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Minireview

The Role of Lymphatic Niches in T Cell Differentiation

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Long-term immunity to many viral and bacterial pathogens requires CD8$^+$ memory T cell development, and the induction of long-lasting CD8$^+$ memory T cells from a naïve, undifferentiated state is a major goal of vaccine design. Formation of the memory CD8$^+$ T cell compartment is highly dependent on the early activation cues received by naïve CD8$^+$ T cells during primary infection. This review aims to highlight the cellularity of various niches within the lymph node and emphasize recent evidence suggesting that distinct types of T cell activation and differentiation occur within different immune contexts in lymphoid organs.

TRADITIONAL VIEWS: NICHES IN STEM CELL BIOLOGY

Successful development and maintenance of tissues within the body relies on the progeny of undifferentiated or less-differentiated stem cells within the population to maintain their lineage (Knoblich, 2008; Morrison and Kimble, 2006; Neumuller and Knoblich, 2009). During the early stages of development, cell division is characterized by the asymmetric segregation of cell fate determinants into daughter cells to promote cellular heterogeneity and differentiation through an evolutionarily conserved mechanism known as asymmetric cell division (ACD). For continuous survival and renewal, it is essential that parent cells retain their stemness after division and daughter cells integrate intrinsic and extrinsic cues from a highly regulated microenvironment referred to as a niche. Niches provide an intimate spatial association surrounded by highly cellular and vascularized stroma that produce extracellular matrix proteins, chemokines, and cytokines to maintain the environment and support cellular identity, survival, and division (Jones and Wagers, 2008). Cellular and chemical borders maintain the anatomy of the niche, and the expression of specific receptors and ligands coordinate interactions and functional processes. Stem cells interact with the niche through integrin-dependent contacts, receiving homeostatic, survival, and proliferation signals. Integrins play a key role in regulating stem cell homing, migration, and retention, as well as a close interaction with the support cells within the niches (Andressen et al., 1998; Benitah et al., 2005; Frye et al., 2003; Fuchs et al., 2004; Hirsch et al., 1996; Watt, 2002; Yang et al., 2015). Furthermore, integrins, specifically those containing a β1 subunit, can modify the axis of cell division in stem cells so that the two daughter cells are located in different microenvironments (Goulas et al., 2012; Gui and Homer, 2012; Metchat et al., 2015; Petridou and Skourides, 2016; Streuli, 2009; Watt, 2002), which has lasting implications for lineage determination.

LYMPHATIC NICHES

The immune system is comprised of primary and secondary lymphoid organs. Both the thymus and the bone marrow are sites of immune cell origin, giving rise to the myeloid and lymphoid lineages. Development within and egress from these sites is dependent on several factors, including cell-cell interactions, cytokine and chemokine signals, and receptor expression level. Lymphoid organs support identity of undifferentiated cells during homeostasis, maintaining quiescence and multipotency, while also giving rise to differentiated cells capable of an immune response upon pathogenic challenge. The primary constituents of niches are stromal cells, providing integrin, chemokine, and cytokine cues. Both the thymus and the lymph node host a number of stromal cells that segregate into specific regions and create macroniches, guiding cell-cell interactions. Lymphatic niches must also stimulate activation, differentiation, and proliferation while retaining a subset of multipotent cells. A successful niche integrates signals to balance a cellular response with the needs of the organism, preventing depletion of low-frequency cells while restricting excessive expansion. Therefore, lymphatic niches must maintain a stable pool of cells poised for an immune response while tightly regulating quiescence and activation to avoid inappropriate immune responses and autoimmunity.

