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Chemokines: Critical Regulators of Memory T Cell Development, Maintenance, and Function

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Abstract

Memory T cells are central to orchestrating antigen-specific recall responses in vivo. Compared to naïve T cells, memory T cells respond more quickly to cognate peptide:MHC with a shorter lag time for entering the cell cycle and exerting effector functions. However, it is now well established that this enhanced responsiveness is not the only mechanism whereby memory T cells are better equipped than naïve T cells to rapidly and robustly induce inflammation. In contrast to naïve T cells, memory T cells are composed of distinct subsets with unique trafficking patterns and localizations. Tissue-resident memory T cells persist in previously inflamed tissue and function as first responders to cognate antigen reexposure. In addition, a heterogeneous group of circulating memory T cells augment inflammation by either rapidly migrating to inflamed tissue or responding to cognate antigen within secondary lymphoid organs and producing additional effector T cells. Defining the mechanisms regulating T cell positioning and trafficking and how this influences the development, maintenance, and function of memory T cell subsets is essential to improving vaccine design as well as treatment of immune-mediated diseases. In this chapter, we will review our current knowledge of how chemokines, critical regulators of cell positioning and migration, govern memory T cell biology in vivo. In addition, we discuss areas of uncertainty and future directions for further delineating how T cell localization influences memory T cell biology.

1. INTRODUCTION

Antigen specificity and immune memory are defining features of adaptive immunity and allow vertebrates to rapidly and specifically respond to recurrent infections. The benefit of immune memory has been recognized since antiquity, when the Greek historian Thucydides noted that individuals who recovered from plague exhibited protection from recurrent disease (Thucydides, 1634). As early as the 16th century, when the technique of variolation for smallpox was first utilized in Asia, there have been attempts to induce antigen-specific immune memory to prevent the morbidity and mortality from infectious diseases (Boylston,

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2012). In contrast to the advantages of immune memory, the development of antigen-specific memory to self or innocuous environmental antigens is central to the pathogenesis of numerous autoimmune and allergic diseases. As a result, the ability to intelligently generate or eliminate immune memory has tremendous implications for human health, yet our ability to do so is very limited. In terms of vaccine development, while successful vaccinations have been one of the great achievements of medicine, safely inducing an effective antigen-specific immune response that leads to long-lasting and effective immune memory in vivo has remained a significant challenge (Lycke, 2012). For immune-mediated diseases, the mainstay of therapy has involved long-term administration of nonspecific immunosuppressive agents. The limitations and side effects of this approach have led to more targeted interventions including antigen-specific immunotherapy and biologic therapy. While both of these approaches have shown benefit in certain inflammatory diseases, each has considerable limitations (Pozsgay, Szekanecz, & Sármy, 2017; Tabatabaian, Ledford, & Casale, 2017; Wolfe & Ang, 2017). In order to develop more effective vaccines as well as therapies for immune-mediated diseases, a greater understanding of the mechanisms regulating immune memory is needed.

Memory T cells are central to orchestrating antigen-specific recall responses in vivo. Compared to naïve T cells, memory T cells respond more rapidly to cognate peptide:MHC with a shorter lag time for entering the cell cycle and exerting effector functions (Sprent & Surh, 2002). However, it is now well established that this enhanced responsiveness is not the only mechanism whereby memory T cells are better equipped than naïve T cells to rapidly and robustly orchestrate inflammation. Over the last 20 years, we have recognized that distinct subsets of memory T cells exhibit unique trafficking patterns compared to naïve T cells in vivo (Carbone, 2015; Schenkel & Masopust, 2014). Tissue-resident memory T cells (Trm) persist in previously inflamed tissue and function as first responders to cognate antigen reexposure. In addition, a heterogeneous group of circulating memory T cells augment inflammation by either rapidly migrating to inflamed tissue or responding to cognate antigen within secondary lymphoid organs (SLO) and producing additional effector T cells (Carbone, Mackay, Heath, & Gebhardt, 2013). Circulating memory T cells were initially characterized as effector memory T cells (Tem) and central memory T cells (Tcm) based on the differential expression of lymph node-homing molecules (Sallusto, Lenig, Forster, Lipp, & Lanzavecchia, 1999). It was proposed that Tem circulate between the blood and nonlymphoid tissue (NLT), poised to rapidly respond to a recurrent infection whereas Tcm preferentially localized within SLO and function to undergo rapid proliferation and produce secondary effector T cells that would subsequently traffic to inflamed NLT to augment the response. However, it is now clear that additional circulating memory T cell populations exist. Specifically, a distinct population of CD8⁺ and CD4⁺ T cells that recirculate between NLT and SLO with greater efficiency than Tem have been identified (Bromley, Yan, Tomura, Kanagawa, & Luster, 2013; Gerlach et al., 2016). The precise functions and contributions of these circulating memory T cell subsets to recall responses are an area of ongoing investigation (Carbone et al., 2013). In this review, we will not distinguish between these different putative subsets and instead refer to them collectively as “circulating memory T cells.” Defining the mechanisms regulating T cell positioning and trafficking and how this influences the development, maintenance, and function of memory

T cells is essential to improving vaccine design as well as treatment of immune-mediated diseases. Here we will review our current knowledge of how chemokines, critical regulators of cell positioning and migration, govern the biology of memory T cells in vivo.

2. CHEMOKINES: MASTER REGULATORS OF CELL POSITIONING IN VIVO

Chemokines are chemotactic cytokines that control the migration of a wide variety of cell types (Griffith, Sokol, & Luster, 2014). In the context of the immune system, chemokines regulate the positioning of all immune cells and play a central role in immune cell development, homeostasis, and the induction of inflammation in vivo. Chemokines are the largest family of cytokines, composed of approximately 50 endogenous chemokine ligands in humans and mice. Chemokine receptors are seven-transmembrane receptors, which are differentially expressed on all cell types within the immune system. They are G protein-coupled receptors, which signal via G_i-type Gproteins. There are also atypical chemokine receptors, which seem to function by scavenging chemokines independently of G protein signaling and, in turn, regulate chemokine gradients in vivo. There are approximately 20 G protein-coupled signaling chemokine receptors and 5 atypical non-G protein-coupled chemokine receptors (Griffith et al., 2014). After synthesis, chemokines are secreted into the extracellular space where they bind heparin-like glycosaminoglycans on cell surfaces and embedded in the extracellular matrix. The resulting immobilized chemokine is believed to form a depot of chemoattractant that maintains a long-lasting directional cue and can also serve as a haptotactic gradient for crawling cells. The specific chemokine produced and the differential chemokine receptors expressed on distinct leukocytes regulate the positioning of immune cells in tissue during homeostasis and immune responses. In addition to regulating immune homeostasis and primary immune responses, a growing literature has demonstrated that chemokines are central to the biology of memory T cells in vivo.

