the subcapsular sinus that contains specialized macrophages that scan afferent lymph for pathogens (14, 24).

Compartmentalization of LNs is largely achieved and maintained by specialized non-hematopoietic stromal cells such as FRC, blood endothelial cells, LEC, follicular dendritic cells and marginal reticular cells (25, 26). These stromal cells contribute crucially to LN homeostasis and T cell responses by producing cytokines and chemokines, survival factors,

and inflammatory mediators in a cross-talk with immune cells. Furthermore, FRCs form scaffolds on which DCs and T cells migrate within the LN, which has important consequences for the efficiency and kinetics of T-DC interactions. Stromal cells can also respond rapidly to infections to reorganize the overall structure of the LN and control LN hypertrophy induced by changes in lymphocyte recruitment (25, 26).

LNs also contain specialized microanatomical niches within the broad areas described above, the best studied of which are the interfollicular regions (IFRs) between B cell follicles. Specialized stromal cells construct a unique IFR niche that is rich in macrophages and DCs, and contains $CXCR6^+$ innate lymphocytes such as $\gamma\delta$ T cells and NKT cells, as well as innate lymphoid cells (ILCs), ILC2s and ILC3s (27-29). Furthermore, IFRs are transition zones for newly arriving migratory DCs as they migrate deeper into the T cell zone (15, 20). Thus, the IFR contains multiple innate and adaptive immune cells and may be important for the initiation of T cell responses against lymph-borne pathogens (27, 30).

Although DCs are present in substantial numbers in T cell zones, it has been shown that DCs are not evenly distributed. Under homeostatic conditions, both resident and migratory cDC1s are mostly localized in the deep paracortex area. Resident cDC2s are found mostly in the medullary regions and around lymphatics while migratory cDC2s are found in IFRs and the outer paracortex (31). The molecular mechanisms responsible for this differential distribution of DCs are not entirely clear but the chemokine receptor CCR7 and the oxysterol receptor EBI2 play key roles in this spatial organization. Migratory cDC1s express higher levels of CCR7 compared to migratory cDC2s, which express higher levels of EBI2 (32). EBI2 expression on DCs was also shown to be essential for localization of cDC2s in IFRs and the outer paracortex where both hematopoietic and stromal cells regulate the availability of EBI2 ligands (33). Whether other signals fine tune DC positioning in SLOs during homeostasis is not known.

Microanatomy of the Spleen

Similar to LNs, the spleen also contains a specialized microanatomy, including the red pulp (RP), white pulp (WP) as well as the marginal zone (MZ) that separates the RP and the WP (24, 34). RP contains several different immune cells such as F4/80$^+$ macrophages, DCs, monocytes, red blood cells and lymphocytes amongst a dense network of stromal cells and endothelial cells that form the RP sinuses. The MZ contains marginal zone B cells and MARCO$^+$ SIGN-R1$^+$ marginal zone macrophages that sit proximal to the RP, and CD169$^+$ marginal metallophilic macrophages on the WP side of the marginal sinus (35). Bridging channels (BCs) form part of the border between the RP and the WP but do not contain CD169$^+$ macrophages. Although details of lymphocyte circulation through the spleen is poorly understood, BCs are considered as the portals for the exit of lymphocytes from the WP. T and B cells reside in organized zones within the WP, similar to LNs. In mice, T cell zones (also known as the periarteriolar lymphoid sheath) occupy the centre of the WP surrounding the central artery (24, 34). As in LNs, heterogeneous populations of stromal cells also form the basis of the different compartments and niches in the spleen. Although less is known about stromal cells in the spleen compared to LNs, they also produce cytokines and chemoattractant gradients for the homeostasis and the organization of the spleen (26).

The spleen does not have extensive afferent lymphatics that drain peripheral non-lymphoid tissues and it is specialized in surveillance of blood for antigens and pathogens. Therefore, unlike LNs, the spleen does not contain migratory DC subsets that arrive from tissues and all DCs in spleen develop from pre-DC precursors that are recruited from blood (8-10). Under homeostatic conditions, most cDC1 cells are found in the RP and the MZ but some of them can also be found in the T cell zone of the WP. The majority of the cDC2s, on the other hand, are found in the BCs. Similar to migratory cDC2s in LNs, the localization and maintenance of cDC2s in BCs depends on expression of the receptor EBI2 on DCs and the availability of the stromal-derived oxysterol ligands in BCs (33, 36, 37). In addition to the role of EBI2, sphingosine-1-phosphate signalling may also be important for the steady state localization of cDC2s to BCs (38).

Surprisingly, a difference in the overall distribution of CD4$^+$ and CD8$^+$ T cells in the T cell zone of the WP has been described (39). Under steady state conditions, the density of CD4+

T cells was higher in the outer regions of the T cell zone while $CD8^+$ T cells were concentrated in the centre. This difference appears to be mirrored by the distribution cDC1s and cDC2s in the WP, with $CD4^+$ T cell densities being highest around cDC2s and $CD8^+$ T cell densities around cDC1s (39). Since most of the T cells found in WP under homeostatic conditions are naïve T cells, this distribution probably reflects a fundamental difference in the localization of T cell subsets. Notably, a similar distribution difference was also suggested for $CD4^+$ and $CD8^+$ T cells in T cell zones of LNs (40). However, it is unclear whether cross-talk between T cells and DCs tunes the positioning of naïve T cells in LNs and spleen, such as via the local production or degradation of specific chemoattractants.

LOCALIZATON OF DENDRITIC CELLS IN LYMPHOID ORGANS DURING INFECTION

The positioning of immune cell subsets in SLOs remains relatively stable over time under homeostatic conditions despite continuous circulation and replenishment. However, after infection or immunization, T cells and DCs can rapidly change their migratory patterns and localization within SLOs. DC positioning within SLOs can have important consequences for T cell responses. Efficient acquisition of antigen by DCs and exposure to locally produced cytokines depends on their position within SLOs. DCs can express several receptors such as CCR7, CXCR5, XCR1, S1PR1 and EBI2 that can direct them to specific niches within SLOs. Therefore, during infection localization of DCs within SLOs is tightly regulated to optimize T cell responses and recent studies shed more light into this reorganization of DCs, especially within spleen.