As primary sites of immune cell maintenance and initiation of immune responses the lymph nodes act as a niche for various immune cells. The lymphatic architecture can be divided into several regions both anatomically and functionally (von Andrian and Mempel, 2003). The outermost layer of the lymph node is the subcapsular sinus (SCS), where the afferent lymph enters the tissue and macrophages reside for access to soluble factors in the lymph (Berney et al., 1999; Carrasco and Batista, 2007; Junt et al., 2007; Kuka and Iannacone, 2014; Phan et al., 2007; 2009). Below this layer is the cortex, which can be divided

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into two regions – the follicular cortex (outer cortex) and the paracortex (inner cortex). The outer cortex, known as the B cell zone, is rich in B cells, $CD4^+$ follicular T helper cells, and follicular dendritic cells (FDCs), while the paracortex, referred to as the T cell zone, includes $CD4^+$ and $CD8^+$ T cells, antigen-presenting cells (APCs) known as dendritic cells (DCs), and fibroblastic reticular cells (FRCs) (Marchesi and Gowans, 1964; Mondino et al., 1996). It is here that adaptive immunity is initiated by interactions between T cells and DCs. Finally, the innermost region of the lymph node is the medulla, comprised of macrophages and plasma cells and containing medullary cords, arterial and venous vessels, and sinusoidal vessels that flow into the efferent lymphatics (Gretz et al., 1997; Kelly, 1975; M'Rini et al., 2003). Expression of specific receptors and ligands by resident stromal and lymphatic cells provides entry and guides interactions within these macroniches. The importance of these cues for niche development and maintenance has been demonstrated in studies where chemokine receptor and chemokine knockout mice exhibit reorganized lymph node structure and altered cell localization and responses (Förster et al., 1999; Junt et al., 2005; Khader et al., 2007; Okada et al., 2002; Voigt et al., 2000).

Over the last 15 years, intravital multi-photon imaging has led to many pivotal studies characterizing the interactions and behavior of cells within the lymph node during immune responses (Bajenoff et al., 2006; Beuneu et al., 2006; Bousso and Robey, 2003; Castellino et al., 2006; Garcia et al., 2007; Henrickson et al., 2008; Hugues et al., 2004; 2007b; Lindquist et al., 2004; Miller et al., 2002; 2004a; 2004b; Stoll et al., 2002; Wei et al., 2007). While these groundbreaking studies have provided insight into important migration patterns and interactions during both homeostasis and infection, their measurements are limited to specific regions and relatively narrow time periods. In addition to its macroniches, the lymph node grows more complex under inflammatory conditions, with global changes to structure and the formation of microniches that guide immune responses. Following sections will focus on the changes in lymphatic niches that regulate T cell differentiation.

STROMAL CELLS

The lymphatic stroma makes up ~1% of the cellularity in the lymph node and comprises several cell types: blood endothelial cells, lymphatic endothelial cells, FRCs, integrin $\alpha7$ pericytes (IAPs), and FDCs (Girard et al., 2012; Malhotra et al., 2012; Turley et al., 2010). These cells are distinguished by expression of podoplanin (gp38) and platelet endothelial cell adhesion molecule-1 (CD31), and they provide structural support and form various venules within the lymph node (Malhotra et al., 2012). Blood endothelial cells form capillaries and high-endothelial venules (HEVs), which are entry sites for leukocytes leaving the blood (Girard and Springer, 1995; Marchesi and Gowans, 1964). Lymphatic endothelial cells construct the afferent and efferent lymphatic vessels, which act as entry sites for DCs exiting the lymph (afferent) and egress sites into the lymphatics (efferent) (Förster et al., 2012; Grigorova et al., 2009; 2010; Sinha et al., 2009). FRCs and FDCs ensheath the reticular collagen network and scaffold the T and B cell zones, respectively. Here, they act as a network of fibers covered in chemokines, exploited as a track for migrating lymphocytes (Bajenoff et al., 2006; Link et al., 2007; Luther et al., 2000). A small fraction of FRC-like cells, known as marginal reticular cells, reside within the SCS and express CXCL13, RANK-L, and MAdCAM-1, molecules that are essential for lymph node development, initial regionalization, and stromal cell growth (Katakai et al., 2008; Roozendaal et al., 2009). Finally, IAPs are the most recently characterized lymphatic stromal cell, composing a small portion of the stroma (~10%). They are located throughout the cortex and medulla and uniquely express integrin $\alpha7$. Their location within the lymph node suggests various interactions and explains their expression of a mix of T cell, B cell, and APC molecules, such as chemokines CCL21/19 and CXCL9/10/13 and APC stimulating factors Flt3L, BAFF, and CSF-1 (Malhotra et al., 2012).

