

# The CD8 memory T cell subsystem: Integration of homeostatic signaling during migration

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## Abstract

The ability of memory CD8 T cells to patrol non-lymphoid tissues represents an effective method whereby proficient immunosurveillance is achieved. From the analysis of memory CD8 T cell migration in vivo, it is clear that tissue-specific factors control trafficking and residence time within tissues. We propose that at least three pools of memory CD8 T cells exist based on migratory capabilities as dictated by their location in the body. Moreover, we hypothesize that the process of acquisition of homeostatic signals in specific tissues, such as the cytokines IL-7 and IL-15, regulates the mobility of memory T cells.

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## 1. Introduction

Naive CD8 T cells patrol secondary lymphoid organs in a circuitous route via blood and lymph until they encounter their cognate antigen (Ag) presented in the context of MHC class I by dendritic cells (DC) [1]. Once the naive T cell is activated, it undergoes a program of blastogenesis and cell division whereby cell numbers are increased dramatically and these cells become armed with effector molecules, such as IFN- $\gamma$ , perforin, and granzyme B, necessary to remove pathogen-infected tissue [2,3]. After the infection wanes, the population of effector cells undergoes significant apoptosis to eventually generate a small population of memory cells that is maintained through low level proliferation, and it is this pool of cells which confers life-long immunity against a particular pathogen [4–7].

The signals that dictate which effector cells are marked to become members of the stable population of CD8 memory T cells has received much attention over the last few years, since enhancing the generation of this pool of cells would greatly aid in current vaccine development. Recent studies have focused on the role of cytokines, particularly those which use

receptors of the common  $\gamma$ -chain family ( $\gamma_c$ ) including IL-2, 4, 7, 15, and 21 as these cytokines are important T cell survival factors [8]. For example, naive cells express high levels of the IL-7 receptor alpha chain (IL-7R $\alpha$ ) which directly influences the expression of anti-apoptotic molecules including bcl-2 [9–11], and IL-7 is essential to naive T cell survival [12,13]. At the peak of an anti-viral response, most responding CD8 effectors downregulate this receptor, suggesting that most of these cells are programmed to die during the contraction phase via cytokine withdrawal [12]. Recent work from Kaech et al. demonstrated that the small proportion (~5–10%) of effector cells which maintain high levels of the IL-7R $\alpha$  at the peak of a viral infection are poised to become memory cells [14]. The exact processes and molecules which maintain or induce re-expression of IL-7R $\alpha$  on this memory cell precursor population is still unknown, however, direct signaling through the IL-7R may not be required as IL7<sup>-/-</sup> mice are capable of generating CD8 memory T cells (unpublished data). Additionally, work from our laboratory using mice devoid of either IL-15 or IL-15R $\alpha$  implicated signaling through the IL-15R as important for the generation and maintenance of memory phenotype as well as virus-specific CD8 memory T cells [15–18]. However, lack of signaling through IL-15 did not completely abolish the development of memory cells, but was compulsory for the low-level turnover

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required for memory cell homeostasis [15]. Thus, it is clear that these two  $\gamma\text{c}$  cytokines play distinct but synergistic roles in the development and maintenance of CD8 memory T cells.

Additionally, other non-cytokine signals, including costimulators such as CD27 [19], may play important roles in the development of memory CD8 T cells. For example, a recent report suggests a role for homodimeric CD8 alpha (CD8 $\alpha\alpha$ ) in generating CD8 memory T cells [20]. CD8 $\alpha\alpha$  is expressed by subsets of CD8 T cells found in the intraepithelial compartment of the intestinal mucosa but is absent from all other resting T cells [21]. The ligand for this dimer is a nonclassical MHC class I molecule, known as thymus leukemia antigen (TL), and TL-tetramers permit tracking of CD8 $\alpha\alpha$  expressing cells [22]. Following polyclonal activation *in vitro* or after infection with lymphocytic choriomeningitis virus (LCMV), a small proportion of responding effector cells transiently express CD8 $\alpha\alpha$  [20]. These responders also coordinately express high levels of IL-7R $\alpha$  and IL-15R $\beta$ , suggesting that this population may also represent memory cell precursors. Indeed, following cell sorting of CD8 $\alpha\alpha^+$  and CD8 $\alpha\alpha^-$  LCMV-specific effectors and subsequent adoptive transfer and reactivation in naive recipients, only the CD8 $\alpha\alpha^+$  transferred population is capable of mounting a robust secondary response [20].