One prominent example of DC relocalization is the migration of cDC2s in the spleen from BCs to WP, which involves upregulation of EBI2 (33, 36, 39, 41, 42). This migration has been observed after red blood cell, alum and LPS immunization as well as after *Listeria* infection, suggesting that this migration is a common feature in diverse immune responses (33, 36, 39, 41, 42). The reorganization of cDC2 distribution has important consequences for $CD4^+$ T cell activation and differentiation (33, 36, 37, 39, 41). After migration into WP, cDC2s do not migrate into the deep T cell zone but rather stay towards the periphery of the T cell zone (33, 36, 39, 41). Notably, cDC1 express the oxysterol-metabolising enzyme Hsd3b7,

enforcing the outer T cell zone positioning of EBI2$^+$ cDC2 that enter the WP upon activation (36). Inflammatory signals including type I interferons (IFN-I) can impact cDC2 migration by reducing EBI2 upregulation, facilitating migration into central T cell zones. Hence, DCs can control the positioning of other DCs, which is controlled by signals from stromal cells and impacted by inflammation.

In the spleen, cDC1s also relocalize in response to inflammatory signals, migrating from the RP and MZ to the T cell zones in the WP (39, 43, 44). Interestingly, migration to WP requires CCR7 expression on cDCs, similar to migration of DCs from non-lymphoid tissues to LNs (39). Despite this prominent role for CCR7, other signals also control DC trafficking and positioning within SLOs. The protein Dock8 impacts the migration of cDC2s to the WP after immunization (39). Furthermore, for both cDC1 and cDC2, this infection- or inflammation-induced positioning in the spleen resembles the localization of migratory cDCs in LNs. Overall, these observations suggest that DC migration from RP and MZ to WP in spleen is functionally analogous to DC migration from non-lymphoid tissues to LNs (45).

Repositioning of DCs within LNs may also support T cell priming. Upon drainage of subcutaneously injected influenza virus into the LN medulla, LN-resident DCs migrate into the medulla and capture antigens for presentation to CD4$^+$ T cells (46). Resident cDC2s were also reported to increase their motility and migrate towards B cell follicles after capturing influenza virus (47). Macrophage death that occurs after virus infection may induce DC repositioning in an IL-1 and CCL2 dependent manner (48). DCs have also been shown to upregulate CXCR5 after *H. polygyrus* infection and these CXCR5$^+$ DCs then migrated to the IFRs and T-B border to promote generation of Th2 cells (49). However, further studies are needed to understand how distinct inflammatory signals regulate differential positioning of DCs within SLOs.

LOCALIZATON OF T CELLS IN LYMPHOID ORGANS DURING IMMUNE RESPONSES

Upon activation, T cells upregulate multiple chemokine receptors that in addition to facilitating migration to tissues, can impact responses within SLOs. T cells upregulate CCR5 after activation, which can direct them towards DCs that upregulate CCL3/4/5 to promote

priming (30, 50, 51). The absence of CCR5 on T cells or treatment of mice with blocking antibodies against CCL3/4/5 can impair $CD8^+$ T cell differentiation and memory formation. $CD8^+$ T cells also produce XCL1 shortly after activation and may attract cDC1s, which express the receptor XCR1 (52). T cells lacking XCL1 showed weaker responses to immunization and infection (52, 53). This chemokine axis helps to bring activated $CD8^+$ T cells and cDC1s together and may facilitate the formation of clusters (54, 55). CCL17 production by DCs was also reported to improve interactions between naïve CCR4-expressing T cells and DCs (56).

T cells also upregulate CXCR3 and respond to CXCL9 and CXCL10 that is produced by stromal cells and DCs in response to interferons (57-59). Many memory $CD8^+$ T cells retain high expression of CXCR3 and can mediate the rapid antigen-independent migration of memory T cells towards infected cells in the outer T cell areas and SCS during infection (57, 59). Therefore, memory cells are recruited towards APCs more rapidly than naïve cells during the early phase of an infection as upregulation of CXCR3 by naïve T cells first requires activation and proliferation (57, 59). However, CXCR3 can also control the differentiation of effector T cell subsets, including $CD4^+$ Th1 cells by positioning of $CD4^+$ T cells to LN IFRs and medulla (46, 58), and recruitment of effector T cells towards infected cells in the splenic MZ and RP that can impact the generation of short-lived effectors at the expense of memory precursors (60, 61). Indeed, CXCR3-deficient T cells can form more memory cells compared to their wild type counterparts (60-62). This may be driven by increased exposure to cytokines, since CXCR3-deficient T cells cannot migrate toward DCs that produce high levels of type I IFN and IL-12 and hence cannot sustain CD25 expression for as long as CXCR3-expressing cells (60). CXCR3-deficient cells are likely also exposed to less antigen (61, 62), and injection of antigen-loaded DCs can normalize the memory differentiation of CXCR3-deficient T cells (62).

Development of Tfh and support for B cell responses also requires controlled positioning of effector $CD4^+$ T cells within SLOs (63, 64). The roles of the chemokine receptors CCR7 and CXCR5 for the differentiation and function of Tfh cells have been described extensively and won't be discussed here in detail (63, 64). Briefly, Tfh precursors that are activated by DCs in the T cell zone downregulate CCR7 while upregulating CXCR5 and migrate to T-B border areas to interact with B cells and complete their differentiation and migrate into the B cell

follicles. Recently, the EBI2 receptor on $CD4^+$ T cells was also shown to be important for the migration of activated T cells to the T-B border (42). CXCR5- or EBI2-deficient T cells cannot efficiently localize to the T-B border and are deficient at differentiating into Tfh cells. Interestingly, this T cell relocalization mirrors the migration of cDC2, which is important for the differentiation of Tfh cells via IL-2 depletion by cDC2s (42). Tfh cells upregulate CXCR5 and ICOS to enter B cell follicles and are recruited into germinal centers (GC) (63, 64). An additional balance of signals controls the entry and residence of Tfh in GC, including repulsive interactions with ephrin-B1 on B cells (65), and expression of PD-1 that suppresses recruitment back into the follicle by reducing CXCR5 signalling and preventing expression of CXCR3 (66). These studies highlight the importance of multiple layers of control for the coordination of T cell positioning in SLOs during immune responses.