Stromal cells are not merely physical support for the lymph node; they serve many functions during homeostasis and inflammation. FRCs and FDCs form a conduit system that channels small molecules under 70 kDa in size from the SCS to HEV, including chemokines, antigens, and cytokines (Gretz et al., 2000; Roozendaal et al., 2009; Sixt et al., 2005). This is especially important considering that chemokine decoration of HEVs leads to leukocyte adhesion and transmigration from the blood into the lymph node, promoting an immune response. Conduits in the lymph node can process soluble antigens provided through the conduit system, supplying APCs with tissue-derived antigens for stimulation. FRCs can produce extracellular matrix (ECM) proteins and CCL21/19 to guide T cell motility as well as cytokines IL-7 and IL-15 to provide homeostatic survival signals (Link et al., 2007; Luther et al., 2000). FRC expression of CCL21/19 also attract DCs through chemokine receptor CCR7, thereby allowing DCs to migrate to and adhere within the T cell zone (Seth et al., 2011). This enhances the probability of successful encounters between naïve T cells and antigen (Ag)-bearing DCs. On the other hand, FDCs express CD35, CD23, and MAdCAM-1 and play and important role in B cell recruitment and antibody production (Ansel et al., 2000). Additionally, FRCs can dampen the T cell response by upregulating transcription of the enzyme inducible nitric oxide synthase (iNOS) in response to T cell-produced interferon-γ (IFNγ) (Lukacs-Kornek et al., 2011; Siegert et al., 2011). This subsequently blocks T cell proliferation, acting as a control mechanism for inflammation (Khan et al., 2011). FRCs secrete factors such as vascular endothelial growth factor (VEGF) to stimulate HEV growth (Webster et al., 2006), increasing lymphocyte access to the lymph node entry points. The lymphatic stromal cells also contribute to peripheral T cell tolerance, as both FRCs and IAPs are capable of presenting self-antigens (Cohen et al., 2010; Fletcher et al., 2010). These cells express Aire and relevant peripheral tissue-restricted antigens under homeostatic conditions, but following inflammation, IAPs strongly alter transcription of peripheral tissue antigens.

Following infection, migratory DCs enter the lymph node from the periphery through the afferent lymphatic vessel, inducing inflammatory signals in the lymph node and triggering lymphatic stromal response. Afferent lymphatic vessels expand to enhance the recruitment of Ag-bearing DCs from the tissue (Mionnet et al., 2011). FRCs detect increase in lymph flow, inflammatory cytokines, and DCs, as well as mechanical stress and hypoxia induced by trapped lymphocytes (Yang et al., 2014). In response, FRCs produce VEGF to increase the permeability, number, and size of HEVs, enabling lymphocyte entry. FRC growth also extends into the medulla, which increases in size to accommodate short-lived plasma cells. Under these conditions, the lymph node expands several folds in size several fold while leaving the dense organization intact. This expansion of the lymphatic stroma is intended to support the impending increase of lymphocytes due to activation and proliferation, as the space available to lymphatic cells remains similar

to homeostatic conditions (Bajenoff et al., 2006).