3. CHEMOKINES AND MEMORY T CELL DEVELOPMENT: LOCATION DETERMINES T CELL FATE

3.1 Models of Memory T Cell Development

To present a framework for how chemokines regulate the development of memory T cells, we will first outline the current models of memory T cell differentiation. Of note, most of our knowledge of memory T cell development stems from studies on memory CD8⁺ T cells. The memory T cell field has focused on CD8⁺ T cells for several reasons. First, compared to CD4⁺ T cells, CD8⁺ T cells exhibit a greater proliferative capacity and more robust numerical stability over time, yielding a larger pool of memory T cells for investigation (Tubo & Jenkins, 2014). Second, CD4⁺ T helper cell activation is associated with tremendous heterogeneity in the differentiation program with a single CD4⁺ T cell giving rise to different effector cell types (O'Shea & Paul, 2010; Tubo et al., 2013). Finally, a landmark study by Kaech and colleagues identified cell surface markers on effector CD8⁺ T cells that allow phenotypic identification of short-lived effector cells (SLEC) and memory precursor effector cells (MPEC) early in the immune response (Kaech et al., 2003). Effector CD8⁺ T cells expressing high levels of KLRG1 and low levels of the IL-7R preferentially die after the peak of the immune response, whereas KLRG1⁻IL-7R⁺ cells preferentially give

rise to long-lived memory cells. The ability to differentiate CD8⁺ SLEC and MPEC with flow cytometry has greatly enhanced the CD8⁺ T cell memory field. SLEC/MPEC markers on T helper type 1 (Th1) cells have been described, but these markers have not been utilized widely in the memory CD4⁺ T cell field and robust markers identifying SLEC and MPEC in other T helper cell lineages have not been identified (Marshall et al., 2011). While there are likely common pathways in memory CD8⁺ and CD4⁺ T cell differentiation, the reader should be aware that the current models are predominantly based on experimental studies on CD8⁺ T cells and there may be unique mechanisms regulating the development of memory CD4⁺ T cells.

Upon recognition of cognate peptide:MHC in the context of appropriate costimulation and cytokines, naïve T cells proceed through three phases that can be characterized as clonal expansion, contraction, and memory formation (Kaeck & Cui, 2012). Approximately 90%–95% of the T cells undergoing clonal expansion die during the contraction phase via apoptosis, but a subset survive to yield long-lived memory cells. Elegant cell tracing studies have demonstrated that an individual naïve T cell can produce both SLECs and MPECs (Buchholz et al., 2013; Gerlach et al., 2013). Interestingly, despite intensive investigation, the mechanisms dictating SLEC vs MPEC cell fate remain unclear. There are at least three nonmutually exclusive models for T cell fate with each supported by experimental evidence (Gerritsen & Pandit, 2016). In the Asymmetrical Division hypothesis, the SLEC vs MPEC fate decision is made during the first cell division via asymmetrical partitioning of factors, such as atypical protein kinase C, mTOR, and c-myc (Arsenio, Metz, & Chang, 2015; Pollizzi et al., 2016; Verbist et al., 2016). Studies have shown that the daughter T cell proximal to the antigen-presenting cell has a greater propensity to differentiate into a SLEC, whereas the distal daughter T cell preferentially differentiates into a MPEC (Amsen, Backer, & Helbig, 2013; Gerritsen & Pandit, 2016). While this early partitioning of cellular factors likely plays a role, studies suggest that subsequent signals can influence T cell fate. In the Effector First hypothesis, naïve T cells must first differentiate into cytokine-producing effector cells and subsequent signals instruct a subset of effector T cells to transition into memory T cells (Gerritsen & Pandit, 2016). In support of this model, both CD8⁺ and CD4⁺ effector T cells expressing cytokines have been shown to give rise to long-lived memory T cells (Harrington, Janowski, Oliver, Zajac, & Weaver, 2008; Opferman, Ober, & Ashton-Rickardt, 1999). In contrast to the concept that effector T cells receive signals to initiate a memory program, the Decreasing Potential hypothesis proposes that activated T cells initially possess memory potential that is subsequently lost in most cells during clonal expansion, possibly due to the “strength” of TCR, costimulatory, and/or inflammatory cytokine signals (Gerritsen & Pandit, 2016). In support of the Decreasing Potential hypothesis, repeated antigenic stimulation increases CD8⁺ effector T cell proliferation at the expense of memory T cell development (Gattinoni et al., 2005). In addition, “latecomer” CD8⁺ T cells during a primary response preferentially differentiate into memory cells (D’Souza & Hedrick, 2006). Finally, blocking effector differentiation pathways increases the propensity for memory T cell development (Yang et al., 2011). As we discuss the role of chemokines in memory T cell development below, we will attempt to place the biology in the context of the prevailing models of memory T cell differentiation.

3.2 Chemokines in the Draining Lymph Node: Setting the Stage for Memory

During a primary immune response, CD4⁺ T cells provide help to CD8⁺ T cells with the former population enhancing the proliferation and functionality of the latter (Laidlaw, Craft, & Kaech, 2016). Furthermore, CD4⁺ T cell help is required for development of long-lived memory CD8⁺ T cells (Laidlaw et al., 2016). The predominant mechanism whereby CD4⁺ T cells provide help to CD8⁺ T cells is via “licensing” of dendritic cells (DC). Activation of naïve CD4⁺ T cells leads to expression of CD40L, which binds CD40 expressed on DC and promotes maturation including enhanced upregulation of costimulatory molecules. Studies from the Germain lab demonstrated that activated CD4⁺ T cells promote DC expression of the chemokines CCL3 and CCL4 (CCR5 ligands) (Castellino et al., 2006). Prior to peptide:MHC I recognition, CD8⁺ T cells within the draining lymph node upregulate expression of CCR5, resulting in colocalization with “licensed” DC. Interestingly, blocking CCL3 or CCL4 expression did not significantly alter the primary CD8⁺ T cell response, but did significantly affect the number and function of memory CD8⁺ T cells. However, the precise mechanisms whereby the CCR5 chemokine system regulates memory CD8⁺ T cell fate remain unclear. Specifically, whether CD4⁺ T cell help influence memory CD8⁺ T cell development by regulating the first cell division or subsequent fate-determining signals is unknown.

