

Minireview

The Role of Lymphatic Niches in T Cell Differentiation

Tara Capece and Minsoo Kim*

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 sig-

nals. 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 $\beta 1$ 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

Department of Microbiology and Immunology, David H. Smith Center for Vaccine Biology and Immunology, University of Rochester, Rochester, NY 14642, USA

*Correspondence: minsoo_kim@urmc.rochester.edu

Received 5 April, 2016; revised 21 May, 2016; accepted 24 May, 2016; published online 16 June, 2016

Keywords: CD8 T cell, chemokine, immune memory, lymph node, T cell

eISSN: 0219-1032

© The Korean Society for Molecular and Cellular Biology. All rights reserved.

© This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>.

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 α 7 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 (Kataikai 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 α 7. 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 attracts 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 an 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 (IFN γ) (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, IFN γ , and IFN α , 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-

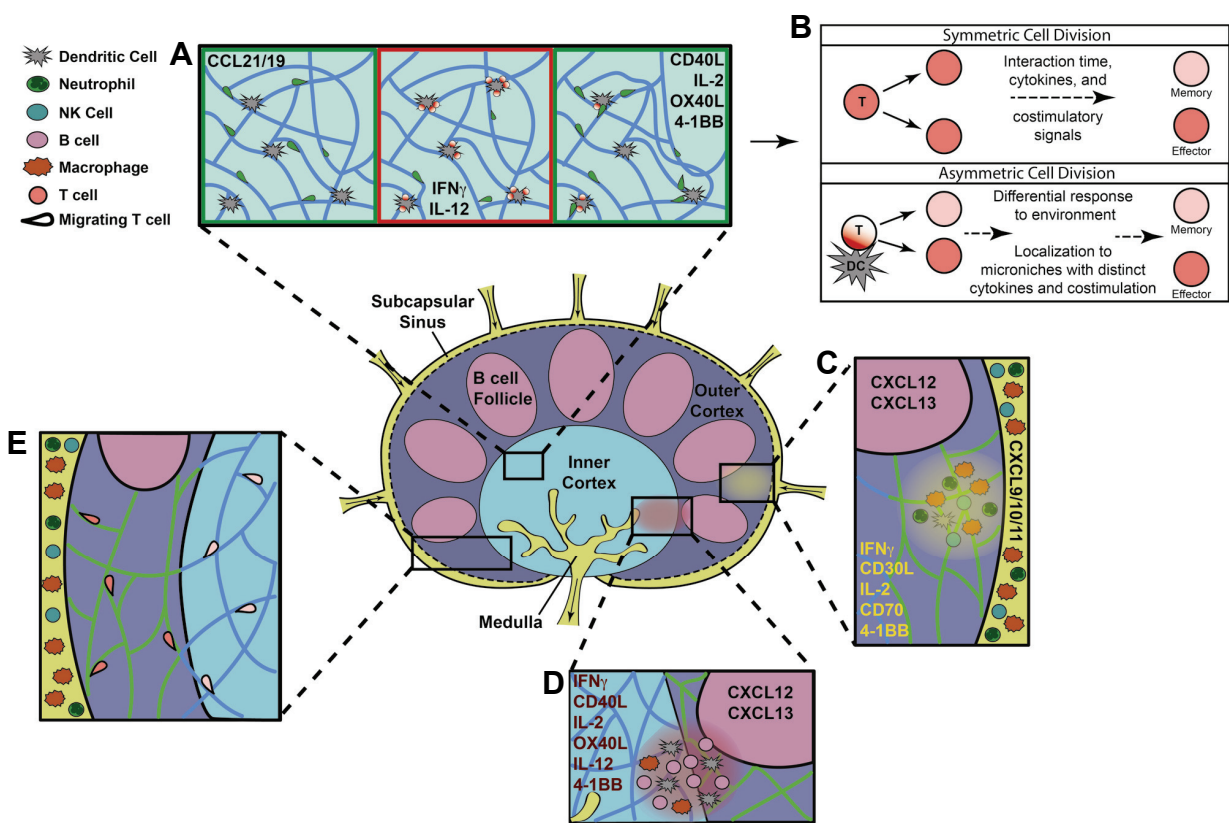


Fig. 1. Localization within the lymph node regulates differentiation. (A) $CCR7^+$ naive $CD8^+$ T cells and Ag-bearing DCs localize in the paracortical region (Inner Cortex, blue) of the lymph node via stromal cell (blue lines) produced CCL21/CCL19 signals. Here, $CD8^+$ T cells undergo three phases 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 $IFN\gamma$ 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 pro-

grams 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; Teixeira 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 micro-niche 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.

ACKNOWLEDGMENTS

We thank members of the Kim laboratory for helpful discussions. This work was financially supported through grants from the National Institute of Health (HL087088 to M.K. and F31AI112257 to T.C.). The authors have no conflicting financial interests.