LYMPHOCYTES

The response orchestrated by the lymphatic stromal cells guides subsequent responses in different lymphatic regions. Chemokines, cytokines, and growth factors normally expressed by APCs and lymphocytes are upregulated to ensure their recruitment and localization (Chyou et al., 2011; Yang et al., 2014). During activation, lymphocytes undergo several changes, including increases in size and altered protein expression patterns. Alterations in chemokine and integrin expression patterns during this process allow the cells to migrate and localize within different regions of the lymph node (Chtanova et al., 2009; Groom et al., 2012; Hickman et al., 2008; 2011; Kastenmuller et al., 2013; Sung et al., 2012). Both the T cell and B cell zones provide excellent examples of lymphatic macro- and microniches. A portion of $CD4^+$ T cells downregulate CCR7 and upregulate CXCR5 expression, allowing them to respond to CXCL13 produced in the B cell zone macroniche and localize at the T-B border to interact with B cells (Breitfeld et al., 2000; Schaerli et al., 2000). Furthermore, regulation of CXCR4 levels on T cells allows them to enter germinal centers, which act as specialized microniches for T-cell-dependent B cell proliferation and differentiation (Victora et al., 2010). In the paracortical macroniche, expression of CCL21/19 recruits both T cells and DCs to adhere and migrate along the FRC network. During T cell activation, multiple T cells can swarm a single DC (Mempel et al., 2004), forming microniches enriched in cytokines produced by both T cells and DC at levels unique to the cluster. Growing evidence suggests that localization within both macro- and microniches regulates cytokine and costimulatory signals and subsequent cell differentiation.

Early *in vivo* imaging studies revealed that T cell activation occurs in distinct phases defined mainly by migratory patterns of the T cell (Mempel et al., 2004) (Fig. 1A). Importantly, the $CD8^+$ T cell response is dependent on the interactions that occur during these phases. The initial phase of high motility allows for antigen scanning, a process where dynamic synapse relocation allows T cells to find an APC bearing a cognate ligand. Upon contact with such an APC, T cells decrease in speed to enable transient, motile encounters referred to as kinapses (Azar et al., 2010; Fooksman et al., 2010; Moreau et al., 2012). During these serial encounters, T cells accumulate signals from different APCs to reach the signaling threshold for immunological synapse (Grakoui et al., 1999) formation and stable conjugation (Pryshchep et al., 2014). T cells enter the second phase, characterized by low-motility T-APC interactions in spatially confined swarms (Mempel et al., 2004; Moreau et al., 2015). After some time of signal accumulation, T cells regain their motility and enter the third phase of activation, a period in which T cells undergo massive proliferation; transient contacts with DCs, other $CD8^+$ T cells, and $CD4^+$ T cells; and cytokine production (Eickhoff et al., 2015; Hor et al., 2015; Mempel et al., 2004).

Differences in initial priming events, such as patterns of transient and stable encounters with Ag-bearing APCs, has lasting implications on T cell activation, cytokine production, and effector function, both qualitatively and quantitatively. Additionally, downstream signaling is essential for the upregulation of integrin affinity, which mediates cell adhesion, costimulation, and actin reorganization critical for T cell activation, proliferation and adhesion, and the mobilization of transcription factors to the nucleus to promote the expression of genes necessary for T cell growth and differentiation. Although costimulation is primarily provided by CD28 and LFA-1 during stable T-APC interactions, transient contacts with APCs and other lymphocytes in the third phase provide $CD8^+$ T cells additional proliferation, differentiation, and survival cues through CD40-, CD27-, CD30-, 4-1BB-, OX40-, and TNFR2-mediated signals (Alzona et al., 1994; Cannons et al., 2001; Hendriks et al., 2003; Redmond et al., 2009; Twu et al., 2011). Importantly, these encounters provide feedback through reciprocal signaling to influence cytokine secretion by DCs, reinforcing the niche milieu.