While CCR5 is induced on CD8⁺ T cells within the draining LN prior to recognition of cognate peptide:MHC I, after T cell activation, additional changes in chemokine receptor expression occur. Specifically, the homeostatic chemokine receptor CCR7, which directs T cells into the T cell zone of SLO, is downregulated, and additional inflammatory chemokine receptors are upregulated (Potsch, Vöhringer, & Pircher, 1999; Sallusto, Kremmer, et al., 1999; Sallusto, Lenig, Mackay, & Lanzavecchia, 1998). CXCR3, a receptor for the inflammatory chemokines CXCL9, CXCL10, and CXCL11, is expressed on activated CD8⁺ T cells and Th1 cells (Griffith et al., 2014). The expression of CXCL10, but not CXCL9, is induced by type I IFN, whereas both ligands are induced by interferon gamma (IFN- γ) (Griffith et al., 2014). Notably, C57BL/6 mice do not have a functional gene for CXCL11. CXCR3 is well demonstrated to promote T cell trafficking into inflamed NLT in certain models (Fadel, Bromley, Medoff, & Luster, 2008; Hancock et al., 2000; Hokeness et al., 2007; Nakanishi, Lu, Gerard, & Iwasaki, 2009; Zhang, Chan, Lu, Diamond, & Klein, 2008). In addition, CXCR3 has been shown to influence T cell differentiation within SLO. Both CD8⁺ T cells and Th1 cells exhibit decreased effector function when activated in vivo in the absence of CXCR3 signaling (Dufour et al., 2002; Groom et al., 2012; Rosenblum et al., 2010; Thapa & Carr, 2009; Whiting et al., 2004). Along with these functions during the primary immune response, CXCR3 also regulates the development of memory CD8⁺ T cells (Hu, Kagari, Clingan, & Matloubian, 2011; Kohlmeier et al., 2011; Kurachi et al., 2011). Three independent groups, utilizing both localized and systemic models of infection in mice, demonstrated that CXCR3 deficiency on CD8⁺ T cells significantly increased the development of memory CD8⁺ T cells (Fig. 1) (Hu et al., 2011; Kohlmeier et al., 2011; Kurachi et al., 2011). Utilizing adoptive transfer and mixed bone marrow chimeras, the authors showed that there was no significant difference in the early expansion or differentiation of WT or CXCR3-deficient CD8⁺ T cells (Hu et al., 2011; Kohlmeier et al., 2011; Kurachi et al., 2011). However, early in the response, CXCR3 deficiency resulted in

fewer SLECs and more MPECs (Hu et al., 2011; Kohlmeier et al., 2011; Kurachi et al., 2011). The increased generation of memory CD8⁺ T cells was not due to increased proliferation during the expansion phase or increased homeostatic proliferation during the memory phase (Kurachi et al., 2011). In fact, CXCR3-deficient CD8⁺ T cells exhibited less proliferation late in the immune response consistent with previous studies, suggesting that CD8⁺ T cells that continue to proliferate during late expansion contribute less to the memory pool (Hu et al., 2011; Kurachi et al., 2011; Sarkar et al., 2008). In addition, there was no evidence that differential trafficking between SLO and NLT explained the differences in the number of WT vs CXCR3-deficient memory CD8⁺ T cells. Of note, the authors did not utilize intravascular staining to determine if the memory CD8⁺ T cells were in the parenchyma or intravascular space of NLT (Anderson et al., 2014, 2012). Nevertheless, in both local and systemic infections, CXCR3-deficient CD8⁺ T cells exhibited a significant decrease in cell death after the peak of the immune response (Hu et al., 2011; Kohlmeier et al., 2011; Kurachi et al., 2011). Upon resolution of inflammation, the CXCR3-deficient memory CD8⁺ T cells contained a greater proportion of CD62L⁺CCR7⁺ T_{cm} and greater expression of markers associated with high memory potential, including CD127, CD27, and CD122 (Kohlmeier et al., 2011; Kurachi et al., 2011). In vitro activation of WT and CXCR3-deficient CD8⁺ T cells, in the absence or presence of CXCR3 ligands, did not result in differences in SLEC or MPEC development, suggesting that this biology only occurs in vivo (Kurachi et al., 2011). Finally, adoptive transfer of in vitro activated WT and CXCR3-deficient CD8⁺ T cells did not result in differences in CD8⁺ T cell contraction, suggesting that the role of CXCR3 in regulating CD8⁺ T cell fate is via T cell positioning in vivo during the expansion phase (Kurachi et al., 2011).

Studies have demonstrated that differential exposure to antigen or inflammatory cytokines, such as IL-2 and IL-12, during early expansion regulates SLEC/MPEC cell fate (Cui, Joshi, Jiang, & Kaech, 2009; Joshi et al., 2007; Kalia et al., 2010; Pipkin et al., 2010). Given the known role of CXCR3 in positioning T cells at the sites of inflammation, the authors hypothesized that CXCR3 was promoting SLEC differentiation by increasing T cell exposure to an inflammatory microenvironment. In models of systemic infection, WT CD8⁺ T cells formed clusters with DC within the marginal zone of the spleen, the location of antigen, and CXCR3 ligand production (Hu et al., 2011; Kurachi et al., 2011). In contrast, CXCR3-deficient CD8⁺ T cells preferentially localized with DC within the T cell zone (Hu et al., 2011; Kurachi et al., 2011). Interestingly, MPEC exhibited less CCR7 downregulation during the early expansion phase than SLEC. Driving persistent CCR7 expression in CD8⁺ T cells resulted in preferential localization within the T cell zone during early expansion, spatially separated from antigen and the inflammatory microenvironment of the marginal zone, and resulted in increased differentiation of MPEC (Hu et al., 2011). In a murine model of influenza infection, WT and CXCR3-deficient CD8⁺ T cells localize to different locations within the lung parenchyma, with WT cells localizing around large conducting airways and CXCR3-deficient CD8⁺ T cells localizing within the interstitium (Kohlmeier et al., 2011). These distinct positions were associated with CXCR3-deficient cells preferentially differentiating into MPEC (Kohlmeier et al., 2011). Interestingly, administration of additional antigen-pulsed DC into the lung resulted in enhanced contraction of CXCR3-deficient CD8⁺ T cells, suggesting that CXCR3 deficiency limits the exposure to antigen, as