REFERENCES

- Ahlers, J.D., Belyakov, I.M., Matsui, S., and Berzofsky, J.A. (2001). Signals delivered through TCR instruct IL-12 receptor (IL-12R) expression: IL-12 and tumor necrosis factor- α synergize for IL-12R expression at low antigen dose. *Int. Immunol.* **13**, 1433-1442.
- Allam, A., Conze, D.B., Giardino Torchia, M.L., Munitic, I., Yagita, H., Sowell, R.T., Marzo, A.L., and Ashwell, J.D. (2009). The CD8⁺ memory T-cell state of readiness is actively maintained and reversible. *Blood* **114**, 2121-2130.
- Alzona, M., Jack, H.M., Fisher, R.I., and Ellis, T.M. (1994). CD30 defines a subset of activated human T cells that produce IFN- γ and IL-5 and exhibit enhanced B cell helper activity. *J. Immunol.* **153**, 2861-2867.
- Andressen, C., Amhold, S., Puschmann, M., Bloch, W., Hescheler, J., Fassler, R., and Addicks, K. (1998). Beta1 integrin deficiency impairs migration and differentiation of mouse embryonic stem cell derived neurons. *Neurosci. Lett.* **251**, 165-168.
- Ansel, K.M., Ngo, V.N., Hyman, P.L., Luther, S.A., Forster, R., Sedgwick, J.D., Browning, J.L., Lipp, M., and Cyster, J.G. (2000). A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* **406**, 309-314.
- Azar, G.A., Lemaitre, F., Robey, E.A., and Bousso, P. (2010). Subcellular dynamics of T cell immunological synapses and kinapses in lymph nodes. *Proc. Natl. Acad. Sci. USA* **107**, 3675-3680.
- Bajenoff, M., Egen, J.G., Koo, L.Y., Laugier, J.P., Brau, F., Gleichenhans, N., and Germain, R.N. (2006). Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* **25**, 989-1001.
- Bannard, O., Kraman, M., and Fearon, D.T. (2009). Secondary replicative function of CD8⁺ T cells that had developed an effector phenotype. *Science* **323**, 505-509.
- Barbi, J., Oghumu, S., Lezama-Davila, C.M., and Satoskar, A.R. (2007). IFN- γ and STAT1 are required for efficient induction of CXC chemokine receptor 3 (CXCR3) on CD4⁺ but not CD8⁺ T cells. *Blood* **110**, 2215-2216.
- Baumgartner, C.K., Yagita, H., and Malherbe, L.P. (2012). A TCR affinity threshold regulates memory CD4 T cell differentiation following vaccination. *J. Immunol.* **189**, 2309-2317.
- Benitah, S.A., Frye, M., Glogauer, M., and Watt, F.M. (2005). Stem cell depletion through epidermal deletion of Rac1. *Science* **309**, 933-935.
- Benvenuti, F., Lagaudriere-Gesbert, C., Grandjean, I., Jancic, C., Hivroz, C., Trautmann, A., Lantz, O., and Amigorena, S. (2004). Dendritic cell maturation controls adhesion, synapse formation, and the duration of the interactions with naive T lymphocytes. *J. Immunol.* **172**, 292-301.
- Berney, C., Herren, S., Power, C.A., Gordon, S., Martinez-Pomares, L., and Kosco-Vilbois, M.H. (1999). A member of the dendritic cell family that enters B cell follicles and stimulates primary antibody responses identified by a mannose receptor fusion protein. *J. Exp. Med.* **190**, 851-860.
- Beuneu, H., Garcia, Z., and Bousso, P. (2006). Cutting edge: cognate CD4 help promotes recruitment of antigen-specific CD8 T cells around dendritic cells. *J. Immunol.* **177**, 1406-1410.
- Bevan, M.J., and Fink, P.J. (2001). The CD8 response on autopilot. *Nat. Immunol.* **2**, 381-382.
- Blattman, J.N., Cheng, L.E., and Greenberg, P.D. (2002). CD8(+) T cell responses: it's all downhill after their prime. *Nat. Immunol.* **3**, 601-602.
- Bose, T.O., Pham, Q.M., Jellison, E.R., Mouries, J., Ballantyne, C.M., and Lefrancois, L. (2013). CD11a regulates effector CD8 T cell differentiation and central memory development in response to infection with *Listeria monocytogenes*. *Infect. Immun.* **81**, 1140-1151.
- Bousso, P., and Robey, E. (2003). Dynamics of CD8⁺ T cell priming by dendritic cells in intact lymph nodes. *Nat. Immunol.* **4**, 579-585.
- Breitfeld, D., Ohi, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., and Forster, R. (2000). Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* **192**, 1545-1552.
- Buentke, E., Mathiot, A., Tolaini, M., Di Santo, J., Zamoyska, R., and Seddon, B. (2006). Do CD8 effector cells need IL-7R expression to become resting memory cells? *Blood* **108**, 1949-1956.
- Cannons, J.L., Lau, P., Ghumman, B., DeBenedette, M.A., Yagita, H., Okumura, K., and Watts, T.H. (2001). 4-1BB ligand induces cell division, sustains survival, and enhances effector function of CD4 and CD8 T cells with similar efficacy. *J. Immunol.* **167**, 1313-1324.
- Carrasco, Y.R., and Batista, F.D. (2007). B cells acquire particulate antigen in a macrophage-rich area at the boundary between the follicle and the subcapsular sinus of the lymph node. *Immunity* **27**, 160-171.
- Castellino, F., and Germain, R.N. (2006). Cooperation between CD4⁺ and CD8⁺ T cells: when, where, and how. *Ann. Rev. Immunol.* **24**, 519-540.
- Castellino, F., Huang, A.Y., Altan-Bonnet, G., Stoll, S., Scheinecker, C., and Germain, R.N. (2006). Chemokines enhance immunity by guiding naive CD8⁺ T cells to sites of CD4⁺ T cell-dendritic cell interaction. *Nature* **440**, 890-895.
- Chang, J.T., Palanivel, V.R., Kinjyo, I., Schambach, F., Intlekofer, A.M., Banerjee, A., Longworth, S.A., Vinup, K.E., Mrass, P., Olliaro, J., et al. (2007). Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science* **315**, 1687-1691.
- Chtanova, T., Han, S.J., Schaeffer, M., van Dooren, G.G., Herzmark, P., Striepen, B., and Robey, E.A. (2009). Dynamics of T cell, antigen-presenting cell, and pathogen interactions during recall responses in the lymph node. *Immunity* **31**, 342-355.
- Chung, K., and Deisseroth, K. (2013). CLARITY for mapping the nervous system. *Nat. Methods* **10**, 508-513.
- Chung, K., Wallace, J., Kim, S.Y., Kalyanasundaram, S., Andalman, A.S., Davidson, T.J., Mirzabekov, J.J., Zalocusky, K.A., Mattis, J., Denisin, A.K., et al. (2013). Structural and molecular interrogation of intact biological systems. *Nature* **497**, 332-337.
- Chyou, S., Benahmed, F., Chen, J., Kumar, V., Tian, S., Lipp, M., and Lu, T.T. (2011). Coordinated regulation of lymph node vascular-stromal growth first by CD11c⁺ cells and then by T and B cells. *J. Immunol.* **187**, 5558-5567.
- Cohen, J.N., Guidi, C.J., Tewalt, E.F., Qiao, H., Rouhani, S.J., Ruddell, A., Farr, A.G., Tung, K.S., and Engelhard, V.H. (2010). Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J. Exp. Med.* **207**, 681-688.
- Cox, M.A., Barnum, S.R., Bullard, D.C., and Zajac, A.J. (2013). ICAM-1-dependent tuning of memory CD8 T-cell responses following acute infection. *Proc. Natl. Acad. Sci. USA* **110**, 1416-1421.
- Cui, W., and Kaeck, S.M. (2010). Generation of effector CD8⁺ T cells and their conversion to memory T cells. *Immunol. Rev.* **236**, 151-166.
- Curtsinger, J.M., Schmidt, C.S., Mondino, A., Lins, D.C., Kedl, R.M., Jenkins, M.K., and Mescher, M.F. (1999). Inflammatory cytokines provide a third signal for activation of naive CD4⁺ and CD8⁺ T cells. *J. Immunol.* **162**, 3256-3262.
- Eickhoff, S., Brewitz, A., Gerner, M.Y., Klauschen, F., Komander, K., Hemmi, H., Garbi, N., Kaisho, T., Germain, R.N., and Kastenmuller, W. (2015). Robust anti-viral immunity requires multiple distinct T cell-dendritic cell interactions. *Cell* **162**, 1322-1337.
- Faroudi, M., Zaru, R., Paulet, P., Müller, S., and Valitutti, S. (2003). Cutting edge: T lymphocyte activation by repeated immunological synapse formation and intermittent signaling. *J. Immunol.* **171**, 1128-1132.
- Fletcher, A.L., Lukacs-Kornek, V., Reynoso, E.D., Pinner, S.E.,