In summary, many researchers have addressed the effect of cytokines and other potential mediators on the generation of memory cells [8]. However, time-course studies analyzing gene expression in effector cells transitioning to memory cells describe numerous ongoing genetic changes prior to the establishment of a stable memory pool and a stable genetic profile [23]. Thus, it is unlikely that expression of a single molecule dictates which cells are destined to become memory cells. A more likely scenario would involve the multifaceted expression of a number of interactive proteins during the transition to memory and beyond to generate and maintain the memory cell pool. Additionally, once memory cells are produced, membership in a particular memory cell subset along with a specific tissue locale may dictate which homeostatic signals are required for maintenance. The focus of this review is to address the acquisition of homeostatic signals by memory CD8 T cells during migration and the resultant influence of these signals on maintaining memory cell pools.

## 2. Memory CD8 T cell localization in tissues

The advent of MHC class I tetramers and adoptive transfer systems allowed researchers to track Ag-specific T cell populations *in situ* during and following Ag challenge or infection [24–26]. The results from such studies inspired the realization that effector and memory T cells in non-lymphoid tissues, rather than in secondary lymphoid tissues, comprise the majority of antigen-specific T cells during all phases of an immune response [27]. For example, mice infected systemically with vesicular stomatitis virus (VSV) or *Listeria monocytogenes* rapidly generate effector cells that migrate

to numerous tissues including the liver, lung, bone marrow, brain and intestinal mucosa [26–29]. The subsequent long-lived memory cell pool derived from these effector cells represents a large proportion of the total T cell population in these sites as well as of the total antigen-specific T cell pool in the body.

While the aforementioned findings were seminal to our understanding of memory CD8 T cell migration, it remained unclear as to whether or not effector cells and their resultant memory cell progeny acquire tissue-specific homing properties. VSV represents a viral pathogen capable of infecting numerous cell types [30], however, do memory cells generated in response to infections with pathogens that replicate in distinct locales exhibit similar migration patterns? To address this question, Masopust et al. infected mice with either rotavirus or Sendai virus which preferentially infect intestinal epithelial cells and type II epithelial cells in the lung, respectively [29]. Using MHC class I tetramers containing each of the specific viral epitopes, the ability of the responding effector population to migrate to numerous tissues was assessed over time. Similar to what occurs following VSV infection, local challenges with Sendai or rotavirus elicit not only a robust response in the target tissue or respective draining lymph node, but in all other tissues analyzed, suggesting that effector cells are capable of migrating to numerous non-lymphoid tissues regardless of the initial site of Ag encounter. Similar results were obtained following influenza virus infection [31]. Thus, as a general rule, we believe that effector CD8 T cells are empowered to enter all tissues of the body. It is also likely that subsets of effector cells exist based on phenotype, function and location and that memory cells spawned by such effectors exhibit similar characteristics.

## 3. Memory cell pools: location, location, location!

To date, it is clear that at least two populations of memory cells exist: central memory cells (T<sub>CM</sub>) and effector memory cells (T<sub>EM</sub>). This subdivision of memory cells was initially based on the differential expression of the molecules CCR7 and CD62L, whose expression pattern directly influences the tissue location of the two populations [32]. CCR7 and CD62L are expressed at high levels on T<sub>CM</sub> and impart to these cells the ability to extravasate through the high endothelial venules (HEVs) of lymph nodes initially into the perivascular region of the medulla [33,34]. In contrast, T<sub>EM</sub> lack CCR7 and CD62L expression, thereby diverting their migration to sites other than lymph nodes. Originally, it was postulated that the T<sub>EM</sub> found in the peripheral tissues represented a pool that was maintained at these sites as the first line of defense against secondary challenge and that the T<sub>CM</sub> provided a reservoir of precursors that would reseed this pool as necessary [32]. However, adoptive transfer studies of memory cells generated following LCMV infection demonstrated that both populations (T<sub>CM</sub> and T<sub>EM</sub>) were capable of rapid killing of target cells in an *in vivo* cytotoxicity assay,