well as an inflammatory microenvironment (Kohlmeier et al., 2011). In this latter study, CXCR3- and CXCR3/CCR5-double deficient CD8⁺ T cells exhibited a greater propensity for memory formation, indicating that these inflammatory chemokine receptors may collaborate to promote SLEC differentiation (Kohlmeier et al., 2011). These findings are supportive of the Decreasing Potential hypothesis with CXCR3 promoting the loss of memory potential by positioning CD8⁺ T cells within an antigen-rich, inflammatory niche. However, the precise mechanism(s) whereby CXCR3 ligands regulate SLEC/MPEC fate in a CXCR3-sufficient host remains unclear. One possibility is that DC in distinct compartments (MZ vs T cell zone or peribronchial region vs lung interstitium) are exposed to different amounts of virus and, as a result, produce different amounts of type I IFN. DC producing high levels of type I IFN would induce more CXCL10 via autocrine/ paracrine signaling. CD8⁺ T cells expressing CXCR3 would be maintained in close contact with the DC, resulting in prolonged TCR, costimulation, and cytokine signaling. The resulting high amounts of IFN- γ produced by the activated CD8⁺ T cell would further induce CXCL10 as well as CXCL9, augmenting this inflammatory niche and promoting SLEC differentiation. In contrast, DC exposed to less virus would produce lower levels of type I IFN and, as a result, less CXCL10. CD8⁺ T cells recognizing cognate antigen in this context or not expressing CXCR3 would be exposed to reduced inflammatory signals and preferentially differentiate into MPEC (Fig. 1). While additional studies are needed to further define the biology whereby CXCR3 regulates SLEC/MPEC cell fate, overall, these studies suggest that positioning of CD8⁺ T cells during the early immune response plays an important role in dictating SLEC/MPEC cell fate and regulating the size of the subsequent memory pool.

3.3 Chemokines in NLT: A Second Memory Checkpoint

The authors of the above studies did not investigate whether CXCR3 influences Trm development in their models since, at the time of these publications, tissue-resident memory T cell biology had not yet been firmly established. The study by Kohlmeier et al. utilizing an intranasal influenza infection model did show an increased number of antigen-specific CXCR3-deficient CD8⁺ T cells relative to WT from the bronchoalveolar lavage (BAL) and lung during the memory phase, suggesting that CXCR3 deficiency leads to an increase in Trm development (Kohlmeier et al., 2011). However, the difference in the number of WT and CXCR3-deficient CD8⁺ T cells within the BAL was small and only statistically significant at one time point. While the differences within the lung were larger, the authors did not convincingly demonstrate whether these memory CD8⁺ T cells were within the lung parenchyma and tissue-resident during the memory phase or were simply circulating memory CD8⁺ T cells trafficking through the extensive vasculature of the lung (Anderson et al., 2012).

The signals that regulate Trm development is an active area of investigation, and over the last several years, pathways have been defined that regulate Trm formation (Carbone, 2015; Schenkel & Masopust, 2014). Studies have demonstrated that chemokine receptor systems are critical in regulating Trm ontogeny in various models (Bergsbaken & Bevan, 2015; Mackay et al., 2013). Utilizing a model of cutaneous HSV infection in mice, Mackay and colleagues demonstrated that chemokine receptor signaling was required for the migration of effector CD8⁺ T cells into the epidermis and upregulation of the Trm marker CD103

(Mackay et al., 2013). Given the known role of the CXCR3 system in recruiting CD8⁺ T cells into the epithelium and the increased keratinocyte expression of CXCL9 and CXCL10 during HSV infection, the authors investigated the role of CXCR3 in regulating CD8⁺ Trm development. Interestingly, within the skin, KLRG1⁺ CD8⁺ T cells, which give rise to Trm, expressed higher levels of CXCR3 than KLRG1⁺ CD8⁺ T cells, which die after the resolution of inflammation. Injection of WT or CXCR3-deficient activated CD8⁺ T cells within the skin demonstrated that CXCR3 is required for efficient CD103⁺ Trm development. The difference between WT and CXCR3-deficient Trm was not due to reduced survival of CXCR3-deficient CD8⁺ T cells as this population was found at greater frequency within SLO, suggesting an enhanced efficiency in returning to the circulation in the absence of CXCR3 signaling. Given the role of CCR7 in promoting T cell egress from NLT into the lymphatics, the authors hypothesized that CCR7-deficient CD8⁺ T cells would give rise to a larger CD103⁺ Trm population. Coinjection of activated WT and CCR7-deficient CD8⁺ T cells demonstrated that CCR7-deficient CD8⁺ T cells yield a larger CD103⁺ Trm population and were present at lower numbers within the SLO. Interestingly, these results suggest that along with regulating CD8⁺ SLEC/MPEC differentiation within SLO, CXCR3 signaling within NLT influences the development of the memory pool by promoting CD103⁺ Trm formation at the expense of CD8⁺ T cell egress to the lymphatics to join the circulating memory CD8⁺ T cell population. Interestingly, utilizing intravital microscopy to investigate CD8⁺ T cell dynamics within the murine skin, Zaid and colleagues provided evidence that CXCR3 deficiency did not alter the cell morphology or motility of CD8⁺ Trm, suggesting that CXCR3 signaling may not be required for Trm trafficking within the epidermis (Zaid et al., 2017).