- Bellemare-Pelletier, A., Curry, M.S., Collier, A.R., Boyd, R.L., and Turley, S.J. (2010). Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J. Exp. Med.* 207, 689-697.
- Fooksman, D.R., Vardhana, S., Vasiliver-Shamis, G., Liese, J., Blair, D.A., Waite, J., Sacristan, C., Victoria, G.D., Zanin-Zhorov, A., and Dustin, M.L. (2010). Functional anatomy of T cell activation and synapse formation. *Ann. Rev. Immunol.* 28, 79-105.
- Förster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Müller, I., Wolf, E., and Lipp, M. (1999). CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99, 23-33.
- Förster, R., Braun, A., and Worbs, T. (2012). Lymph node homing of T cells and dendritic cells via afferent lymphatics. *Trends Immunol.* 33, 271-280.
- Frye, M., Gardner, C., Li, E.R., Arnold, I., and Watt, F.M. (2003). Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. *Development* 130, 2793-2808.
- Fuchs, E., Tumber, T., and Guasch, G. (2004). Socializing with the neighbors: stem cells and their niche. *Cell* 116, 769-778.
- Garcia, Z., Pradelli, E., Celli, S., Beuneu, H., Simon, A., and Bousso, P. (2007). Competition for antigen determines the stability of T cell-dendritic cell interactions during clonal expansion. *Proc. Natl. Acad. Sci. USA* 104, 4553-4558.
- Gattinoni, L., Zhong, X.S., Palmer, D.C., Ji, Y., Hinrichs, C.S., Yu, Z., Wrzesinski, C., Boni, A., Cassard, L., Garvin, L.M., et al. (2009). Wnt signaling arrests effector T cell differentiation and generates CD8⁺ memory stem cells. *Nat. Med.* 15, 808-813.
- Gattinoni, L., Lugli, E., Ji, Y., Pos, Z., Paulos, C.M., Quigley, M.F., Almeida, J.R., Gostick, E., Yu, Z., Carpenito, C., et al. (2011). A human memory T cell subset with stem cell-like properties. *Nat. Med.* 17, 1290-1297.
- Ghosh, S., Chackerian, A.A., Parker, C.M., Ballantyne, C.M., and Behar, S.M. (2006). The LFA-1 adhesion molecule is required for protective immunity during pulmonary Mycobacterium tuberculosis infection. *J. Immunol.* 176, 4914-4922.
- Girard, J.P., and Springer, T.A. (1995). High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunology Today* 16, 449-457.
- Girard, J.P., Moussion, C., and Forster, R. (2012). HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. *Nat. Rev. Immunol.* 12, 762-773.
- Goulas, S., Conder, R., and Knoblich, J.A. (2012). The Par complex and integrins direct asymmetric cell division in adult intestinal stem cells. *Cell Stem Cell* 11, 529-540.
- Grakoui, A., Bromley, S.K., Sumen, C., Davis, M.M., Shaw, A.S., Allen, P.M., and Dustin, M.L. (1999). The immunological synapse: a molecular machine controlling T cell activation. *Science* 285, 221-227.
- Gretz, J.E., Anderson, A.O., and Shaw, S. (1997). Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol. Rev.* 156, 11-24.
- Gretz, J.E., Norbury, C.C., Anderson, A.O., Proudfoot, A.E., and Shaw, S. (2000). Lymph-borne chemokines and other low molecular weight molecules reach high endothelial venules via specialized conduits while a functional barrier limits access to the lymphocyte microenvironments in lymph node cortex. *J. Exp. Med.* 192, 1425-1440.
- Grigorova, I.L., Schwab, S.R., Phan, T.G., Pham, T.H., Okada, T., and Cyster, J.G. (2009). Cortical sinus probing, S1P1-dependent entry and flow-based capture of egressing T cells. *Nat. Immunol.* 10, 58-65.
- Grigorova, I.L., Panteleev, M., and Cyster, J.G. (2010). Lymph node cortical sinus organization and relationship to lymphocyte egress dynamics and antigen exposure. *Proc. Natl. Acad. Sci. USA* 107, 20447-20452.
- Groom, J.R., Richmond, J., Murooka, T.T., Sorensen, E.W., Sung, J.H., Bankert, K., von Andrian, U.H., Moon, J.J., Mempel, T.R., and Luster, A.D. (2012). CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4⁺ T helper 1 cell differentiation. *Immunity* 37, 1091-1103.
- Gui, L., and Homer, H. (2012). Spindle assembly checkpoint signalling is uncoupled from chromosomal position in mouse oocytes. *Development* 139, 1941-1946.
- Gunzer, M., Schafer, A., Borgmann, S., Grabbe, S., Zanker, K.S., Brocker, E.B., Kampgen, E., and Friedl, P. (2000). Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity* 13, 323-332.