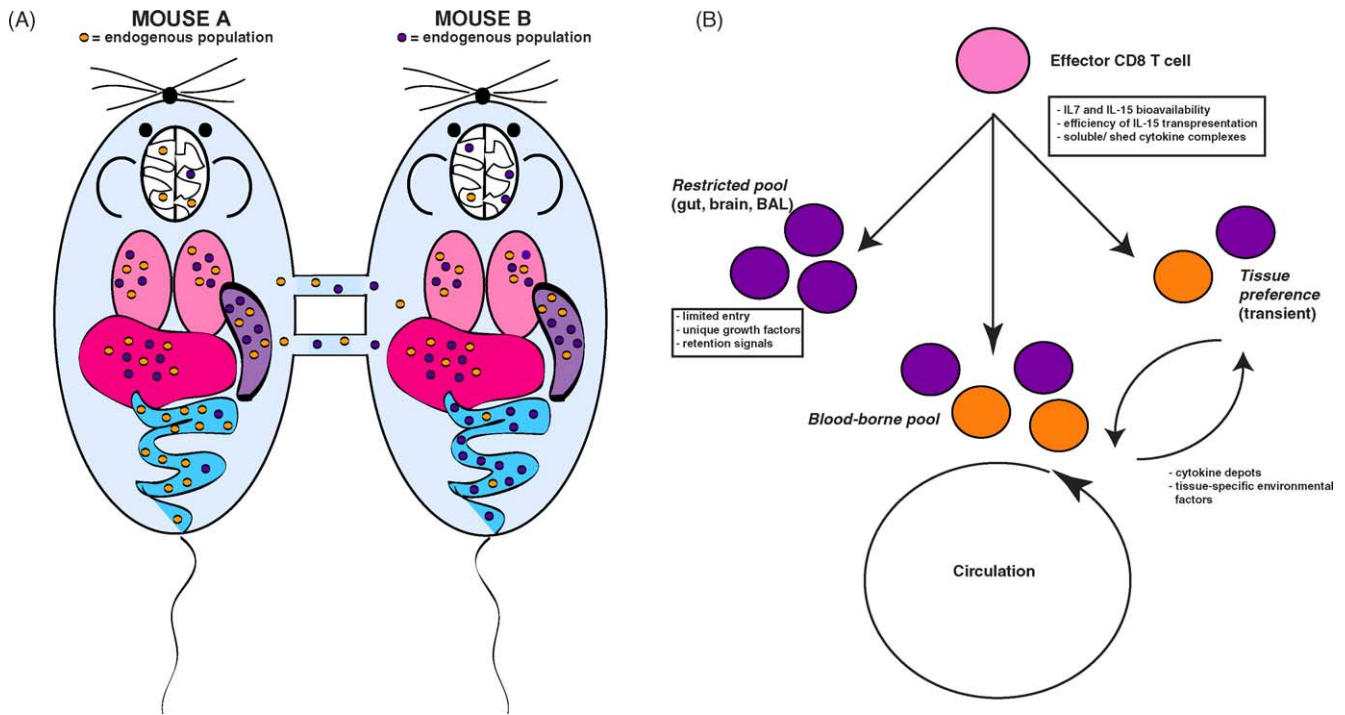


Fig. 1. The three pools of CD8 memory T cells and the influence of homeostatic signals on their tissue localization and migration patterns. (A) Analysis of parabiotic mice demonstrates at least three pools of memory cells: (1) a blood–borne pool, (2) a tissue–resident pool, and (3) a memory cell pool with preference for a particular tissue. (B) The differentiation of effector cells results in the development of three populations of memory cells whose tissue localization and migration patterns can be modulated by the indicated signals.

although when compared on a per cell basis, the  $T_{CM}$  were slightly more effective killers [35]. Long-term transfer experiments of LCMV-specific memory cells expressing low levels of CD62L ( $T_{EM}$ ) demonstrated that over time this population of cells converts to CD62L<sup>hi</sup>  $T_{CM}$ , suggesting that it is this population of memory cells that confers long-term protective immunity [36]. In contrast to protection against systemic LCMV infection,  $T_{EM}$  were more effective than  $T_{CM}$  at providing immunity to pulmonary infection with Sendai virus [37]. Thus, the neighborhood in which Ag is encountered can direct involvement of a particular memory cell subset.

While the precise lineage relationship between the  $T_{CM}$  and  $T_{EM}$  populations remains unclear, an additional layer of complexity is added when one considers further heterogeneity within these subpopulations of memory cells. Analysis of circulating human CD8 memory T cells demonstrates that disparate T cell receptor  $\beta$  chain repertoires [38] and distinct cell surface markers [39] can further distinguish the  $T_{CM}$  and  $T_{EM}$  subsets. Additional data indicates considerable phenotypic and functional heterogeneity among human CD8 memory T cell subsets [40]. These findings suggest that the two subsets may represent distinct lineages, but additional fate mapping studies are needed. In any case, these and other differences may influence trafficking of memory cells, as migration across tissue-specific endothelium or basement membranes may favor retention of cells with a certain phenotype or promote migration through a tissue. Our laboratory has used *in vivo* systems to ascertain the migration patterns of

memory CD8 T cells [27,29,41]. Extrapolation of the data unveils three potential pools of CD8 memory T cells based on their migratory capacity: (1) a common blood–borne pool with broad migratory ability, (2) a restricted population with strict tissue-specific tropism, and (3) an intermediate pool that is capable of moving into the blood–borne pool but temporarily may acquire preference for a distinct tissue (Fig. 1). This multilayered migratory capability of pools of CD8 memory cells may represent an underappreciated intrinsic ability of the adaptive immune system to patrol numerous sites in an extremely efficient manner.