While the study by Mackay and colleagues suggests a role for CXCR3 in regulating CD8⁺ Trm development, it was unclear whether this chemokine receptor system would exhibit similar or distinct biology in different NLT or models of inflammation. In contrast to the skin, where the majority of CD8⁺ Trm express CD103, some organs possess both CD103⁺ and CD103⁻ Trm (Bergsbaken & Bevan, 2015; Casey et al., 2012; Wakim et al., 2012). A study utilizing a murine model of gastroenteritis and mesenteric lymphadenitis with the Gram-negative bacteria *Yersinia pseudotuberculosis* (Yptb) demonstrated that antigen-specific CD8⁺ Trm within the intraepithelial compartment were predominantly CD103⁺ whereas only half of the CD8⁺ Trm within the lamina propria expressed CD103 (Bergsbaken & Bevan, 2015). Among CD8⁺ Trm in the lamina propria, CD8⁺CD103⁺ Trm required TGF- β signaling, whereas CD8⁺CD103⁻ Trm did not require TGF- β signaling and were restricted to clusters with CX3CR1-expressing antigenpresenting cells (macrophages and/or DC) as well as CD4⁺ T cells within the lamina propria. The authors speculated that during the primary response the subset of effector CD8⁺ T cells within the lamina propria may be exposed to a distinct inflammatory microenvironment that promotes CD103 Trm development. They found that Yptb infection led to increased expression of CXCL10, but not CXCL9, in CX3CR1⁺ cells within the intestine. Interestingly, CXCR3-deficient CD8⁺ T cells were able to traffic into the intestine, but poorly formed clusters with CX3CR1⁺ cells and exhibited preferential differentiation into CD103⁺ Trm within both the intraepithelial and lamina propria compartments at the expense of CD103⁻ Trm. Finally, CXCR3deficient

CD8⁺ T cells less efficiently controlled bacterial replication within the intestine, suggesting a biologic relevance of the CD103 Trm to host defense.

These findings suggest that CXCR3 may regulate effector CD8⁺ T cell fate during two temporally and spatially distinct processes. First, CXCR3 regulates the positioning of CD8⁺ T cells during initial activation in SLO, localizing cells to a microenvironment rich in cognate antigen and inflammatory cytokines, thus driving SLEC development. Second, upon egress from SLO and trafficking to the infected NLT, CXCR3 exhibits distinct functions in different organs. Within the skin, the relative balance between CXCR3 and CCR7 signaling determines whether CD8⁺ T cells traffic in the epidermis and develop into CD103⁺ Trm or egress into the lymphatics and contribute to the circulating memory CD8⁺ T cell population. Within the intestine, CXCR3 guides CD8⁺ T cells to a niche in the lamina propria occupied by CXCL10-producing CX3CR1⁺ antigen-presenting cells and CD4⁺ T cells where signals are provided to promote CD103 Trm development at the expense of the CD103⁺ Trm population. While the role of CXCR3 in regulating CD8⁺ SLEC/MPEC cell fate within SLO is consistent with the Decreasing Potential hypothesis, the function of CXCR3 in CD8⁺ T cell fate within NLT is more supportive of the Effector First hypothesis. Specifically, CXCR3 signaling seems required to instruct CD8⁺ T cells to differentiate into Trm. These findings demonstrate the complexity of T cell fate decisions and underscore the difficulty of creating a unifying model for memory T cell development.

The role of chemokine receptor signaling in memory CD8⁺ T cell development does not appear to be restricted to the CXCR3 system. In a model of cutaneous HSV infection, Zaid et al. investigated the role of CCR10 in memory CD8⁺ T cell biology (Zaid et al., 2017). CCR10 has been shown to promote T cell homing to the skin during inflammation, and the CCR10 ligand CCL27 is highly expressed in the epidermis (Homey et al., 2002). Utilizing an adoptive transfer approach, the authors demonstrated that CCR10-deficient CD8⁺ T cells only show a modest and nonsignificant reduction in trafficking to the skin compared to WT cells. In addition, utilizing intravital microscopy they demonstrate that the morphology and velocity of CCR10-deficient cells were similar to WT cells. However, 30 days after HSV infection there was a significant decline in antigen-specific CD8⁺ T cells within the skin and spleen, suggesting CCR10 is required for efficient development of both CD8⁺ Trm and circulating memory T cells. The loss of Trm was greater with a 12-fold reduction in cell number compared to WT cells. This effect did not appear to be due to initial activation or expansion of CCR10-deficient CD8⁺ T cells. Interestingly, while the expression of CCR10 is induced early after activation and during the expansion phase, the expression is lost during the memory phase, suggesting that during the immune response CCR10 positions effector CD8⁺ T cells into a niche that promotes memory T cell differentiation.

In a murine model of malaria, CD8⁺ T cells within the liver upregulate the chemokine receptor CXCR6 as well as CXCR3 and CCR5. While CXCR6 was not required for trafficking of effector CD8⁺ T cells to the liver, there was a significant loss of CD8⁺ Trm in the absence of CXCR6 (Tse, Radtke, Espinosa, Cockburn, & Zavala, 2014). In support of the importance of CD8⁺ Trm in malaria, CXCR6-deficient mice did not inhibit the development of *Plasmodium* liver stages. Interestingly, phenotypic analysis of WT and CXCR6-deficient CD8⁺ T cells during the memory phase demonstrated that WT CD8⁺ Trm

within the liver are KLRG1^{lo}, whereas CXCR6-deficient cells expressed high levels of KLRG1, suggesting CXCR6 may be influencing the differentiation from effectors to Trm. However, the source of the ligands and the mechanisms for this lack of Trm differentiation were not investigated.

While these studies demonstrate that chemokine receptors play a critical role in memory CD8⁺ T cell development, numerous questions remain. Specifically, are the cell fate decisions regulated by chemokines within SLO irreversible or can signals within inflamed NLT reeducate a SLEC into a MPEC or vice versa? While CXCR3 regulated the size of the circulating memory T cell pool, does CXCR3 or other chemokine receptor systems regulate the size of specific memory T cell subsets? Within inflamed NLT, what signals determine the relative balance of inflammatory chemokine receptor vs CCR7 signaling that influence the size of the Trm and circulating memory CD8⁺ T cell pools? More specifically, what factors regulate CXCR3 signaling within the intestine to determine the relative size of the CD103⁺ and CD103 Trm populations? Is it simply the amount of CXCR3 ligands produced or are there additional antagonizing signals? Within the intestine, what additional signals regulate the trafficking of CD8⁺ T cells into the epithelium and subsequent differentiation into CD103⁺ Trm? Lastly, given that CCR10 is a skin-specific homing chemokine receptor, are other organ-specific homing chemokine receptors critical in CD8⁺ memory T cell fate decisions? Future investigation will be needed to further define the mechanisms whereby location of memory T cells within both SLO and NLT influences cell fate programs.