FACTORS LEADING TO CELL DIFFERENTIATION

Recent observations have demonstrated that $CD8^+$ T cells fully activate and expand with as little as 24 hours of antigen stimulation (Bevan and Fink, 2001; Blattman et al., 2002; Kaech and Ahmed, 2001; van Stipdonk et al., 2001). Unlike $CD4^+$ T cells, proliferation and differentiation into effector T cells could occur without the need for additional antigen, and this fate was inherited by daughter cells without additional antigenic stimulation. These findings suggest that $CD8^+$ T cell fate may be imparted during early T cell activation through T-APC interactions (Fig. 1B). Although a limited number of factors influence $CD8^+$ T cell differentiation, APCs produce inflammatory cytokines IL-12, IFNγ, and IFNα, which regulate expansion, cytokine production, and effector programs (Joshi and Kaech, 2008). Additionally, IL-2 exposure enhances $CD8^+$ T cell proliferation and expansion. The role of cytokines is especially apparent considering that the presence of these inflammatory cytokines during weak antigen stimulation can rescue the response to a similar level as that obtained with strong antigenic exposure (Ahlers et al., 2001; Schurich et al., 2013). Furthermore, there are several cell-intrinsic factors affected by T-APC dwell time, signal accumulation, and cytokine exposure, including T-bet, Blimp-1, Bcl-3, Bcl-6, ID2, p27kip, and Bmi-1 (Cui and Kaech, 2010; Hand et al., 2010; Heffner and Fearon, 2007; Lu et al., 2014; Xin et al., 2016; Yeo and Fearon, 2011). Alternatively, localization of activating $CD8^+$ T cells in microniches within the lymph node may regulate their differentiation (Figs. 1C and 1D). In this case, $CD8^+$ T cells are exposed to varied costimulation, cytokines, chemokines, ECM, $CD4^+$ T cell help, and APC and stroma interactions, providing cues for cell fate and differentiation.

These scenarios are not mutually exclusive, and an integrated model best fits the experimental data. The presence of inflammatory cytokines during stable T-APC interactions can induce cell fate programs in $CD8^+$ T cells. The amount of cytokine exposure and costimulation received during these interactions can change chemokine receptor and integrin expression patterns. T cells can be guided to different microenvironments due to their sensitivity to various external cues, enabling localization to microniches within the lymph node that reinforce or modify the initial activation and fate signals. For instance, APCs and both activated $CD4^+$ and $CD8^+$ T cells migrate to the interfollicular region of the lymph node. This is in part due to CCR5 upregulation on newly activated $CD8^+$ T cells and their subsequent recruitment to CCL3/4-rich microenvironments created by $CD4^+$ T cell-APC interactions (Castellino and Germain, 2006; Hugues et al., 2007a). During this time, FRCs downregulate the expression of CCL21/19, shifting motility patterns from a random walk to chemotactic migration. Additionally, studies have demonstrated that terminal effector differentiation occurs in the outer regions of the lymph node. CXCL9/10 attract newly activated CXCR3-expressing $CD8^+$ T cells to Ag-rich DC and macrophage microenvironments that favor commitment to a termi-