### 3.1. A common blood–borne memory pool

Memory cells retain the ability to move into many lymphoid and non-lymphoid tissues, including the spleen, lymph nodes, lung, and liver [27]. Yet, the extent to which these cells move between tissues is unknown. The use of adoptive transfer systems and parabiotic mice has shed light on this topic and the results suggest that a common pool of memory cells exists that is capable of continuous migration through the blood to a number of tissues. For example, in one such study memory cells generated in VSV-infected mice were isolated from the spleen, lung, and liver and transferred into naive recipients [29] and their ability to migrate to other tissues was assessed using MHC class I tetramers. Results from this experiment demonstrate that while the memory cells exhibit a slight preference to home back to their tissue of origin, they

were also able to effectively migrate to all three sites, suggesting the existence of a pool of memory cells capable of moving between these tissues via the blood.

An independent set of experiments assessed the role of Gi-protein coupled receptors in splenic memory cell migration to these tissues [41]. Pertussis toxin (Ptx) inhibits Gi protein signaling [42,43], a component of the signaling pathway used by chemokine receptors, which have long been appreciated to mediate lymphocyte arrest on vascular endothelium and mediate extravasation into underlying tissue [44,45]. The expression of chemokine receptors in conjunction with distinct integrins and selectins was believed to potentiate the tissue-specificity hypothesized for memory cells [46,47]. However, pretreatment of VSV-specific memory cells with Ptx and subsequent transfer into naive recipients results in equivalent migration to the spleen, lung, and liver, further suggesting that a common pool of cells possesses the inherent ability to marginate to these tissues [41]. In contrast, Ptx treatment abrogates memory cell migration to the peritoneal cavity indicating that entry into certain tissues is chemokine receptor dependent [41].

While these experiments collectively established that memory cells from the spleen, lung, or liver could traffic to any tissue of this triad, they fell short of illustrating whether or not these cells are in constant recirculation through the blood *in vivo*. Removal and adoptive transfer of memory cell populations from tissues may influence their resultant migration pattern. For example, isolation techniques could result in the stripping of various cell surface molecules by enzymatic digestion from tissues and transfer of cells into the blood by *i.v.* injection may force these populations into an inappropriate migration route. To overcome these obstacles and directly assess the migration patterns of unmanipulated memory cell populations *in vivo*, our laboratory employed the use of parabiotic mice. Since as early as the 1920s researchers have used this model to directly assess the effects of circulating proteins on various physiological processes [48]. In this system, mice are surgically joined resulting in anastomoses of blood vessels within a few days. This model is powerful in that careful kinetic analysis of memory cell migration can be performed directly *in vivo* without perturbing the natural migration routes of established memory cell pools. In these experiments, memory mice generated from prior infection with VSV were surgically joined to uninfected naive mice and the migration of memory cells from the infected partner to various tissues of the conjoined recipient was assessed over time. These experiments demonstrate that as early as 8 days after joining, memory CD8 T cells in the spleen, lung, and liver have equally partitioned themselves between both parabionts [41]. That is, while the VSV-immune mouse lost half of its endogenous memory cell population, the naive partner acquired a memory cell pool in the corresponding tissue equivalent in size and functionality compared to the VSV-infected mouse. While these experiments are incapable of addressing whether or not the memory cells trafficked exclusively between the tissue of origin and its counterpart in

the naive mouse, they clearly demonstrate the ability of CD8 memory T cells to leave these sites and traffic between tissues as part of a common blood-borne pool.

### 3.2. *Restricted migration in specialized tissues*

While a common pool of blood-borne memory cells exists, there are examples of tissue-specific migration whereby effector cells are restricted in the initial migration to or retention within certain tissues. Much of this tropism to migrate to a particular tissue can be attributed to a variety of factors including the environment of initial T cell priming or the expression of certain integrins or chemokine receptors. Over the years, several groups have documented tissue-specific migration of both effector and memory CD8 T cells to particular sites, especially the skin and intestinal mucosa [45,46]. The heightened interest in these two compartments stemmed from the fact that certain known homing molecules were differentially expressed on cells isolated from these sites. For example, T cells isolated from the skin preferentially express E-selectin which binds the cutaneous lymphocyte antigen (CLA) expressed in the dermis [49]. The integrin  $\alpha 4\beta 7$  is differentially required for migration of CD8 T cells with different activation status to specific regions of the intestinal mucosa [26,41,49–51]. In addition, cells in the intestinal mucosa preferentially express the chemokine receptor CCR9 whose ligand CCL25 is expressed by small intestinal epithelial cells [52,53].