4. CHEMOKINES IN MEMORY MAINTENANCE: STORING MEMORY IN THE RIGHT PLACE

4.1 Maintaining Memory Clusters

The discovery of CD4⁺ Trm raised the question of how these cells rapidly find cognate peptide:MHCII when the number of CD4⁺ Trm and MHCII-expressing cells is relatively low in NLT. In both humans and mice, CD4⁺ Trm have been found to form clusters with antigen-presenting cells, maintaining these two populations within close proximity (Clark et al., 2006; Collins et al., 2016; Iijima & Iwasaki, 2014). Two independent groups have shown that chemokine signaling is required to maintain the colocalization of CD4⁺ Trm with antigen-presenting cells. Utilizing a model of genital HSV-2 infection in mice, Iijima and Iwasaki demonstrated that a population of antigen-specific CD4⁺ Trm persists in the genital mucosa, localized to clusters containing macrophages that the authors termed memory lymphocyte clusters (MLCs) (Fig. 2) (Iijima & Iwasaki, 2014). The presence of CD4⁺ Trm, compared with circulating CD4⁺ T cells alone, provided hosts with superior protection to lethal HSV-2 challenge. Treatment of HSV-2 immunized mice with topical pertussis toxin resulted in a significant loss of antigen-specific CD4⁺ Trm as well as the disappearance of MLCs, which was associated with a lack of protection against HSV-2 rechallenge. The loss of the MLCs did not appear to be due to increased cell death but rather expulsion of CD4⁺ Trm into the vaginal lumen, suggesting that chemokine receptor signaling was required for MLC maintenance. The authors found that genital mucosa from HSV-2 immunized mice exhibited increased expression of CCL5 and CXCL9.

In support of these chemokines regulating MLC persistence, CD4⁺ Trm within the genital mucosa expressed CCR1 and CCR5, which bind CCL5, and CXCR3, which binds CXCL9. Antibody neutralization of CCL5 resulted in a decrease in the number of antigen-specific CD4⁺ Trm as well as CD8⁺ Trm within the vaginal mucosa of immune mice. In addition, the loss of constitutive CCL5 signaling led to partial loss of MLCs and decreased protection to HSV-2 rechallenge that was similar to CD4⁺ T cell depletion. Complementary with the previous studies suggesting CXCR3 signaling regulates CD8⁺ Trm development, CXCL9 neutralization reduced the number of established CD8⁺ Trm, suggesting that ongoing CXCR3 signaling is required for CD8⁺ Trm maintenance within the genital mucosa. Macrophages were found to be the cellular source of constitutive CCL5 and depletion of CD11b⁺ cells resulted in loss of CCL5 expression as well as CD4⁺ Trm and MLCs. Interestingly, CCL5 expression required constitutive IFN- γ signaling from CD4⁺ Trm. While the authors demonstrate that HSV viral DNA was not detectable in the memory phase, the observation that constitutive CCL5 expression required basal IFN- γ expression raises the question of whether low levels of persistent antigen are required for MLC maintenance. The authors presented evidence that CD4⁺ Trm from the genital mucosa of immune mice can produce IFN- γ ex vivo without restimulation. These CD4⁺ Trm may be producing basal IFN- γ in response to low, persistent HSV antigen or through low avidity interactions with self-peptide:MHCII. An alternative possibility is that a subset of Th1 Trm are programmed to produce low levels of IFN- γ constitutively. Future investigations are needed to define the mechanisms maintaining MLCs.

In contrast to the MLCs observed in the genital mucosa, memory CD4⁺ T cells within the skin form clusters around hair follicles and dispersed throughout the interfollicular region (Clark et al., 2006; Collins et al., 2016). After viral infection with HSV or inflammation with the contact sensitizing agent DNFB, the total number of clusters as well as the number of CD4⁺ T cells within each cluster increases (Collins et al., 2016). Intravital microscopy revealed that CD4⁺ T cells within each compartment exhibit similar velocities, but the subset of CD4⁺ T cells within the perifollicular clusters exhibit a constrained migration pattern. Similar to the findings by Iijima and Iwasaki, the chemokine CCL5 was constitutively expressed within the memory CD4⁺ T cell clusters and contributed to the increase in perifollicular memory CD4⁺ T cell clusters as well as the number of memory CD4⁺ T cells within each cluster after infection or inflammation.

These studies suggest that constitutive CCL5 signaling within NLT is one mechanism promoting the colocalization of CD4⁺ Trm with antigenpresenting cells, enhancing the ability of CD4⁺ Trm to rapidly respond upon cognate antigen reexposure (Fig. 2).

4.2 Tissue-Resident Memory Within SLO

Along with the strong evidence that infection or inflammation establishes Trm within NLT, there is also evidence that Trm develop within SLO (Marriott, Dutton, Tomura, & Withers, 2017; Schenkel, Fraser, & Masopust, 2014; Ugur, Schulz, Menon, Krueger, & Pabst, 2014). Utilizing parabiotic mice, Schenkel and colleagues demonstrated that a subset of viral-specific memory CD8⁺ T cells in SLO are in disequilibrium with the circulation and exhibit phenotypic signatures of tissue residency including CD69 expression (Schenkel et al.,

2014). Furthermore, SLO-resident memory CD8⁺ T cells were localized within entry points for peripheral antigens. Specifically, they were localized to the splenic marginal zone, red pulp, and lymph node sinuses, suggesting they were poised to rapidly respond to antigens draining into SLO. This subpopulation of antigen-specific memory CD8⁺ T cells were enriched in IL-15-deficient mice, suggesting distinct mechanisms of persistence compared to circulating memory CD8⁺ T cells. In addition to the evidence that CD8⁺ Trm within SLO exist, two independent groups, utilizing in vivo cell labeling via photoconversion, have demonstrated that antigen-specific memory CD4⁺ T cells can establish residency within SLO (Marriott et al., 2017; Ugur et al., 2014). However, it remains unclear whether constitutive chemokine signaling regulates Trm maintenance within SLO. Future investigations are needed in this area to understand the mechanisms regulating Trm positioning within SLO.