KINETICS OF ANTIGEN DISTRIBUTION AND PRESENTATION IN LYMPHOID ORGANS

During an infection, the type, dose, and route of antigen entry into SLOs can have important implications for cognate T-DC interactions and effective T cell responses. Depending on the type of infection or immunization, antigens can reach to LNs via different routes. Antigens can be picked up by DCs in the periphery and then brought to LNs as these DCs migrate to the LN via lymphatics. Similarly, pathogens can infect migratory DCs and be transported to LNs in the same manner, although evidence suggests that this is not an important route of priming (67). Once these antigen-laden DCs enter the LN SCS they can migrate into the LN parenchyma and interact with T cells. Pathogens and antigens can also enter lymphatics and drain directly to LNs. Particulate antigens and pathogens are phagocytosed by macrophages in the SCS and medulla. Certain pathogens infect macrophages and DCs, notably in models of sub-cutaneous injection of viruses and bacteria (30, 68). Soluble antigen can also enter the conduit system and be picked up by LN-resident APCs (69, 70). Different infection/immunization models usually result in different antigen distribution and presentation kinetics and may also result in different DC subsets engaging in cognate interactions with T cells.

While infected APCs can directly present antigens to $CD8^+$ T cells, antigens from infected or antigen-laden APCs can also be picked up by LN-resident cDC1s and cross-presented to $CD8^+$

T cells. During cutaneous HSV infection, antigen is initially transported by dermal DCs and then transferred to resident cDC1s (71). This requirement for antigen transfer results in differential kinetics of $CD4^+$ and $CD8^+$ T cell activation during cutaneous HSV infection as $CD4^+$ T cells are activated by migratory DCs prior to $CD8^+$ T cell activation by LN-resident DCs (72). Antigen transfer from migratory to resident DCs can boost $CD8^+$ and $CD4^+$ T cell responses when DCs are infected (73, 74), potentially overcoming suppression of antigen presentation by pathogens. The molecular and cellular details of this antigen transfer to resident DCs is not known. One simple possibility is that migratory DCs carrying pathogen-derived components die in the LN and the antigen in these cells is picked up by resident DCs. More specialized alternative mechanisms such as exosomes and gap junctions have also been suggested to be required for antigen transfer (73, 75-77). Furthermore, there may be different mechanisms depending on the type of antigen that is transferred. Intact proteins can be taken up DCs and then degraded and loaded onto MHC-I molecules. Peptides generated in donor cells can also be transferred to DCs which can then be loaded onto MHC-I molecules. Finally, pMHC-I complexes from a cell can be presented on another cell, a process termed cross-dressing (78-80), although in one study this was shown to boost memory but not naïve T cell responses (80). Such mechanisms require close proximity or direct contact between antigen donor and recipient cells, further emphasizing the importance of DC positioning in SLOs. The relative contributions of each of these processes to activation of $CD8^+$ T cells probably depends on particular infection models and require further investigation.

Interestingly, antigen transfer to resident DCs and subsequent cross-presentation appears to be context-dependent instead of being a general rule for activation of $CD8^+$ T cells. In models of constitutive antigen presentation from peripheral tissues, $CD8^+$ T cells activation was shown to be restricted to migratory cDC1s (81-83). When antigen is abundant in tissues, including following subcutaneous injection of antigen, migratory cDC1s also activate T cells in the LNs (20). Theoretically, once antigen reaches the LN, it should be available to resident cDC1s for presentation to $CD8^+$ T cells via at least one of the mechanisms discussed above. However, specific inflammatory signals might be required to drive antigen transfer to resident cDC1s for the activation of $CD8^+$ T cells.

Antigen dose and its distribution within SLOs also has important consequences for the magnitude of $CD4^+$ and $CD8^+$ T cell responses. After protein immunization, LN-resident cDC2s acquire more antigen compared LN-resident cDC1s due to their localization in the proximity of lymphatic sinuses (69). This difference in antigen loading resulted in a more dramatic decrease in $CD8^+$ T cell responses compared to $CD4^+$ T cell responses when antigen was limiting (69).

In addition, the efficiency of antigen uptake and presentation by different cells may also influence T cell activation. Although in most infections, cDCs are the major cell population that is presenting antigens to T cells, other cells such as B cells, macrophages and stromal cells can also present antigens (8-10, 84). Such APCs may present fewer peptide-MHC complexes and lack co-stimulatory signals. Yet, it is possible that these less potent encounters may cumulatively contribute to efficient T cell priming. Furthermore, sub-optimal T-DC interactions can usually be compensated by an increase in the availability of antigen (85, 86). Further work is required to understand how T cells acquire signals from multiple APCs during the process of activation and expansion in SLOs.

DYNAMICS OF T CELL INTERACTIONS WITH DENDRITIC CELLS DURING PRIMING

Chemotactic cues direct the positioning of DCs and T cells in uninflamed SLOs and control attraction and repositioning of cells during immune responses. Recognition of pathogen-derived pMHC complexes controls interactions between T cells and DCs during the early phases of immune responses. T cells can progressively accumulate, or sum activation signals through serial brief interactions with multiple APC, in part by progressive NFAT activation (87), and potentially also via the summation of other signals and the reorganization of TCR-CD3 complexes into nanoclusters (88). This scanning behavior can last for hours and is referred to as phase 1 interactions, which are subsequently followed by stable T-DC interactions (phase 2) (89). How long T cells spend in stable contact with DCs is unclear, given the difficulty of reliably following such interactions over long periods via intravital microscopy. Following stable interactions with DCs, T cells break away and resume motility (phase 3) before beginning to divide. This appears to be supported by a phase of reduced responsiveness to TCR-mediated signals that presumably promotes T cell proliferation by

preventing continued interactions with DCs (90). After dividing T cells might continue to interact with DCs, although these interactions are presumably transient and not stable. Interactions with DCs during this late phase of T cell priming may be important for the receipt of help signals by $CD8^+$ T cells. During this phase, LFA-1-dependent homotypic interactions between activated $CD8^+$ T cells also facilitates expansion and differentiation (91).