Based on the ability of certain gut-seeking populations of CD8 T cells to upregulate  $\alpha 4\beta 7$  and migrate to CCL25 *in vitro* [54–57], it was hypothesized that the initial site of T cell priming may influence the expression of  $\alpha 4\beta 7$  and CCR9 and mediate homing to the intestinal mucosa [58,59]. Recent studies have evaluated the contribution of DC isolated from distinct sites to evoke the expression of skin-homing (E-selectin) or gut-tropic ( $\alpha 4\beta 7$ ) molecules on responding CD8 T cells and promote their subsequent migration *in vivo* [59,60]. In one study, LCMV-specific P14 transgenic CD8 T cells were adoptively transferred into naive recipients which received DC loaded with GP33 (the P14 specific peptide) and administered *i.v.*, *i.p.*, or *s.c.* [60]. Depending upon the route of DC administration, the P14 T cells expressed different cell surface markers indicative of their homing preference. When DC were administered *s.c.*, P14 cells in the blood expressed high levels of E-selectin while those responding to DC administered *i.p.* preferentially expressed  $\alpha 4\beta 7$ . Although *i.p.* immunization would not be expected to prime T cells in a mucosal tissue, the results nevertheless suggest that factors present in the local lymph node environment instruct effectors to express tissue-specific homing molecules. Additional experiments using dendritic cells isolated from either Peyer's patch (PP) or peripheral lymph nodes demonstrated that only those effectors generated *in vitro* in the presence of PP DC were capable of upregulating gut-tropic molecules and trafficking to the intestinal mucosa *in vivo* [59]. Work by Iwata et al. implicates retinoic acid production

by mesenteric lymph nodes (MLN) or PP DC in imprinting  $\alpha 4\beta 7$  expression on effector T cells [61]. Thus, at least for the initial homing of CD8 T cell effectors to specialized sites, the local tissue environment of T cell priming directly affects resultant trafficking patterns. It should be noted however that tissue-specific imprinting is not required for tissue-specific homing of T cells, since T cell activation solely in the spleen results in migration of CD8 T cells to the intestinal mucosa as well as to other non-lymphoid tissues (unpublished results).

While the bias of effector T cells primed in gut-associated lymphoid tissue to traffic back to the intestinal mucosa has been well addressed, the ability of CD8 memory T cells to dynamically move into and out of this compartment has received little attention. Experiments using parabiotic mice demonstrate that unlike migration to other non-lymphoid sites such as the lung or liver, the migration of memory CD8 T cells to the intestinal mucosa is highly restricted [41]. Few CD8 T cells are capable of penetrating the intraepithelial lymphocyte (IEL) compartment while entry of lymphocytes into the lamina propria (LP) is only slightly less restrictive. Similarly, while no Ag-specific memory cells localized in the IEL compartment of the naive parabiont, few could be found in the LP 15 days after joining. Analysis of the migrants in the LP demonstrates that as CD8 T cells enter this space, they gradually upregulate expression of CD69, a marker constitutively present on resident CD8 T cells in the gut [62,63]. Therefore, it is conceivable that while these cells were not necessarily primed in the gut, a small portion of the blood-borne pool of memory cells is capable of colonizing the site. Further, after entry, intestinal memory cells may receive cues from the environment of the LP that regulate retention signals (perhaps CD69) which restrict migration out of the tissue. Niche size could also directly affect migration, as homeostatic mechanisms in the LP may support the maintenance of a finite memory pool. Thus, feedback mechanisms could be in place to “gate” entry into this tissue. Alternatively, perhaps only a small subset of circulating memory cells are capable of migration into the LP by virtue of expression of the necessary pattern of homing molecules. It is also possible that a population of LP memory cells from the infected mouse exits to the blood and preferentially homes to the LP of the naive partner. However, this scenario is unlikely considering that memory cells in the LP express low to undetectable levels of  $\alpha 4\beta 7$  and CCR9, and the  $\alpha 4\beta 7$  integrin is required for CD8 memory T cells to access the LP [41].

The experiments using parabiotic mice also demonstrate limited entry of memory cells into the brain [41]. The brain is an immunoprivileged site whereby the blood-brain barrier prevents potentially pathogenic T cells from migrating into the site and inducing neurological disease. Thus, it is imperative that migration of lymphocytes to this site should be tightly regulated as lymphocytes are not normally present in the brain in the absence of inflammation. Several studies using neurotropic strains of either influenza or dengue virus have demonstrated that CD8 memory cells resulting from these infections can generate long-term pools of mem-

ory cells in situ [64,65]. Additionally, VSV-immune mice are capable of generating effectors and resultant CD8 memory T cells at this site [27]. The parabiotic studies, however, demonstrate that once these memory cells are generated, they inefficiently exit the site as three times more VSV-specific memory cells are localized in the brain of the immune mice compared to the naive partners [41]. Thus, it seems likely that the memory cell pool found in this site, as well as perhaps the pool present in the intestinal LP, are derived from the initial effector populations that gain access to these specialized sites during the primary response (Fig. 1). However, the ability of a small number of blood-borne memory cells to enter these sites in the naive parabionts in the absence of overt inflammation suggests that the memory populations in these tissues can be slowly and perhaps partially replenished by the circulating common memory T cell pool.