5. CHEMOKINES DURING MEMORY RESPONSES: RAPIDLY ORCHESTRATING INFLAMMATION

5.1 Frontline Defenders Call in the Troops

One of the defining characteristics of memory T cells is the ability to rapidly respond during antigen rechallenge. The established model of a primary immune response involves the initial activation of innate immunity leading to expansion and differentiation of antigen-specific T and B cells. These effector lymphocytes can subsequently provide help to the innate immune system to orchestrate inflammation. However, memory T cells, as well as memory B cells, can rapidly respond to antigen and lead to early cross talk between the adaptive and innate systems. The discovery of Trm and subsequent demonstration of their critical importance in recall responses raised the question of how a relatively small number of T cells could have such a significant effect on inflammation. Several elegant studies have demonstrated that Trm can act as frontline defenders and upon activation rapidly produce cytokines that transform the surrounding microenvironment, activating local innate immunity and recruiting both innate cells and circulating memory T cells to the site of antigen reencounter (Fig. 3) (Ariotti et al., 2014; Glennie et al., 2015; Jiang et al., 2012; Schenkel, Fraser, Vezy, & Masopust, 2013; Stary et al., 2015).

Utilizing systemic LCMV infection in mice with subsequent rechallenge within the female reproductive tract, the Masopust group demonstrated that CD8⁺ Trm rapidly activate local chemokine expression upon antigen reexposure (Schenkel et al., 2013). Interestingly, this response occurred upon addition of antigen alone without the context of recurrent viral infection. CD8⁺ Trm induced various inflammatory chemokines, including CCL2 and CXCL9 within DC, CCL3 and CCL4 from CD8⁺ T cells, and CXCL10 and CX3CL1 from the endothelium. The activation of CD8⁺ Trm resulted in an increase in total CD8⁺ T cells within the NLT. To determine whether this was due to local proliferation of Trm or recruitment of circulating T cells, the authors used a congenic adoptive transfer system and demonstrated that activation of CD8⁺ Trm led to recruitment of circulating memory CD8⁺ T cells in an IFN- γ -dependent manner. Interestingly, this accumulation of circulating memory CD8⁺ T cells was associated with a decline in circulating memory CD8⁺ T cells within the blood and spleen and no increase in the draining or nondraining lymph nodes. These results

suggest that CD8⁺ Trm are able to recruit resting, circulating memory CD8⁺ T cells directly from the blood. Interestingly, a previous study demonstrated that topical application of the chemokines CXCL9 and CXCL10 to the vaginal mucosa in mice was capable of inducing recruitment of CD8⁺ T cell effectors (Shin & Iwasaki, 2012). However, resting memory T cells were not recruited into the tissue in this model despite CXCR3 expression. This raises the question of what additional signals beyond CXCR3 ligands are promoting the recruitment of resting, circulating memory CD8⁺ T cells. Similar mechanisms have been demonstrated for CD4⁺ Trm in murine models of *Chlamydia* and *Leishmania* infection, whereby CD4⁺ Trm rapidly produce IFN- γ , induce CXCL9 and CXCL10 expression that recruits circulating memory CD4⁺ T cells to the site of pathogen reencounter (Fig. 3) (Glennie et al., 2015; Stary et al., 2015).

5.2 Recalling Memories in SLO

Along with Trm inducing local chemokine expression to regulate circulating memory T cell recruitment, there is evidence that circulating memory T cells within LN rapidly induce local chemokine expression to further enhance the LN response (Fig. 4). Utilizing viral models in mice, two groups independently demonstrated that viral-specific memory CD8⁺ T cells and naïve CD8⁺ T cells (Tn) exhibit distinct trafficking patterns (Kastenmüller et al., 2013; Sung et al., 2012). Sung et al. demonstrated that both adoptively transferred LCMV-specific memory CD8⁺ T cells and Tn localize to the deep T cell area at steady state, but within hours after LCMV infection, 90% of memory CD8⁺ T cells had trafficked to the medulla, interfollicular area (IFA), and subcapsular sinus (SCS) whereas Tn trafficked to the periphery of the LN with reduced kinetics and efficacy. This initial, rapid trafficking of memory CD8⁺ T cells to the LN periphery occurred as soon as subcapsular sinus and medullary macrophages within the LN became infected and was CXCR3 dependent. LCMV infection of macrophages resulted in rapid production of type I IFN that induced local expression of CXCL10. Memory CD8⁺ T cells, which expressed CXCR3, were able to follow this chemokine gradient into the LN periphery whereas Tn could not. The rapid trafficking of antigen-specific memory CD8⁺ T cells to the periphery of the LN allowed them to interact with virally infected cells leading to IFN- γ production, which increased CXCL9 expression and promoted peripheral trafficking of additional memory CD8⁺ T cells. CXCR3-deficient memory CD8⁺ T cells exhibited a delayed response and reduced ability to clear virus. Kastenmüller et al. supported these findings, but the authors found that memory CD8⁺ T cells were prepositioned within the IFA and cortical ridge (Kastenmüller et al., 2013). During viral infection, “scout” memory CD8⁺ T cells residing in the LN periphery rapidly produced IFN- γ , which induced local CXCL9 expression, recruiting additional CXCR3⁺ memory CD8⁺ T cells in a feed-forward amplification loop. The differences found between these two studies regarding the prepositioning of memory CD8⁺ T cells within the LN during steady state may be due to technical differences. While Sung et al. predominantly used adoptive transfer of in vitro generated memory CD8⁺ T cells (via treatment with IL-15), Kastenmüller et al. investigated in vivo generated memory CD8⁺ T cells (Kastenmüller et al., 2013; Sung et al., 2012). The prepositioning described by Kastenmüller et al. is consistent with the report by Schenkel et al. demonstrating that a subset of in vivo generated memory CD8⁺ T cells in SLO are tissue-resident (Schenkel et al., 2014). Taken together, these studies suggest that CD8⁺ Trm within the SLO as well as circulating memory

CD8⁺ T cells are capable of augmenting the antigen-specific memory response to infection via a feed-forward circuit whereby IFN- γ -induced CXCL9 further recruits resting memory CD8⁺ T cells to the periphery of the LN to enhance T cell antigen-presenting cell interactions (Fig. 4).