- Haining, W.N., Ebert, B.L., Subramanian, A., Wherry, E.J., Eichbaum, Q., Evans, J.W., Mak, R., Rivoli, S., Pretz, J., Angelosanto, J., et al. (2008). Identification of an evolutionarily conserved transcriptional signature of CD8 memory differentiation that is shared by T and B cells. *J. Immunol.* 181, 1859-1868.
- Hand, T.W., Cui, W., Jung, Y.W., Sefik, E., Joshi, N.S., Chande, A., Liu, Y., and Kaech, S.M. (2010). Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. *Proc. Natl. Acad. Sci. USA* 107, 16601-16606.
- Harrington, L.E., Janowski, K.M., Oliver, J.R., Zajac, A.J., and Weaver, C.T. (2008). Memory CD4 T cells emerge from effector T-cell progenitors. *Nature* 452, 356-360.
- Heffner, M. and Fearon, D.T. (2007). Loss of T cell receptor-induced Bmi-1 in the KLRG1(+) senescent CD8(+) T lymphocyte. *Proc. Natl. Acad. Sci. USA* 104, 13414-13419.
- Hendriks, J., Xiao, Y., and Borst, J. (2003). CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *J. Exp. Med.* 198, 1369-1380.
- Henrickson, S.E., Mempel, T.R., Mazo, I.B., Liu, B., Artyomov, M.N., Zheng, H., Peixoto, A., Flynn, M.P., Senman, B., Jung, T., et al. (2008). T cell sensing of antigen dose governs interactive behavior with dendritic cells and sets a threshold for T cell activation. *Nat. Immunol.* 9, 282-291.
- Hickman, H.D., Takeda, K., Skon, C.N., Murray, F.R., Hensley, S.E., Loomis, J., Barber, G.N., Bennink, J.R., and Yewdell, J.W. (2008). Direct priming of antiviral CD8⁺ T cells in the peripheral interfollicular region of lymph nodes. *Nat. Immunol.* 9, 155-165.
- Hickman, H.D., Li, L., Reynoso, G.V., Rubin, E.J., Skon, C.N., Mays, J.W., Gibbs, J., Schwartz, O., Bennink, J.R., and Yewdell, J.W. (2011). Chemokines control naive CD8⁺ T cell selection of optimal lymph node antigen presenting cells. *J. Exp. Med.* 208, 2511-2524.
- Hickman, H.D., Reynoso, G.V., Ngudiankama, B.F., Cush, S.S., Gibbs, J., Bennink, J.R., and Yewdell, J.W. (2015). CXCR3 chemokine receptor enables local CD8(+) T cell migration for the destruction of virus-infected cells. *Immunity* 42, 524-537.
- Hirsch, E., Iglesias, A., Potocnik, A.J., Hartmann, U., and Fessler, R. (1996). Impaired migration but not differentiation of haematopoietic stem cells in the absence of beta1 integrins. *Nature* 380, 171-175.
- Hor, J.L., Whitney, P.G., Zaid, A., Brooks, A.G., Heath, W.R., and Mueller, S.N. (2015). Spatiotemporally Distinct Interactions with Dendritic Cell Subsets Facilitates CD4⁺ and CD8⁺ T Cell Activation to Localized Viral Infection. *Immunity* 43, 554-565.
- Hu, J.K., Kagari, T., Clingan, J.M., and Matloubian, M. (2011). Expression of chemokine receptor CXCR3 on T cells affects the balance between effector and memory CD8 T-cell generation. *Proc. Natl. Acad. Sci. USA* 108, E118-127.
- Hugues, S., Fetter, L., Bonifaz, L., Helft, J., Amblard, F., and Amigorena, S. (2004). Distinct T cell dynamics in lymph nodes during the induction of tolerance and immunity. *Nat. Immunol.* 5, 1235-1242.
- Hugues, S., Scholer, A., Boissonnas, A., Nussbaum, A., Combadiere, C., Amigorena, S., and Fetter, L. (2007a). Dynamic imaging of chemokine-dependent CD8⁺ T cell help for CD8⁺ T cell responses. *Nat. Immunol.* 8, 921-930.
- Hugues, S., Scholer, A., Boissonnas, A., Nussbaum, A., Combadiere, C., Amigorena, S., and Fetter, L. (2007b). Dynamic imaging of chemokine-dependent CD8⁺ T cell help for CD8⁺ T cell responses. *Nat. Immunol.* 8, 921-930.
- Huppa, J.B., Gleimer, M., Sumen, C., and Davis, M.M. (2003). Continuous T cell receptor signaling required for synapse maintenance and full effector potential. *Nat. Immunol.* 4, 749-755.
- Huster, K.M., Stemberger, C., Gasteiger, G., Kastenmuller, W., Drexler, I., and Busch, D.H. (2009). Cutting edge: memory CD8 T cell compartment grows in size with immunological experience but nevertheless can lose function. *J. Immunol.* 183, 6898-6902.
- Iezzi, G., Karjalainen, K., and Lanzavecchia, A. (1998). The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* 8, 89-95.
- Jones, D.L., and Wagers, A.J. (2008). No place like home: anatomy