T cell migration to the lung airways is also highly restricted. Under steady-state conditions, few T cells are present at this site. However, following infection with pathogens that preferentially infect lung epithelium, such as influenza or Sendai virus, a substantial proportion of memory cells can localize in the lung airways and can be isolated by bronchoalveolar lavage (BAL) [66]. BAL memory T cells proliferate poorly in the lung airways [67,68] despite the fact that they express markers suggestive of a heightened state of activation [67]. Notwithstanding their inefficient ability to divide in situ, BAL memory cells when transferred i.v. confer protection against secondary infection [67]. The same population of memory cells transferred intratracheally (i.t.), however, are incapable of clearing a secondary viral infection. One explanation for the lack of protection following i.t. transfer might be that cells in the lung airways are incapable of transversing back into the lung tissue and into the circulation, as migration to the mediastinal lymph node or the bronchus associated tissue (BALT) is necessary for mounting effective primary responses against influenza virus [69,70] and may also be required for recall responses. An outstanding question is whether memory CD8 T cells in the lung airways are continuously replenished by recruitment of blood-borne memory cells into this tissue.

### 3.3. Memory CD8 T cells which preferentially localize within specific tissues

While a common pool of blood-borne CD8 memory T cells exists, it is not unlikely that a small population of these cells acquire tropism for a given tissue based on homeostatic signals received following extravasation into a specific site. That is, following migration of effector cells to specific non-lymphoid sites, resultant memory cells may be instructed to acquire a residence preference for a distinct locale based on environmental cues. This population would be an intermediary between the blood-borne pool and the specialized tissues, and membership in this pool may be transient. Results from experiments in which VSV-specific memory cells from distinct anatomical locations are adoptively transferred into

naive recipients suggest that this may be the case [29]. Thus, memory cells isolated from the lung or liver, while capable of migrating to multiple sites, show a slight preference for homing back to their tissue of origin, suggesting that a subset of memory cells may have tissue-specificity. Whether such cells acquire this capability during the primary response or following differentiation into memory T cells remains to be determined.

#### 4. Regulation of memory pool homeostasis

While differences in pathogen or route of infection may perturb the balance of cells allocated to each memory T cell subset, homeostatic signals may also contribute to the distribution and/or reprogramming of the migration patterns of CD8 memory T cells. These signals could be generated during the initial priming event or during the subsequent localization and continued differentiation of memory cells during residence in specific non-lymphoid tissues. Although little experimental evidence exists which directly implicates specific homeostatic signals in differential maintenance of each of the previously discussed memory cell pools, we hypothesize that a combination of signals via cytokines and other costimulatory molecules may directly affect the migration into, dwell time within, and exit from specific non-lymphoid tissues.

##### 4.1. Regulation of memory cells by IL-7 and IL-15: maintaining a moving target

Since a large proportion of memory CD8 T cells are continually recirculating between blood, lymphoid and non-lymphoid tissues, the system must provide anatomical and cellular cues which direct acquisition of homeostatic signals by transiting memory cells. Distinct pools of memory cells either transiently or permanently present within certain tissues may require different combinations of cytokines for growth, survival, or maintenance, and the availability or expression pattern of growth factors may also influence memory cell trafficking. While additional cytokines may affect the migration of memory cells *in vivo*, we will focus our attention on IL-7 and IL-15 as their role in maintaining memory CD8 T cell survival and maintenance is well documented [12,16–18,71]. These cytokines could influence the migration and compartmentalization of memory pools in three ways: (1) through the levels of cytokine available in a given tissue, (2) in the case of IL-15, by the efficiency of transpresentation by IL-15R $\alpha$ , or (3) by soluble or shed cytokine/cytokine receptor complexes which could be present in tissues, blood, or lymph.