Multiple factors influence the type and duration of T-DC contacts (**Figure 1**). Low levels of antigen or low affinity T cell receptor-pMHC interactions result in transient contact between T cells and DCs. Stable T-DC interactions, but not transient interactions, require ICAM-1 on DCs (92). High dose antigen can facilitate rapid induction of phase 2 interactions, effectively bypassing transient interactions (93). In contrast, low dose antigens can induce the initial activation and proliferation of $CD8^+$ T cells in the absence of stable T-DC interactions (86). Low affinity TCR-pMHC interactions result in brief interactions with APCs, as opposed to stable T-APC synapses induced by high affinity interactions (94). Moreover, elegant work demonstrated that low affinity interactions resulted in shorter duration phase 2 interactions between T cells and DCs (95). Additionally, clonal competition can reduce the duration of T-DC interactions when antigen is limiting and the T cell precursor frequency is high (85). Terminating TCR signals by blocking MHC-II was also found to induce $CD4^+$ T cells to detach from DCs (96). Notably, DCs appear to be able to sustain both transient and stable interactions with T cells of different specificities (93), suggesting that DCs may simultaneously contribute to multiple phases of T cell priming. Since much of what we know about this process stems from experiments involving immunization with antigen-loaded APCs, it will be important to ascertain whether the same rules apply during infections.

Although it was initially proposed that stable T-DC interactions were required for proper activation of T cells, initial activation and proliferation of T cells can occur following multiple brief T-DC interactions (86, 92). In experiments where DCs were pulsed with low dose antigen, resulting in only transient T-DC interactions, $CD8^+$ T cell differentiation and memory formation was impaired (86). This suggests that strong TCR triggering that induces arrest and adhesion to DCs is important for full T cell activation. Notably, signals that prevent strong TCR signalling, including the co-inhibitory receptors CTLA-4 and PD-1, can also reduce

stable interactions with DCs (97, 98). It is unclear whether transient phase 1 interactions are also insufficient to induce fully functional T cells during the context of infection, where additional signals from different APC subsets, inflammation, $CD4^+$ T cell help, and other factors contribute to effector and memory T cell differentiation.

The clustering of T cells around DCs that is observed in SLOs during the early phase of immune responses is a convenient measure of cognate T-APC interactions. Although most obvious under experimental conditions where TCR transgenic T cells are adoptively transferred into mice prior to infection, it is clear that such cluster formation is also a feature of endogenous T cell responses to infection (99). T cell-DC clusters provide a concentration of signals, including cytokines and chemokines that attract T cells to increase the likelihood of activation and may expose T cells to an optimized microenvironment for priming. Other immune cells can also be recruited to T-DC clusters to support priming, including pDCs, NK cells, neutrophils and monocytes. During modified vaccinia virus Ankara (MVA) infection, $CCR5^+$ pDCs were recruited to T cell clusters, providing type I IFN for efficient DC activation (54). NK cells are also observed within T cell clusters and IFNγ produced by NK cells was shown to be important for the formation of these cluster as well as CXCL9 and IL-12 production by DCs (55). Furthermore, NK cells are also potent producers of XCL1, which might contribute to formation of $CD8^+$ T cell clusters by reinforcing the recruitment of $XCR1^+$ DCs (100). These observations support the concept that $CD8^+$ T cell clusters around DCs provide a specialized microenvironment that assists the differentiation of effector T cells.

COMPLEXITY OF T CELL-APC INTERACTIONS INFLUENCED BY INFECTION ROUTE AND ANTIGEN ABUNDANCE

T cells can acquire activation signals from DCs across a spectrum of cellular interactions that are influenced by the route of infection or immunization and the abundance of antigen in the tissues (**Figure 2**). Numerous studies have demonstrated the specialization of DC subsets for the priming of T cells. cDC1 were shown to dominate presentation to CD8+ T cells after infection with viral and bacterial pathogens (101, 102). Following infection or immunization of the skin, cDC2 migrate to the draining LNs where these DCs mediate the transfer of

antigens to LN-resident cDC1s for presentation to CD8+ T cells (71). In this context, it was shown that migratory cDC1s contribute minimally to early antigen presentation (83). Yet, when antigen is abundant in the skin, such as following viral spread during zosteriform HSV infection or immunization of the skin with soluble antigen, migratory CD103+ XCR1+ cDC1s can activate CD8+ T cells (20, 83). In contrast, cDC2s dominate the presentation of antigen to CD4+ T cells for the priming of effector subsets (32, 72, 103, 104).

Many of these studies relied upon the *ex vivo* presentation of antigens for the identification of DC subsets capable of stimulating T cells, thereby removing the *in vivo* context of the cellular interactions involved in T cell priming. Nonetheless, $XCR1^+$ cDC1s have been shown to be the main DC subset present in clusters of $CD8^+$ T cells during T cell priming in several infection and immunization models (54, 69, 72, 105). During skin infection with HSV, where virus is excluded from the LNs, $CD4^+$ T cells were primed first by migratory cDC2s and $CD8^+$ T cells were found to cluster exclusively with cDC1s later in the response (72). We do not know if transient interactions with migratory APCs precede stable interactions with cDC1s and facilitate priming.

When viruses directly infect cells in the LNs, CD8+ T cells will rapidly interact with virus infected cells as well as with DCs recruited to regions of infection (30, 72, 105, 106). CD8+ T cells subsequently also interact with uninfected DCs, presumably promoting optimal priming. Removal of DCs did not prevent T cell activation and expansion when directly infected cells were present to stimulate the T cells. However, T cell activation was demonstrated to be impaired in the absence of DCs, either due to poor priming by infected macrophages or lack of CD4+ T cell help that is delivered via DCs (30, 105).