- and function of the stem cell niche. *Nat. Rev. Mol. Cell Biol.* 9, 11-21.
- Joshi, N.S., and Kaech, S.M. (2008). Effector CD8 T cell development: a balancing act between memory cell potential and terminal differentiation. *J. Immunol.* 180, 1309-1315.
- Joshi, N.S., Cui, W., Chande, A., Lee, H.K., Urso, D.R., Hagman, J., Gapin, L., and Kaech, S.M. (2007). Inflammation directs memory precursor and short-lived effector CD8(+) T cell fates via the graded expression of T-bet transcription factor. *Immunity* 27, 281-295.
- Junt, T., Fink, K., Forster, R., Senn, B., Lipp, M., Muramatsu, M., Zinkernagel, R.M., Ludewig, B. and Hengartner, H. (2005). CXCR5-dependent seeding of follicular niches by B and Th cells augments antiviral B cell responses. *J. Immunol.* 175, 7109-7116.
- Junt, T., Moseman, E.A., Iannacone, M., Massberg, S., Lang, P.A., Boes, M., Fink, K., Henrickson, S.E., Shayakhmetov, D.M., Di Paolo, N.C., et al. (2007). Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* 450, 110-114.
- Kaech, S.M., and Ahmed, R. (2001). Memory CD8⁺ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. *Nat. Immunol.* 2, 415-422.
- Kaech, S.M., Tan, J.T., Wherry, E.J., Konieczny, B.T., Surh, C.D., and Ahmed, R. (2003). Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. *Nat. Immunol.* 4, 1191-1198.
- Kalia, V., Sarkar, S., and Ahmed, R. (2010). CD8 T-cell memory differentiation during acute and chronic viral infections. *Adv. Exp. Med. Biol.* 684, 79-95.
- Kastenmuller, W., Brandes, M., Wang, Z., Herz, J., Egen, J.G., and Germain, R.N. (2013). Peripheral prepositioning and local CXCL9 chemokine-mediated guidance orchestrate rapid memory CD8⁺ T cell responses in the lymph node. *Immunity* 38, 502-513.
- Katakai, T., Suto, H., Sugai, M., Gonda, H., Togawa, A., Suematsu, S., Ebisuno, Y., Katagiri, K., Kinashi, T., and Shimizu, A. (2008). Organizer-like reticular stromal cell layer common to adult secondary lymphoid organs. *J. Immunol.* 181, 6189-6200.
- Kelly, R.H. (1975). Functional anatomy of lymph nodes. I. The paracortical cords. *Int. Arch. Allergy Appl. Immunol.* 48, 836-849.
- Khader, S.A., Bell, G.K., Pearl, J.E., Fountain, J.J., Rangel-Moreno, J., Cilley, G.E., Shen, F., Eaton, S.M., Gaffen, S.L., Swain, S.L., et al. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat. Immunol.* 8, 369-377.
- Khan, O., Headley, M., Gerard, A., Wei, W., Liu, L., and Krummel, M.F. (2011). Regulation of T cell priming by lymphoid stroma. *PLoS One* 6, e26138.
- Knoblich, J.A. (2008). Mechanisms of asymmetric stem cell division. *Cell* 132, 583-597.
- Kuka, M., and Iannacone, M. (2014). The role of lymph node sinus macrophages in host defense. *Ann. N.Y. Acad. Sci.* 1319, 38-46.
- Lindquist, R.L., Shakhari, G., Dudziak, D., Wardemann, H., Eisenreich, T., Dustin, M.L., and Nussenzweig, M.C. (2004). Visualizing dendritic cell networks *in vivo*. *Nat. Immunol.* 5, 1243-1250.
- Link, A., Vogt, T.K., Favre, S., Britschgi, M.R., Acha-Orbea, H., Hinz, B., Cyster, J.G., and Luther, S.A. (2007). Fibroblastic reticular cells in lymph nodes regulate the homeostasis of naive T cells. *Nat. Immunol.* 8, 1255-1265.
- Lohning, M., Hegazy, A.N., Pinschewer, D.D., Busse, D., Lang, K.S., Hofer, T., Radbruch, A., Zinkernagel, R.M., and Hengartner, H. (2008). Long-lived virus-reactive memory T cells generated from purified cytokine-secreting T helper type 1 and type 2 effectors. *J. Exp. Med.* 205, 53-61.
- Lu, P., Youngblood, B.A., Austin, J.W., Mohammed, A.U., Butler, R., Ahmed, R., and Boss, J.M. (2014). Blimp-1 represses CD8 T cell expression of PD-1 using a feed-forward transcriptional circuit during acute viral infection. *J. Exp. Med.* 211, 515-527.
- Lukacs-Kornek, V., Malhotra, D., Fletcher, A.L., Acton, S.E., Elpek, K.G., Tayalia, P., Collier, A.R., and Turley, S.J. (2011). Regulated release of nitric oxide by nonhematopoietic stroma controls expansion of the activated T cell pool in lymph nodes. *Nat. Immunol.* 12, 1096-1104.
- Luther, S.A., Tang, H.L., Hyman, P.L., Farr, A.G., and Cyster, J.G. (2000). Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the *plf/plt* mouse. *Proc. Natl. Acad. Sci. USA* 97, 12694-12699.