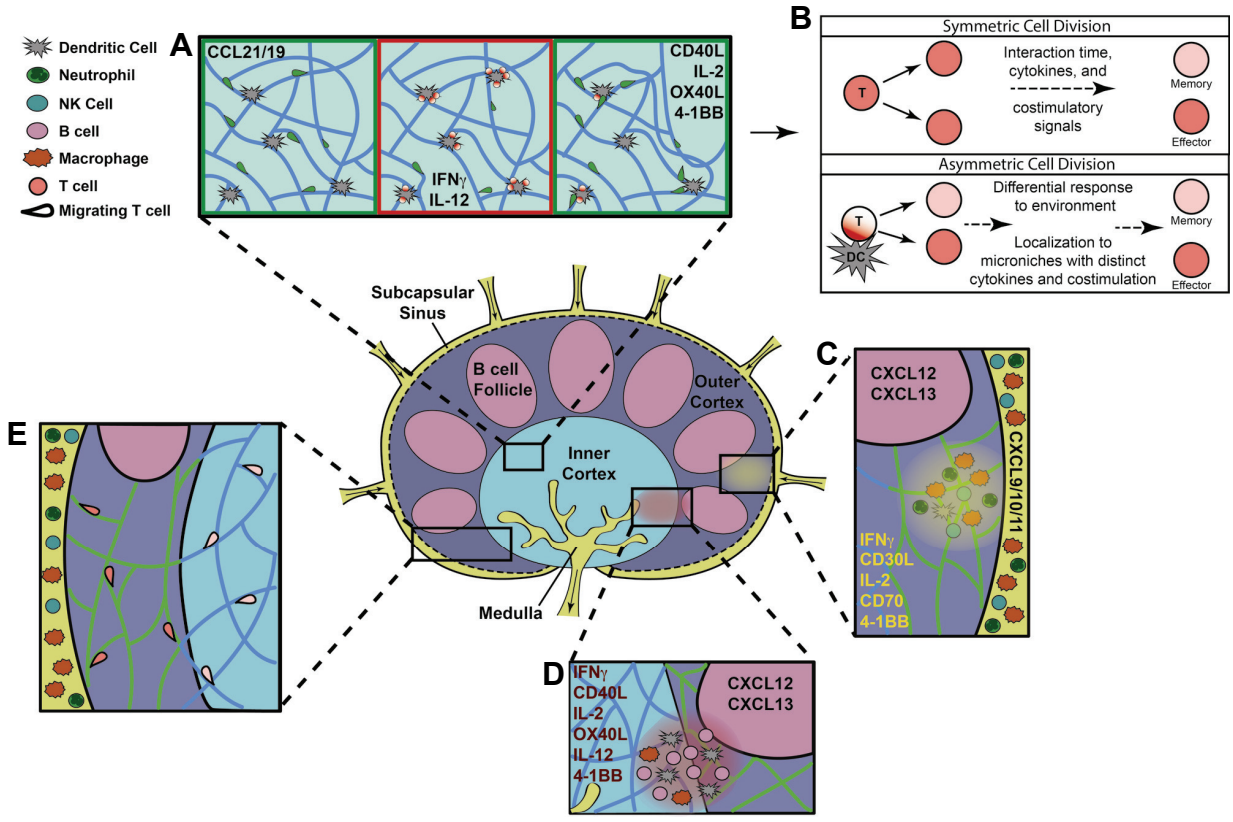

Fig. 1. Localization within the lymph node regulates differentiation. (A) CCR7$^+$ naïve CD8$^+$ T cells and Ag-bearing DCs localize in the paracortical region (Inner Cortex, blue) of the lymph node via stromal cell (blue lines) produced CCL21/CCL19 signals. Here, CD8$^+$ T cells undergo three phases of activation characterized by their motility and DC interactions. Key signals guiding cell behavior and differentiation are highlighted. (B) After initial activation signals, T cells undergo cell division. Symmetric cell division (upper panel) accounts for a majority of cell division and yields daughter cells with similar surface and intracellular protein content. Current dogma indicates that signals received during early activation, such as initial T-DC signal duration and cytokine exposure, regulate cell differentiation. Additionally, later in the immune response, activated T cells may receive additional signals to drive differentiation towards memory phenotypes. A small subset of recently activated T cells undergoes cell division while in contact with a DC (lower panel), allowing intracellular polarity dictated by signaling at the T-APC contact site to be maintained throughout division. Daughter cells proximal to the DC inherit surface and intercellular proteins that give rise to effector cells, while distal daughter cells acquire a memory phenotype. (C, D) During the inflammatory response, the lymph node undergoes chemical and physical changes that give rise to specialized cellular niches with unique cytokine and chemokine profiles. Microniche composition is determined by existing stromal cells and cells residing in the macroniche. Expression of chemokine receptors and integrins on recently activated T cells guide entry to microniches, allowing cells to receive distinct cytokine and costimulatory signals (highlighted in each panel). For example, recently activated T cells reduce CCR7 and increase CXCR3, CXCR4, and CXCR5 expression to various degrees. Diverse expression levels allow T cells to respond to chemokines expressed in the outer cortex (purple), B cell follicles (pink), and SCS (yellow), providing access to distinct microniches. Activated CD8$^+$ T cells localize through CXCL9/10/11 signals to the SCS, which contains macrophages, neutrophils, natural killer cells, and marginal reticular cells. Additionally CD4$^+$ T cells and DCs migrate to the outer cortex, which is rich in B cells, FDCs, and chemokines CXCL12 and CXCL13. While simplified, the schematic highlights the complexity of lymphatic organization and microniches (C-D), which should not be viewed as discrete entities, but rather overlapping gradients and cues. (E) Surface expression levels arising from early activation signals and cell division guide effector and memory cells into distinct regions of the lymph node. Interactions within distinct microniches provide diverse costimulation and cytokine exposure, reinforcing or altering early differentiation programs.