In contrast to $CD8^+$ T cells, $CD4^+$ T cells predominantly cluster with cDC2s, and hence $CD4^+$ and $CD8^+$ T cell generally form clusters on different DCs during the early phase of immune responses (69, 72, 105, 107). This division of labor is striking but may be important to help promote appropriate T cell activation and differentiation. Notably, both Tfh and Th2 responses require interactions with cDC2s (32, 104) and this potentially occurs via specialization within the cDC2 subset (108). Although resident DCs that pick-up antigens from conduits can rapidly present antigen to CD4+ T cells after immunization, migratory

APCs that arrive later are required for an optimal T cell response (109, 110). Preferential clustering of $CD4^+$ and $CD8^+$ T cells with different cDC subsets may also be promoted by the differential localization of T cells and DC subsets within T cell zones of LNs and spleen (39, 40, 69). CXCR3 expression on $CD4^+$ T cells and CXCL10 produced by DCs is also important for the formation $CD4^+$ T cell clusters during priming (46, 111).

$CD4^+$ T cells also need to interact with the DCs that stimulate $CD8^+$ T cells to provide help for $CD8^+$ T cell responses (112, 113). The spatiotemporal kinetics of the interaction between $CD4^+$ T cell and DC in the context of DC licensing remain elusive. Some aspects of these dynamic interactions have been described and $XCR1^+$ cDC1s emerged as the crucial stage for the delivery of $CD4^+$ T cell help to $CD8^+$ T cells (72, 105). $CD4^+$ T cells were shown to interact transiently with T cell-$XCR1^+$ cDC1 clusters during the late phase of priming (72). This occurred subsequent to CD4+ T cell activation by cDC2s suggesting that CD4 help is provided by activated $CD4^+$ T cells. Despite these observations suggesting that pre-activated $CD4^+$ T cells interact transiently with $XCR1^+$ cDC1s to provide help, it is not clear whether cDC1s provide further differentiation signals to $CD4^+$ T cells during this phase, especially for the development of Th1 cells. Whether $CD8^+$ T cells also require pre-activation or can simultaneously receive priming and help signals from cDC1s is unclear. A likely scenario is that both possibilities occur during infections. A recent elegant study demonstrated that early CD40-CD40L interactions involve cognate recognition of DCs, whereas at later stages cognate activated CD4+ T cells appeared to be able to provide help signals wholesale, presumably via transient non-cognate interactions with DCs while navigating through the T cell zone (114). This mode of help delivery by $CD4^+$ T cells would increase the number of licensed DCs that are available for priming of $CD8^+$ T cells. Considerably more insight into the dynamics of how $CD4^+$ T cell help is delivered during T cell priming is required to fully understand this process.

REGULATORY T CELLS AND REORGANIZATION OF LYMPHOID ORGAN MICROANATOMY

Reorganization of T cells and DCs in SLOs during infection also poses unique challenges in terms of regulation of immune responses. For example, increased migration of cDC1s into T cell zones, combined with inflammation potentially increases the risk of bystander

activation of autoreactive T cells. Foxp3^{+} regulatory CD4^{+} T cells (Tregs) are master regulators of T cell responses due to their suppressive abilities in both homeostatic and inflammatory conditions (115, 116). Tregs are equipped with both contact dependent and independent suppressive mechanisms. For example, contact dependent mechanisms such as engagement of the costimulatory molecules CD80/86 with CTLA4 requires interactions between DCs and Tregs. Additionally, contact independent mechanisms such as IL-2 depletion due to high CD25 expression requires close proximity of Tregs to activated conventional T cells and DCs. Therefore, Treg-based regulation of conventional T cell responses also requires cellular reorganization and correct positioning in SLOs.

Although Tregs are competent suppressors of undesired T cell activation, TCR signalling in Tregs is required to fully perform their suppressive functions (117, 118). It has been shown that when DCs cannot form cognate interactions with Tregs, these DCs can activate autoreactive T cells (119). Further evidence of localized Treg function comes from the observation of STAT5^{+} Treg clusters in steady state LNs(120). These Treg clusters form around rare IL-2 producing T cells and depend on cognate interactions of Tregs (120). It was been proposed that these clusters suppress undesired sporadic activation of autoreactive conventional T cells by decreasing IL-2 availability.

Tregs have also been shown to engage T cell clusters around DCs while interacting with both activated T cells and DCs in a CTLA4-dependent manner (121). Disruption of these interactions increased the size of T-DC clusters and enhanced the proliferation of T cells (121). TCR transgenic Tregs were also observed to form clusters around DCs thereby reducing the formation of clusters of conventional T cell and DCs (122, 123).

Tregs may also play important roles in shaping of the T cell repertoire and diversity during T cell responses. One study showed that the presence of Tregs increased the overall avidity of responding T cells as Tregs limited the production of CCL3/4/5 chemokines by DCs. The authors proposed that this reduction in chemokine production reduced the interaction of low avidity T cells with DCs (124). This may reflect the ability of Tregs to control chemokine expression by DCs in SLOs (including CXCL10 and CCL2), which allows effector T cells to egress and migrate to infected tissues (125). Similarly, low affinity T cells undergo shorter

duration interactions with DCs and are the first to exit the SLOs (95, 126). Hence, by altering the microenvironment in SLOs during infection, Tregs can influence the duration or quality of interactions with DCs and may also impact migration and positioning of responding cells.

PATHOGENS AND REORGANIZATION OF LYMPHOID ORGAN MICROANATOMY

Organization of T cells and DCs in SLOs creates specialized niches by bringing cells together and can provide a means to access certain anatomical compartments. It is not surprising that some pathogens try to use this machinery to their advantage. One example is the spread of *Listeria* in the spleen. Migration of *Listeria*-infected DCs from MZ to WP is required for the transport and proliferation of *Listeria* in the WP (127). When this migration was blocked by pertussis toxin treatment, bacterial growth was reduced while initial capture of bacteria was unaffected. In this infection, DCs coordinate clustering and activation of myeloid cells and NK cells that together help to contain the pathogen (128). Interestingly, initial infection of DCs with *Listeria* may require clustering of cDC1s with $CD169^+$ macrophages that capture *Listeria* in the MZ (129). Another example is the systemic spread of *Toxoplasma gondii* in T cells (130). *T. gondii* is initially captured by SCS macrophages and DCs in the LN. The parasite directly invades T cells during the clustering of T cells around infected macrophages and DCs.