IL-7 is produced primarily by stromal cells, including intestinal epithelial cells, but monocytes and subsets of human DC may also produce this cytokine [72–75]. These cellular sources of IL-7 are well represented in most tissues suggesting that regulation of this cytokine may be achieved independent from production, which may also be limiting in specific tissues. IL-7 has been found to be associated with heparin sul-

fate, a macromolecule which binds laminin, a constituent of basement membranes (BM) [76,77]. As lymphocytes transverse blood vessels prior to entry into tissue parenchyma, cells contact BM and may encounter repositories of IL-7. In addition, IL-7 can also associate with the soluble molecule heparin at an even higher affinity [78]. Heparin is found in normal serum and also intracellularly in many tissues [79]. It is believed that heparin can act as a carrier molecule, capable of either preventing molecular degradation of IL-7 by plasmin or blocking interaction of IL-7 with its receptor on cells [79]. Thus, the availability of IL-7 can be regulated on several levels as the amount and distribution of heparin, heparin sulfate, and plasmin could be differentially regulated in distinct tissues. While the relevance of IL-7 regulation by these mechanisms is untested *in vivo*, *in vitro* studies using IL-7-expressing stromal lines demonstrate that the addition of heparin blocks the IL-7 dependent maturation of B cell precursors [80] and degradation of IL-7 by plasmin [79]. Taken together these findings suggest that regulation of IL-7 bioavailability could directly affect memory cell migration. Since memory cells require IL-7 for survival [12], those cells migrating through certain tissues may sense reservoirs of IL-7 and remain temporarily tissue-resident, until they are reassimilated into the blood-borne pool. In addition, as the sources of IL-7 are diverse and represented in numerous tissues, broad expression of soluble or BM-bound IL-7 may promote continual migration through tissues. Enhanced adhesive properties of memory cells based on increased levels of molecules such as CD44 and LFA-1 are likely to also mediate increased dwell time in tissues thereby enhancing acquisition of homeostatic signals.

Messenger RNA encoding IL-15 can be detected in numerous tissues, however, IL-15 protein is rarely detected in tissues due to poor translation or a very short half-life *in vivo* [81,82]. The receptor for IL-15 is heterotrimeric, comprised of the  $\gamma$ C, a  $\beta$  chain shared with IL-2 receptor (IL-2/15R $\beta$ ), and a private IL-15R alpha chain (IL-15R $\alpha$ ). Memory CD8 T cells express high levels of IL-15R $\alpha$  and  $\beta$ , which led to the belief that the ability of memory cells to proliferate in response to IL-15 was mediated via the trimolecular receptor [15,83]. However, work from Dubois et al describes a novel model of cytokine signaling whereby bystander cells expressing IL-15R $\alpha$  could bind IL-15 *in vitro* and present the cytokine *in trans* to opposing cells expressing IL-2/15R $\beta$  and  $\gamma$ C [84]. Indeed, this mechanism of transpresentation of IL-15 was discovered to be functional *in vivo* since when IL-15R $\alpha$ <sup>-/-</sup> CD8 memory T cells are present in a normal host, they undergo normal proliferation [71,85,86]. Moreover, signaling is directed through the IL-15R since CD8 memory T cells deficient in IL-2/15R $\beta$  are incapable of proliferating. Additionally, when normal memory cells are transferred into IL-15R $\alpha$ <sup>-/-</sup> recipients, and exogenous IL-15 is provided, the memory cells are unable to divide. Recent data also indicate that the same cell type producing IL-15 also must express IL-15R $\alpha$  to mediate CD8 memory T cell and NK cell homeostasis [86,87]. This novel pathway of cytokine function adds an

additional twist to the process of memory T cell homeostasis. Such a system may be important for decreasing the biosynthetic requirements for IL-15 production, for increasing IL-15 half-life, and for localizing IL-15 to specific anatomical niches [86–88].

The limited bioavailability of IL-7 or IL-15 within a given tissue could evoke intense competition among memory cells for access to survival and growth signals. This finite supply of resources may impart a distinct homing pattern on memory cells in that those cells unable to acquire a survival signal *in situ* would move on, entering the afferent lymphatics to eventually return to the bloodstream via the lymphatic ducts and continue the search for resources. It is also possible, though untested, that lymphatic fluid or lymphatic vessels may be rich in homeostatic cytokines, thereby providing a ready source of nutritional factors as lymphocytes travel through the body. Alternatively, some memory cells which may have initially been part of the blood-borne pool may find themselves in tissues or specific niches within an organ where IL-7 and/or IL-15 is abundant. Perhaps these memory cells, striking cytokine gold, will either remain resident in a given tissue or prolong residence time at a particular site until the resource becomes low and warrants the memory cell to seek another cytokine source. This notion of limited cytokine availability within distinct tissues is relevant as some extracellular matrices may be less efficient at binding IL-7 while a quantitation of IL-15/IL-15R $\alpha$  expression *in situ* is currently lacking. Thus, regulation of the synthesis and stability of IL-7 and IL-15 *in vivo* could influence memory T cell migration. Experiments in which tissue-specific expression levels of these cytokines can be manipulated may address whether supply and demand of these cytokines can perturb the dynamics of the memory cell pools.