- M'Rini, C., Cheng, G., Schweitzer, C., Cavanagh, L.L., Palframan, R.T., Mempel, T.R., Warnock, R.A., Lowe, J.B., Quackenbush, E.J., and von Andrian, U.H. (2003). A novel endothelial L-selectin ligand activity in lymph node medulla that is regulated by alpha(1,3)-fucosyltransferase-IV. *J. Exp. Med.* 198, 1301-1312.
- Malhotra, D., Fletcher, A.L., Astarita, J., Lukacs-Kornek, V., Tayalia, P., Gonzalez, S.F., Elpek, K.G., Chang, S.K., Knoblich, K., Hemler, M.E., et al. (2012). Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. *Nat. Immunol.* 13, 499-510.
- Marchesi, V.T., and Gowans, J.L. (1964). The Migration of Lymphocytes through the Endothelium of Venules in Lymph Nodes: An Electron Microscope Study. *Proc. R. Soc. Lond. B Biol. Sci.* 159, 283-290.
- Maris, C.H., Miller, J.D., Altman, J.D., and Jacob, J. (2003). A transgenic mouse model genetically tags all activated CD8 T cells. *J. Immunol.* 171, 2393-2401.
- Masopust, D., Vezys, V., Usherwood, E.J., Cauley, L.S., Olson, S., Marzo, A.L., Ward, R.L., Woodland, D.L., and Lefrancois, L. (2004). Activated primary and memory CD8 T cells migrate to nonlymphoid tissues regardless of site of activation or tissue of origin. *J. Immunol.* 172, 4875-4882.
- Mempel, T.R., Henrickson, S.E., and Von Andrian, U.H. (2004). T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature* 427, 154-159.
- Metchat, A., Eguren, M., Hossain, J.M., Politi, A.Z., Huet, S., and Ellenberg, J. (2015). An actin-dependent spindle position checkpoint ensures the asymmetric division in mouse oocytes. *Nat. Commun.* 6, 7784.
- Miller, M.J., Wei, S.H., Parker, I., and Cahalan, M.D. (2002). Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. *Science* 296, 1869-1873.
- Miller, M.J., Hejazi, A.S., Wei, S.H., Cahalan, M.D., and Parker, I. (2004a). T cell repertoire scanning is promoted by dynamic dendritic cell behavior and random T cell motility in the lymph node. *Proc. Natl. Acad. Sci. USA* 101, 998-1003.
- Miller, M.J., Safrina, O., Parker, I., and Cahalan, M.D. (2004b). Imaging the single cell dynamics of CD4⁺ T cell activation by dendritic cells in lymph nodes. *J. Exp. Med.* 200, 847-856.
- Mionnet, C., Sanos, S.L., Mondor, I., Jorquera, A., Laugier, J.P., Germain, R.N., and Bajenoff, M. (2011). High endothelial venules as traffic control points maintaining lymphocyte population homeostasis in lymph nodes. *Blood* 118, 6115-6122.
- Mondino, A., Khoruts, A., and Jenkins, M.K. (1996). The anatomy of T-cell activation and tolerance. *Proc. Natl. Acad. Sci. USA* 93, 2245-2252.
- Moreau, H.D., Lemaitre, F., Terriac, E., Azar, G., Piel, M., Lennon-Dumenil, A.M., and Bousso, P. (2012). Dynamic *in situ* cytometry uncovers T cell receptor signaling during immunological synapses and kinapses *in vivo*. *Immunity* 37, 351-363.
- Moreau, H.D., Lemaitre, F., Garrod, K.R., Garcia, Z., Lennon-Dumenil, A.M., and Bousso, P. (2015). Signal strength regulates antigen-mediated T-cell deceleration by distinct mechanisms to promote local exploration or arrest. *Proc. Natl. Acad. Sci. USA* 112, 12151-12156.
- Morrison, S.J., and Kimble, J. (2006). Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 441, 1068-1074.
- Murray, E., Cho, J.H., Goodwin, D., Ku, T., Swaney, J., Kim, S.Y., Choi, H., Park, Y.G., Park, J.Y., Hubbert, A., et al. (2015). Simple, Scalable Proteomic Imaging for High-Dimensional Profiling of Intact Systems. *Cell* 163, 1500-1514.
- Neumuller, R.A., and Knoblich, J.A. (2009). Dividing cellular asymmetry: asymmetric cell division and its implications for stem cells and cancer. *Genes Dev.* 23, 2675-2699.
- Okada, T., Ngo, V.N., Eklund, E.H., Forster, R., Lipp, M., Littman, D.R., and Cyster, J.G. (2002). Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. *J. Exp. Med.* 196, 65-75.
- Parameswaran, N., Suresh, R., Bal, V., Rath, S., and George, A. (2005). Lack of ICAM-1 on APCs during T cell priming leads to poor generation of central memory cells. *J. Immunol.* 175, 2201-2211.
- Pearce, E.L., and Shen, H. (2007). Generation of CD8 T cell memory is regulated by IL-12. *J. Immunol.* 179, 2074-2081.
- Petridou, N.I., and Skourides, P.A. (2016). A ligand-independent integrin beta1 mechanosensory complex guides spindle orientation.