In addition to hijacking immune cell migration, pathogens have developed mechanisms to counteract the reorganization in SLOs, as part of their arms race with the host. For example, *Plasmodium* pigment hemozoin reduces T cell clustering around DCs (131), and *Leishmania donovani* can prevent the migration of DCs from MZ to WP by downregulating their CCR7 expression (132). Further studies are required to improve our understanding on the roles of pathogens in intra-organ migration of immune cells.

T CELL MEMORY AND RESIDENCE IN LYMPHOID ORGANS

Memory T cells can provide enhanced protection against re-exposure to pathogens due to the localization of memory T cells within most tissues around the body and the capacity of

these cells to exert rapid effector functions. Circulating memory T cells include central memory T cells (T_{CM}) that express receptors including CCR7 and CD62L that facilitate entry into lymphoid organs, and effector memory T cells (T_{EM}) that are primarily found in blood and peripheral tissues. Recent evidence suggests that T_{EM} can be divided into multiple subsets: high expression of the chemokine receptor CX3CR1 identifies T_{EM} cells that are predominant in the blood, whereas lower expression was observed on T_{EM} cells that enter peripheral tissues (133). In SLOs, though the majority of T cells are antigen-inexperienced, subsets of memory T cells are also present. Under homeostatic conditions, $CD8^+$ T_{CM} cells were shown to reside in the outer paracortex and medullary areas of LNs, while the deeper regions of the T cell area contain naive T cells (59, 130). Memory T cells often express higher levels of CXCR3 compared to naive T cells (61), which may direct these cells to the outer regions of the paracortex (59). In contrast, a separate study showed that naïve and memory $CD8^+$ T cells localize in the paracortex (57). This discrepancy is possibly due to use of different memory T cells for comparison as the former studies used *in vivo* generated memory T cells while the latter used *in vitro* generated cells (57, 59, 130). Yet, $CD8^+$ T_{CM} cells in LNs were shown to recirculate with slower kinetics and express lower levels of migration-related genes such as *Ccr7*, *S1pr1* and *Klf2* compared to naïve cells, which might contribute to their distinct localization in LNs (134).

A distinct difference between LNs and the spleen is that spleen contains a substantial pool of T_{EM} cells, in addition to T_{CM} and naïve T cells. T_{EM} cells in the spleen are mostly found in the RP while T_{CM} and naïve cells are enriched in the WP (55, 135, 136). This difference in localization is probably due to lower CCR7 expression by T_{EM} cells compared to T_{CM} and naïve T cells (135), as CCR7 binds CCL21 and CCL19 and is required for efficient homing to T cell zones. However, this separation has been most clearly demonstrated for $CD8^+$ T cells, and whether subsets of memory $CD4^+$ T cells, including Th2 cells, reside in preferential niches in the spleen is less clear.

Memory T cells that enter non-lymphoid tissues can form populations of tissue-resident T cells (T_{RM}) that may persist for long periods without recirculating around the body (137). T_{RM} have been shown to recognise antigens and respond rapidly in situ, producing cytokines that can recruit other immune cells (including circulating memory T cells) while proliferating

to maintain local densities of memory T cells within tissues (138, 139). The localization of T_{RM} cells within tissues plays key roles in protective immunity, with $CD8^+$ T_{RM} cells constrained to epithelial layers in tissues such as the skin where they can control infections including Herpes Simplex virus and Vaccinia virus (140, 141). Expression of the chemokine receptors CXCR6, CXCR3 and CCR10 are required for recruitment of effector T cells into the skin where they develop and persist as T_{RM} cells (142, 143). Similarly, CXCR6 is required for the formation of T_{RM} cells within the blood-rich sinusoids of the liver that can provide protective immunity against malaria infection (144). Conversely, T_{RM} cells in all tissues appear to downregulate expression of receptors required for exit from the tissues, including CCR7 and S1PR1 (143). T_{RM} cells are also highly motile within tissues, including the highly constrained environment of the skin epidermis where these cells utilise cytoskeletal components including microtubules and the protein DOCK8 to navigate the tissues and maintain cell shape (142, 145). This enables T_{RM} to form a highly protective immune surveillance network at key sites of pathogen entry.

Conspicuously, populations of tissue-resident $CD8^+$ T cells are also found in SLOs in mice and in humans (134, 146-148). $CD8^+$ T_{RM} cells in LNs express CXCR3 and mainly reside around subcapsular and medullary sinuses, suggesting that they contribute to early responses against infections due to their strategic positioning, similar to T_{RM} cells in non-lymphoid organs (146, 147). Although SLO-resident $CD8^+$ T cells share some phenotypic properties with T_{RM} cells in non-lymphoid tissues, including CD69 expression (146), the precise ontogeny and functional properties of these cells remains to be established. Importantly, local restimulation may increase numbers of SLO T_{RM} cells to boost regional immunity (146).

A substantial proportion of $CD62L^{lo}CD44^{hi}$ memory $CD4^+$ T cells have also been shown to stay in SLOs as resident cells in several contexts (149-153). These cells often have a T_{FH} phenotype and reside in B cell follicles, though some don't express CXCR5 and can be found in T cell areas (149). Furthermore, resident memory $\gamma\delta$ T cells were also found in LNs after oral *Listeria monocytogenes* infection and were located in medullary regions and the IFRs and these cells formed clusters around bacteria after rechallenge (154). Hence, naïve and memory T cells occupy different niches within LNs, yet the implications of these differences for protective immune responses are yet to be fully explored. It will be important to

determine if memory T cells resident in SLOs coordinate recall responses and whether pools of T_{RM} increase with age and possibly redirect where early T cell-DC interactions occur during secondary infections.