5.3 Memory T Cells Augment Innate Responses

In addition to activated memory CD8⁺ T cells regulating the localization of resting memory T cells, there is evidence that memory T cells accelerate activation of the innate immune system. Utilizing a murine model of *Listeria monocytogenes* (LM), Soudja and colleagues demonstrated that immunized mice rechallenged with LM exhibited rapid activation of multiple innate cell types (Soudja et al., 2014). In vaccinated mice, there was more rapid activation of neutrophils, tissue macrophages, DC, NK cells, and NKT cells, which was associated with early and robust induction of several inflammatory chemokines, including CCL2, CXCL1, CXCL9, and CXCL10. This response was dependent on IFN- γ production by T cells, and this augmentation of the innate immune response was important for a protective recall response. However, whether tissue-resident or circulating memory T cells are the important subset was not investigated. In a murine model of *Leishmania*, the Scott group demonstrated that pathogen-specific CD4⁺ Trm recruit inflammatory monocytes to the site of local infection via regulation of CCR2 ligands (Glennie, Volk, & Scott, 2017). The recruitment of these inflammatory monocytes was necessary to mount an effective recall response. However, the mechanism whereby inflammatory monocytes promote protection remains unclear.

These studies demonstrate that memory T cells quickly and robustly induce a recall response in vivo via regulation of chemokine expression. Specifically, cognate antigen recognition by memory T cells induces rapid cytokine production, which, in turn, induces local chemokine expression that augments the recruitment and activation of resting memory T cells as well as components of the innate immune system to orchestrate inflammation.

6. FUTURE DIRECTIONS

As outlined in this review, we have made great progress in defining the mechanisms regulating memory T cell biology. However, numerous questions remain. For memory T cell development, a more comprehensive model for the SLEC/MPEC fate decision in vivo is needed. Specifically, given the significant heterogeneity in circulating memory T cell subset trafficking, identifying the overlapping and distinct mechanisms controlling the development of these individual subsets requires further investigation. Furthermore, while we are beginning to define the mechanisms maintaining Trm with NLT, it remains unclear which factors dictate the maintenance of Trm within SLO. In addition, although significant work has been performed to define the biology of memory CD8⁺ T cells, our understanding of memory CD4⁺ T cells remains limited. Among the multiple reasons for our paucity of understanding of memory CD4⁺ T cells, the extensive heterogeneity of T helper subsets is a major contributor. Elegant MHC class II tetramer experiments have clearly demonstrated that CD4⁺ T cells recognizing a particular peptide:MHCII complex differentiate into different T helper subsets (Tubo et al., 2013). Future studies will need to more clearly define

which T helper subsets contribute to the memory pool and identify markers that will allow identification of SLEC and MPEC early in the immune response. The ability to identify and track CD4⁺ SLEC and MPEC for a particular T helper subtype would enhance our ability to identify factors and mechanisms regulating this fate decision. Finally, additional investigation will be needed to leverage our understanding of memory T cell differentiation to design vaccines that induce a T cell response with a high propensity for MPEC formation as well as development of Trm in the proper location. In contrast, developing therapeutic agents that inhibit MPEC formation to treat autoimmune and allergic diseases has potential to prevent future exacerbations. Such advancements would yield tremendous opportunities to intervene in a vast array of infectious and inflammatory diseases.

7. CONCLUSIONS

Despite significant advancements in our understanding of primary T cell responses, the mechanisms regulating memory T cell development, maintenance, and function are not as well understood. Chemokines play a critical role in regulating memory T cell biology by influencing cell positioning in vivo. T cell localization influences the differentiation and maintenance of memory T cells in the appropriate niche, poised to rapidly respond to recurrent antigen exposure. In addition, memory T cells, which reside in low numbers, induce local chemokine expression to rapidly recruit numerous inflammatory cell types in order to orchestrate a robust memory response. Given the importance of memory T cells in infectious and inflammatory diseases, a greater understanding of the mechanisms regulating memory T cell biology has tremendous potential to improve human health.

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Fig. 1.

CXCR3 promotes CD8⁺ T cell differentiation into short-lived effector cells. DC recognition of virus induces the expression of IFN- α/β , which upregulates production of CXCL10. Priming of antigen-specific CD8⁺ T cells leads to expression of CXCR3 with the level of CXCL10 production facilitating CD8⁺ T cell–DC interactions. CD8⁺ T cell-derived interferon gamma (IFN- γ) increases DC expression of CXCL9 and CXCL10. High expression of the CXCR3 ligands maintains activated CD8⁺ T cells expressing CXCR3 within a niche of cognate antigen, costimulation, and inflammatory cytokines, including IL-12 and IFN- α/β . CD8⁺ T cell exposure to this inflammatory microenvironment preferentially promotes differentiation of short-lived effector cells (SLEC) at the expense of memory precursor effector cells (MPEC). CD8⁺ T cells not expressing CXCR3 receive less activating signals and preferentially differentiate into MPEC.

Fig. 2.

IFN- γ -dependent CCL5 production by macrophages maintains CD4⁺ Trm in memory lymphocyte clusters within nonlymphoid tissue. A subset of CD4⁺ resident memory T cells (Trm) constitutively express IFN- γ , inducing macrophages (M Φ) to express CCL5 and, in turn, maintain CD4⁺ Trm within clusters. Whether constitutive expression of IFN- γ by CD4⁺ Trm requires ongoing TCR signaling, either via persistent viral antigen or weak signals from self-peptide:MHCII, remains unclear.

Fig. 3.

Trm recruit resting, circulating memory T cells via local induction of CXCR3 ligand expression. Both CD4⁺ and CD8⁺ Trm are capable of rapidly producing IFN-γ upon reexposure to cognate antigen. IFN-γ induces the local expression of the CXCR3 ligands CXCL9 and CXCL10, which recruits resting, circulating memory T cells (Tmem) to the site of antigen encounter.

Fig. 4.

Memory CD8⁺ T cells within lymph nodes augment the recall response via a CXCR3-dependent feed-forward circuit. Memory CD8⁺ T cells may be within the T cell zone or prepositioned within the lymph node periphery. (1) Lymph-borne virus infects and/or activates subcapsular (SCS) macrophages, leading to local type I IFN production and, consequently, (2) CXCL10 expression. (3) CXCL10 preferentially recruits CXCR3⁺ memory CD8⁺ T cells (Tmem), but not naïve T cells (Tn), from the periphery and T cell zone to the sites of antigen presentation where (4) recognition of cognate antigen and inflammatory signals (5) induces IFN-γ expression. (6) IFN-γ subsequently induces CXCL9 expression, resulting in (7) recruitment of additional memory CD8⁺ T cells.