- Nat. Commun. 7, 10899.
- Phan, T.G., Grigorova, I., Okada, T., and Cyster, J.G. (2007). Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8, 992-1000.
- Phan, T.G., Green, J.A., Gray, E.E., Xu, Y., and Cyster, J.G. (2009). Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. *Nat. Immunol.* 10, 786-793.
- Pryshchep, S., Zarnitsyna, V.I., Hong, J., Evavold, B.D., and Zhu, C. (2014). Accumulation of serial forces on TCR and CD8 frequently applied by agonist antigenic peptides embedded in MHC molecules triggers calcium in T cells. *J. Immunol.* 193, 68-76.
- Redmond, W.L., Ruby, C.E., and Weinberg, A.D. (2009). The role of OX40-mediated co-stimulation in T-cell activation and survival. *Crit. Rev. Immunol.* 29, 187-201.
- Rohr, J.C., Gerlach, C., Kok, L., and Schumacher, T.N. (2014). Single cell behavior in T cell differentiation. *Trends Immunol.* 35, 170-177.
- Rozenendaal, R., Mempel, T.R., Pitcher, L.A., Gonzalez, S.F., Verschoor, A., Mebius, R.E., von Andrian, U.H., and Carroll, M.C. (2009). Conduits mediate transport of low-molecular-weight antigen to lymph node follicles. *Immunity* 30, 264-276.
- Sallusto, F., Lenig, D., Forster, R., Lipp, M., and Lanzavecchia, A. (1999). Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401, 708-712.
- Sarkar, S., Kalia, V., Haining, W.N., Konieczny, B.T., Subramaniam, S., and Ahmed, R. (2008). Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. *J. Exp. Med.* 205, 625-640.
- Schaerli, P., Willmann, K., Lang, A.B., Lipp, M., Loetscher, P., and Moser, B. (2000). CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* 192, 1553-1562.
- Scholer, A., Hugues, S., Boissonnas, A., Fetter, L., and Amigorena, S. (2008). Intercellular adhesion molecule-1-dependent stable interactions between T cells and dendritic cells determine CD8⁺ T cell memory. *Immunity* 28, 258-270.
- Schulz, E.G., Mariani, L., Radbruch, A., and Hofer, T. (2009). Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-gamma and interleukin-12. *Immunity* 30, 673-683.
- Schurich, A., Pallett, L.J., Lubowiecki, M., Singh, H.D., Gill, U.S., Kennedy, P.T., Nastouli, E., Tanwar, S., Rosenberg, W., and Maini, M.K. (2013). The third signal cytokine IL-12 rescues the anti-viral function of exhausted HBV-specific CD8 T cells. *PLoS Pathogens* 9, e1003208.
- Seder, R.A., Darrah, P.A., and Roederer, M. (2008). T-cell quality in memory and protection: implications for vaccine design. *Nat. Rev. Immunol.* 8, 247-258.
- Seth, S., Oberdorfer, L., Hyde, R., Hoff, K., Thies, V., Worbs, T., Schmitz, S., and Forster, R. (2011). CCR7 essentially contributes to the homing of plasmacytoid dendritic cells to lymph nodes under steady-state as well as inflammatory conditions. *J. Immunol.* 186, 3364-3372.
- Siegert, S., Huang, H.Y., Yang, C.Y., Scarpellino, L., Carrie, L., Essex, S., Nelson, P.J., Heikenwalder, M., Acha-Orbea, H., Buckley, C.D., et al. (2011). Fibroblastic reticular cells from lymph nodes attenuate T cell expansion by producing nitric oxide. *PLoS One* 6, e27618.
- Sinha, R.K., Park, C., Hwang, I.Y., Davis, M.D., and Kehr, J.H. (2009). B lymphocytes exit lymph nodes through cortical lymphatic sinusoids by a mechanism independent of sphingosine-1-phosphate-mediated chemotaxis. *Immunity* 30, 434-446.
- Sixt, M., Kanazawa, N., Selg, M., Samson, T., Roos, G., Reinhardt, D.P., Pabst, R., Lutz, M.B., and Sorokin, L. (2005). The conduit system transports soluble antigens from the afferent lymph to resident dendritic cells in the T cell area of the lymph node. *Immunity* 22, 19-29.
- Stoll, S., Delon, J., Brotz, T.M., and Germain, R.N. (2002). Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. *Science* 296, 1873-1876.
- Streuli, C.H. (2009). Integrins and cell-fate determination. *J. Cell Sci.* 122, 171-177.