CONCLUDING REMARKS AND OUTLOOK

We still know relatively little about the type, duration and scope of interactions that occur between T cells and subsets of DC during the process of T cell priming in SLOs, particularly during the context of infections. What we have learnt from models using DCs pulsed with antigens provides critical insight into these basic processes. Yet, it is clear that T cells can interact with multiple APCs during the process of priming (**Figure 2**). $CD8^+$ T cells that are primed by $XCR1^+$ cDC1s may also be initially activated by infected cells if pathogens gain entry to the SLOs. $CD4^+$ T cells are predominantly activated by cDC2s, but then require access to cDC1s to provide help signals for proper $CD8^+$ T cell priming. A major technical hurdle to dissecting these events is the challenge of following individual cells for days in order to discern, for instance, if $CD8^+$ T cells that undergo initial interactions with infected cells can then transition to stable interactions with non-infected cDC1s, or if distinct naïve T cells are primed by infected cells and cDC1s. Additionally, low antigen levels, low affinity T cell populations and pre-existing memory populations that can compete for pMHC molecules can alter the duration of stable T-DC interactions. Further studies are needed to determine the importance of transient interactions for T cell priming, and if inflammation and $CD4^+$ T cell help signals contribute to T cell priming via summation of signals obtained through brief interactions with multiple APCs during the context of infection.

T cell activation and interactions with APCs also occurs upon the underlying stromal cell architecture of the SLOs (26). The heterogeneity of the cells that construct the stromal network influences the positioning of T cells and DCs that presumably increases the efficiency of T cell priming. To what degree cross-talk between immune cells and the stromal cells fine-tunes cellular positioning during immune responses will be important to determine. Equally, disruption of this process or the chemoattractant gradients that control cell positioning by pathogens could impact on immune outcomes. Sophisticated techniques

including optical clearing and imaging of whole SLOs and histocytometry approaches are needed to appropriately dissect the complexity of the lymphoid tissues, cellular localization and interactions during immune responses to infection.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

FIGURES

Figure 1. Mode and duration of interactions between T cells and DCs during priming.
T cells undergo brief interactions (phase 1) with DCs presenting low levels of antigen and in the context of low affinity TCR-pMHC interactions. Via the summation of activation signals these T cells may progress directly to cell division or undergo sustained interactions and clustering with DCs (phase 2), followed by disengagement and proliferation of the T cells (phase 3). When DCs present high levels of antigen T cells undergo shorter duration phase 1 interactions and can rapidly undergo stable interactions and clustering that result in proliferation. Low affinity TCR-pMHC interactions induce earlier disengagement of T cells from DCs for proliferation. Subsequent to cell division, activated T cells may undergo multiple further interactions with DCs that are likely to be transient and may facilitate tuning of effector differentiation and delivery of help.

Figure 2. Potential interactions between T cells and APCs during T cell priming following infection or immunization. A, Pathogens that enter the LNs can infect LN-resident DCs or macrophages and induce $CD8^+$ T cell activation via direct presentation of antigens on MHC-I. LN-resident cDC2s that acquire antigen can induce clustering and activation of $CD4^+$ T cells. i) $CD8^+$ T cells pre-activated by infected DCs might then interact with LN-resident cDC1s to receive help signals by pre-activated CD4+ T cells. Subsequent acquisition of antigen by LN-resident cDC1s may facilitate ii) the priming of additional naïve T cells. B, During natural infections where low levels of antigen are present in the tissues, migratory cDC2s transport antigen to LNs where they first activate CD4+ T cells. Whether these DCs can also stimulate CD8+ T cells via brief interactions is unknown. The transfer of antigen to LN-resident cDC1s facilitates the subsequent priming of naïve CD8+ T cells. Activated CD4+ T cells deliver help signals to cDC1s, likely via transient T-DC interactions. C, During immunization abundant antigen is delivered to the tissues and migratory cDC1s and cDC2s that capture antigens can induce clustering and activation of CD8+ and CD4+ T cells, respectively. LN-resident DCs also acquire antigen and can contribute to T cell priming. D, When soluble antigens drain to the LNs and are captured by DCs, CD4+ T cells are activated by LN-resident cDC2s and CD8+ T cells by LN-resident cDC1s. How CD4+ T cells deliver help and which DCs are involved when antigen is abundant (C and D) is unclear. Curved dashed arrows are shown to depict transient as opposed to stable interactions between T cells and DCs.