nal effector phenotype and IFNγ production (Barbi et al., 2007; Hickman et al., 2015; Hu et al., 2011). Cumulatively, these data suggest that signals arising from both long-lasting and transient T-APC interactions and exposure to different microenvironments regulate cell differentiation programming. The balance of cytokine exposure and costimulatory interactions coordinates the expression of surface receptors for localization (Fig. 1E). Subsequent signals activate transcription factors, inducing differentiation and effector programs. While strong effector programs are beneficial for pathogen clearance, programs for memory cell differentiation must also initiate to provide long-lasting protection. Microniches provide varied exposure levels for a range of memory and effector fate programs.

DECIDING TO REMEMBER

The expansion phase of a CD8$^+$ T cell response gives rise to a large number of effector cells at its peak (Kalia et al., 2010;

Wherry and Ahmed, 2004). During the contraction phase, over 90% of effector $CD8^+$ T cells undergo apoptosis-mediated cell death. A small fraction of the cells, referred to as memory $CD8^+$ T cells, survive. Memory T cells include central memory (T_{CM}) cells, residing in lymphatic tissues; effector memory (T_{EM}) cells, inhabiting non-lymphoid tissues and the spleen (Sallusto et al., 1999); and tissue-resident memory (T_{RM}), located in peripheral non-lymphoid tissues. While T_{CM} cells lack immediate effector function and require stimulation to produce IL-2 and proliferate extensively, T_{EM} and T_{RM} cells possess immediate effector function but lack proliferative abilities. Under homeostatic and inflamed conditions, T_{CM} cells proliferate and differentiate to maintain both T_{CM} and T_{EM} repertoires. Early in the response, $CD8^+$ T cells are defined as memory precursor effector cells (MPECs), which go on to generate T_{EM} and T_{CM} cells, and short-lived effector cells (SLECs) (Masopust et al., 2004).

The generation of memory cells can be affected by many factors, including the strength and duration of antigenic stimulus, inflammatory milieu, and the modulation of chemokine and homing receptors. There is an abundance of studies aiming to determine whether priming and the subsequent migratory cues of $CD8^+$ T cells effects the differentiation of memory and effector cells. Some models suggest that a portion of effector $CD8^+$ T cells receive additional signals at the end of the primary infection that allow them to differentiate into memory cells, while other models suggest that the effector $CD8^+$ T cells that survive the contraction phase simply adapt to their new non-inflammatory microenvironment and lose their effector programs due to a lack of further differentiation signals enforcing the new phenotype. Another model stipulates that all cells become memory precursor cells to maintain the plasticity found in this population and that further differentiation is required to program different effector functions (Allam et al., 2009; Bannard et al., 2009; Baumgartner et al., 2012; Buentke et al., 2006; Chang et al., 2007; Haining et al., 2008; Harrington et al., 2008; Huster et al., 2009; Joshi et al., 2007; Kaech et al., 2003; Lohning et al., 2008; Maris et al., 2003; Pearce and Shen, 2007; Sarkar et al., 2008; Schulz et al., 2009; Seder et al., 2008; Teixeiro et al., 2009; Vezys et al., 2009; Wherry and Ahmed, 2004; Williams et al., 2008). One of the more controversial models states that effector and memory differentiation are two divergent pathways. Recent evidence showed that early and brief exposure to antigen is sufficient to direct both long-lived and short-lived effector $CD8^+$ T cell fates (Bevan and Fink, 2001; Blattman et al., 2002; Kaech and Ahmed, 2001; van Stipdonk et al., 2001). Additional studies have revealed that the formation of MPECs and SLECs begin to diverge from a common pool of $KLRG1^{low}$ effector cells after 7-10 cell divisions (Joshi and Kaech, 2008) and that ACD is a mechanism for $CD8^+$ memory T cell development (Chang et al., 2007).