- Sung, J.H., Zhang, H., Moseman, E.A., Alvarez, D., Iannacone, M., Henrickson, S.E., de la Torre, J.C., Groom, J.R., Luster, A.D., and von Andrian, U.H. (2012). Chemokine guidance of central memory T cells is critical for antiviral recall responses in lymph nodes. *Cell* 150, 1249-1263.
- Teixeiro, E., Daniels, M.A., Hamilton, S.E., Schrum, A.G., Bragado, R., Jameson, S.C., and Palmer, E. (2009). Different T cell receptor signals determine CD8⁺ memory versus effector development. *Science* 323, 502-505.
- Thaunat, O., Granja, A.G., Barral, P., Filby, A., Montaner, B., Collinson, L., Martinez-Martin, N., Harwood, N.E., Bruckbauer, A., and Batista, F.D. (2012). Asymmetric segregation of polarized antigen on B cell division shapes presentation capacity. *Science* 335, 475-479.
- Turley, S.J., Fletcher, A.L., and Elpek, K.G. (2010). The stromal and haematopoietic antigen-presenting cells that reside in secondary lymphoid organs. *Nat. Rev. Immunol.* 10, 813-825.
- Twu, Y.C., Gold, M.R., and Teh, H.S. (2011). TNFR1 delivers pro-survival signals that are required for limiting TNFR2-dependent activation-induced cell death (AICD) in CD8⁺ T cells. *Eur. J. Immunol.* 41, 335-344.
- van Stipdonk, M.J., Lemmens, E.E., and Schoenberger, S.P. (2001). Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. *Nat. Immunol.* 2, 423-429.
- Vezys, V., Yates, A., Casey, K.A., Lanier, G., Ahmed, R., Antia, R., and Masopust, D. (2009). Memory CD8 T-cell compartment grows in size with immunological experience. *Nature* 457, 196-199.
- Victoria, G.D., Schwickert, T.A., Fooksman, D.R., Kamphorst, A.O., Meyer-Hermann, M., Dustin, M.L., and Nussenzweig, M.C. (2010). Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* 143, 592-605.
- Voigt, I., Camacho, S.A., de Boer, B.A., Lipp, M., Forster, R., and Berek, C. (2000). CXCR5-deficient mice develop functional germinal centers in the splenic T cell zone. *Eur. J. Immunol.* 30, 560-567.
- von Andrian, U.H., and Mempel, T.R. (2003). Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.* 3, 867-878.
- Watt, F.M. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J.* 21, 3919-3926.
- Webster, B., Ekland, E.H., Agle, L.M., Chyou, S., Ruggieri, R., and Lu, T.T. (2006). Regulation of lymph node vascular growth by dendritic cells. *J. Exp. Med.* 203, 1903-1913.
- Wei, S.H., Safrina, O., Yu, Y., Garrod, K.R., Cahalan, M.D., and Parker, I. (2007). Ca²⁺ signals in CD4⁺ T cells during early contacts with antigen-bearing dendritic cells in lymph node. *J. Immunol.* 179, 1586-1594.
- Wherry, E.J., and Ahmed, R. (2004). Memory CD8 T-cell differentiation during viral infection. *J. Virol.* 78, 5535-5545.
- Williams, M.A., Ravkov, E.V., and Bevan, M.J. (2008). Rapid culling of the CD4⁺ T cell repertoire in the transition from effector to memory. *Immunity* 28, 533-545.
- Xin, A., Masson, F., Liao, Y., Preston, S., Guan, T., Gloury, R., Olshansky, M., Lin, J.X., Li, P., Speed, T.P., et al. (2016). A molecular threshold for effector CD8 T cell differentiation controlled by transcription factors Blimp-1 and T-bet. *Nat. Immunol.* 17, 422-432.
- Yang, C.Y., Vogt, T.K., Favre, S., Scarpellino, L., Huang, H.Y., Tacchini-Cottier, F., and Luther, S.A. (2014). Trapping of naive lymphocytes triggers rapid growth and remodeling of the fibroblast network in reactive murine lymph nodes. *Proc. Natl. Acad. Sci. USA* 111, E109-118.
- Yang, J., Plikus, M.V., and Komarova, N.L. (2015). The Role of Symmetric Stem Cell Divisions in Tissue Homeostasis. *PLoS Comput. Biol.* 11, e1004629.
- Yeo, C.J., and Fearon, D.T. (2011). T-bet-mediated differentiation of the activated CD8⁺ T cell. *Eur. J. Immunol.* 41, 60-66.
- Zumwalde, N.A., Domae, E., Mescher, M.F., and Shimizu, Y. (2013). ICAM-1-dependent homotypic aggregates regulate CD8 T cell effector function and differentiation during T cell activation. *J. Immunol.* 191, 3681-3693.