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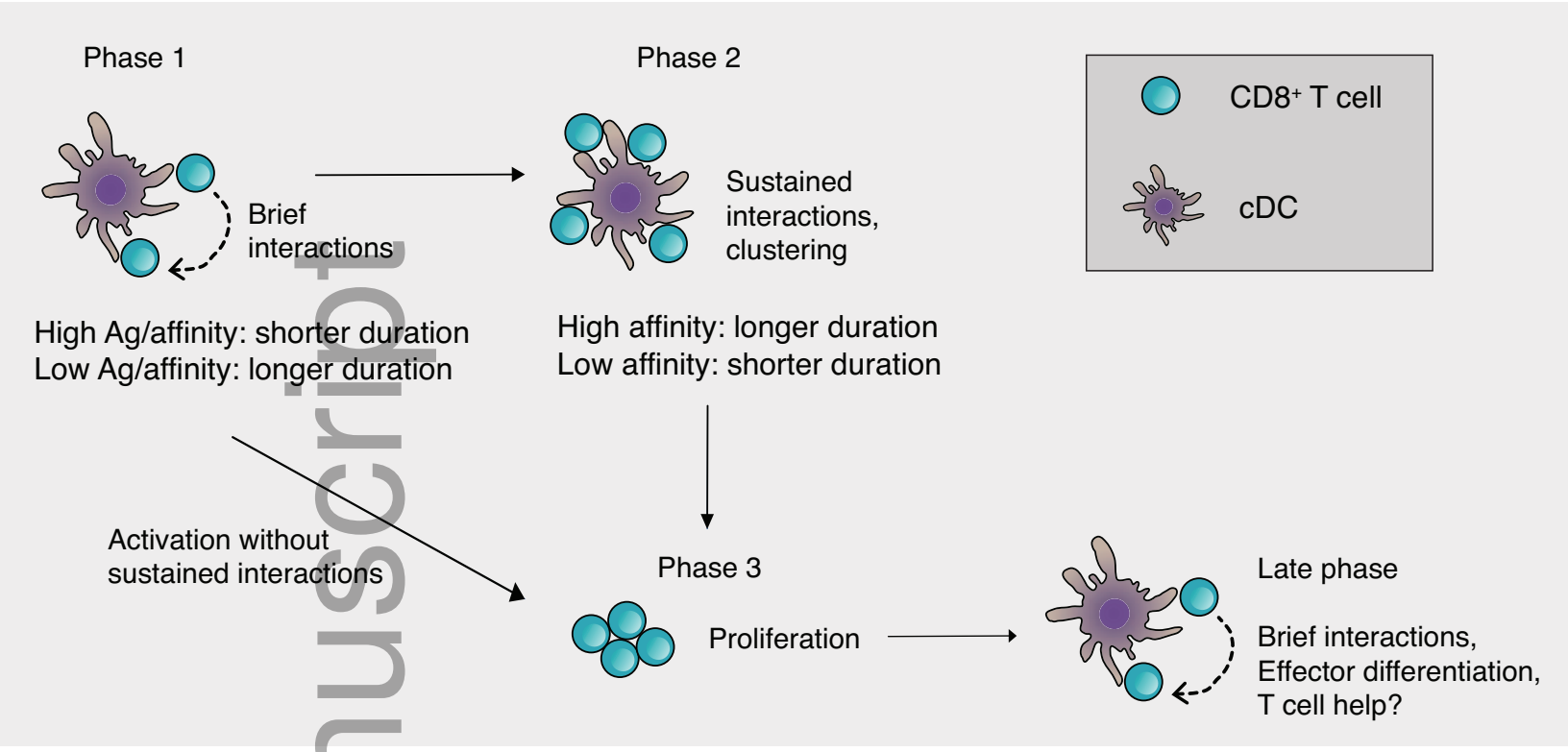

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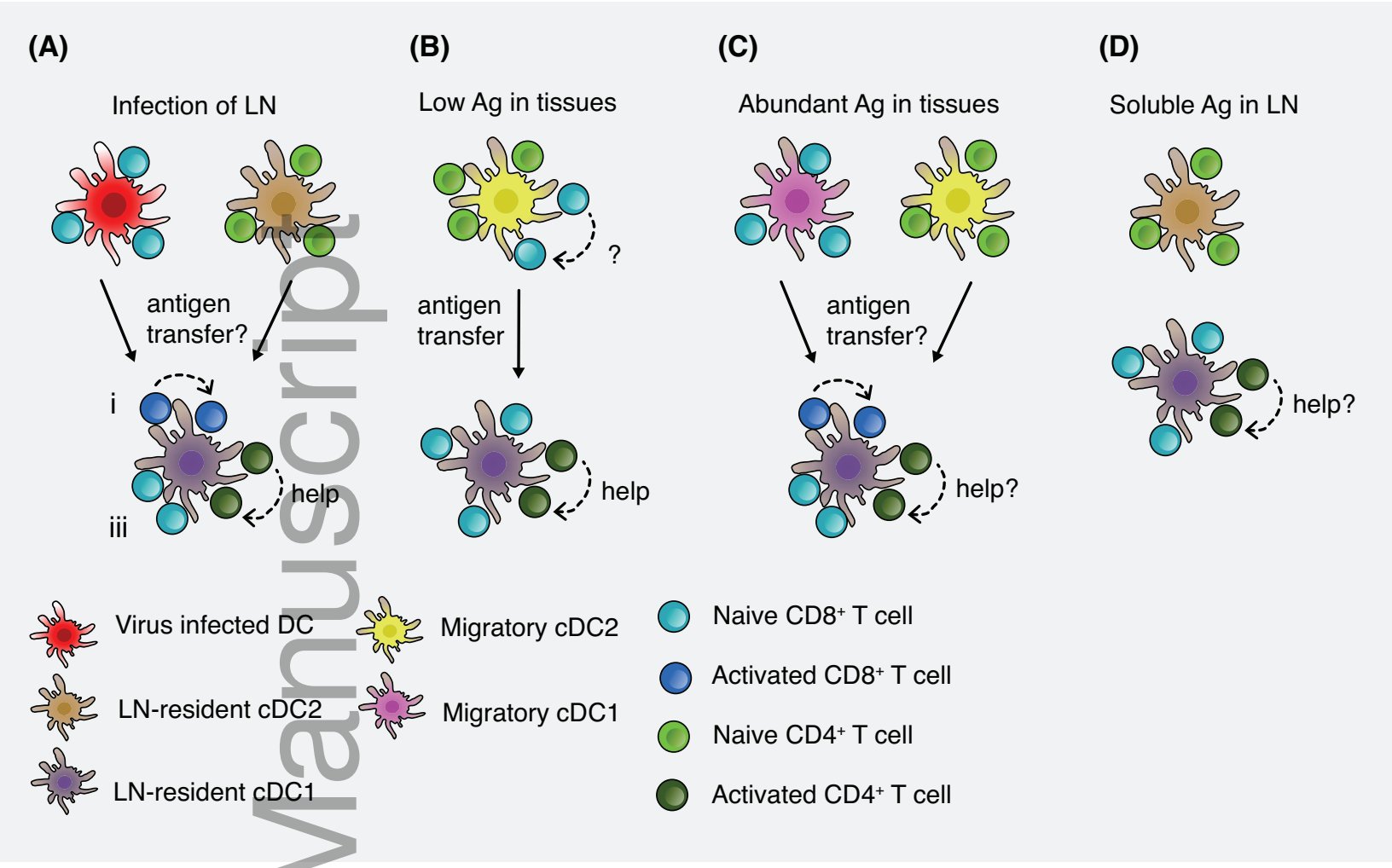

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