Data from our studies (in preparation) and others have shown that one set of daughter cells arising from ACD are capable of forming memory cells and clearing secondary infection (Chang et al., 2007). Taken together, these data suggest that one set of daughter T cells contains a specialized memory precursor cell that is capable of giving rise to long-lasting, less differentiated stem-cell-like memory cells. Recent studies have identified T_{CM} cells as adult stem cells of the immune system that are capable of repopulating the $CD8^+$ T cell compartment in response to pathogenic exposure as well as naïve $CD8^+$ T cells (Gattinoni et al., 2009; 2011). We would hypothesize that ACD gives rise to this stem-cell-like population of T_{CM} cells, which can go on to give rise to T_{RM} and T_{EM} cells to clear a secondary response while maintaining their plasticity through self-renewal and differentiation.

It is important to emphasize that these models are not mutually exclusive. In fact, the divergent differentiation model does not require complete asymmetry in the first division, and computational models have shown that asymmetric inheritance only occurs a fraction of the time (Thaunat et al., 2012). Our hypothesis does not exclude the possibility that T_{RM} and T_{EM} cells can develop from symmetric cell division. While these cells provide some level of protection at the tissue site, they lack the ability to mount a complete secondary response and clear infections. The process of ACD is an evolutionarily conserved mechanism of stem cells in various tissues in the body. Our data and those of others suggest that ACD is also a conserved mechanism in the immune system, used to ensure that a less-differentiated, stem-cell-like memory precursor cell is established early on during primary infection. We would propose an inclusive model, suggesting that memory development incorporates a range of differentiation methods. The factors that affect the development of a memory compartment occur at each step of T cell activation. A combination of signals, T-APC dwell time, and microniche localization alter differentiation and cell fate, and it is tempting to speculate that different types of memory cells arise from various differentiation mechanisms.

CONCLUSION

The existing dogma states that a local milieu created by T cell-APC interactions during T cell activation dictates T cell differentiation and cell fate (Curtsinger et al., 1999). The effects of T-APC interaction duration and frequency on T cell activation are still under debate, as some studies show that long-lived interactions are required for full activation (Benvenuti et al., 2004; Huppa et al., 2003; Iezzi et al., 1998), while others show that T cell activation can occur as a result of transient interactions (Faroudi et al., 2003; Gunzer et al., 2000). While these signals are integral to T cell activation and differentiation, our recent work demonstrates that Rab27-mediated asymmetric inheritance of LFA-1 during $CD8^+$ T cell division results in an unequal distribution of migratory and cellular adhesion factors into proximal and distal daughter cells. These factors dictate the localization of daughter T cells to distinct lymph node microenvironments, exposing them to different determinants (i.e., antigens, cytokines, interactions) in the lymph node to ensure T cell diversity during an immune response. While previous studies have noted the importance of LFA-1-ICAM-1 interactions in $CD8^+$ T cell memory development (Bose et al., 2013; Cox et al., 2013; Ghosh et al., 2006; Parameswaran et al., 2005; Scholer et al., 2008; Zumwalde et al., 2013), a direct link between LFA-1 and memory formation had yet to be reported.

Recent work highlights the importance of studying $CD8^+$ T cell crosstalk with the stroma, other lymphocytes and APCs, the ECM, and the chemotactic and cytokine factors present in lymphatic niches during activation and differentiation. Unfortunately, the current methodology for studying T cell localization uses immunohistochemistry of thin tissue sections or whole tissue mounts. This leads to disruption of the tissue structure and cellularity, a loss of spatial relations between important epitopes due to extensive mechanical processing, and restrictions on the number of antibodies/fluorophores used in each imaging. To address these issues, a technique was recently developed that allows for antibody staining and imaging penetration into an intact organ through a process known as CLARITY (Chung and Deisseroth, 2013; Chung et al., 2013). Further developments have optimized the procedure (Murray et al., 2015) to allow

intact organs to be chemically processed and stained with different antibodies up to 22 times. Investigation of the lymphocyte localization within different cytokine and chemokine microenvironments through multiplexed labeling and imaging of intact lymph nodes will shed light on localization signals and differentiation programs.

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