Tissue-specific mechanisms may also be in place with regard to transpresentation of IL-15, which may be disparate between tissues. This may indeed be the case as suggested by experiments from our laboratory which sought the identity of the cell type capable of presenting IL-15 to opposing cells using bone marrow chimeras to restrict IL-15R $\alpha$  expression to either bone marrow-derived or parenchymal cells [71]. These experiments reveal that proliferation of memory cells found in the spleen requires bone marrow-derived cells for transpresentation, while memory cell proliferation in the lung is mediated by both bone marrow-derived and parenchymal cells, the exact cell types still unknown. Understanding the role of specific cells in the control of transpresentation in a given tissue will require a detailed analysis of IL-15/IL-15R $\alpha$  expression on the various cell types in conjunction with *in situ* imaging. Further, the stoichiometry of the  $\gamma$ C receptors should be considered. That is, memory CD8 T cells may express several  $\gamma$ C-utilizing receptors whose expression pattern may be distinct depending on subset or location so competition may exist at the molecular level for receptor signaling components linked to IL-7R or IL-15R.

Finally, recent experiments from Mortier et al. suggested an additional mechanism that may regulate the biological

functions of IL-15 *in vivo* [89]. This group transfected IL-15R $\alpha$  incompetent cells with the human IL-15R $\alpha$  gene and detected expression of the protein using radiolabeled human IL-15 *in vitro*. Over time, these receptor complexes were spontaneously shed as soluble proteins with certain stimuli (PMA and ionomycin) inducing a more rapid cleavage. This IL-15/IL-15R $\alpha$  complex is generated via a natural proteolytic cleavage site in the extracellular domain of the IL-15R $\alpha$  protein. Further, this cleavage product promotes T cell proliferation [89]. Whether this soluble receptor exists and has function *in vivo* is unclear, but such a complex could have profound consequences for memory cell homeostasis. Indeed, transpresentation by a soluble IL-15/IL-15R $\alpha$  complex could be an effective means of driving IL-15 mediated functions, including proliferation. Thus, this shed receptor complex could be beneficial in maintaining the blood-borne pool of memory cells. While this idea is attractive, determining the expression pattern of the proteases responsible for production of the soluble complex as well learning the biological half-life of this complex and its functional effect on memory cell function and migration requires further investigation. In summary, only after determining the relative contribution of tissue-specific presentation of IL-7/IL-15 and other cytokines/chemokines to memory T cell homeostasis can we begin to understand the effects of cytokine acquisition on memory cell migration.

#### 4.2. *Distinct homeostatic signals in memory “privileged” tissues*

While the common migrating pool of memory cells may have multiple opportunities to acquire homeostatic cytokine signals in a number of sites, this may not be the case for tissue-resident memory cells. Our results suggest that memory cells in the brain and intestinal mucosa and perhaps other “privileged” tissues may be largely derived from primary effectors in those sites, implying that the memory population is maintained in large part by self-renewal and survival *in situ*, with limited input from “external” memory CD8 T cells. Thus, regulation of IL-7 or IL-15 presentation in such tissues may require additional constraints. Moreover, there are likely tissue-specific factors that come into play to control the migration/retention and perhaps function of resident CD8 and CD4 memory T cells. In the intestinal mucosa, the fact that the memory cell pool seems to be static over time, permitting few memory cells from the blood to enter, would suggest that the size of the memory cell pool in this niche is restricted. Indeed, when one considers that, unlike other tissues such as the lung and liver, the vast majority of T cells in the LP and intestinal epithelium, as well as in the brain, appear to be memory or effector cells, it is likely that additional levels of control are in place.

Memory CD8 T cells in the intestinal mucosa are distinct from their counterparts in other non-lymphoid tissues in a number of ways. For example, intestinal memory CD8 T cells express lower levels of IL-7R $\alpha$  than memory cells in

other tissues (unpublished data). As mentioned previously, intestinal epithelial cells express high levels of IL-7 and IL-15, in part due to the requirement for these cytokines in the thymus-independent development of the TCR $\gamma\delta$  subset of T cells in the IEL compartment [88,90]. Thus, these developing CD8 $\alpha\alpha$  TCR $\gamma\delta$  cells, as well as the IL-15-dependent CD8 $\alpha\alpha$  TCR $\alpha\beta$  IEL, may directly compete with mucosal memory cells for cytokine access. Recent work from Park, et al, suggests that the limited levels of IL-7 present in vivo, can be shared by the greatest number of cells vying for IL-7-mediated survival signals, by downregulating IL-7R $\alpha$  through transcriptional repression [91]. While the relevance of this mechanism in vivo is unknown, the lower levels of IL-7R $\alpha$  present on memory cells in the intestinal mucosa may validate this theory. Whether lower IL-7R expression by mucosal memory cells results in decreased survival is not known. It is also possible that IL-7R downregulation is the result of a more chronic, rather than a transient, IL-7 driven signaling. In addition, while memory CD8 T cells in other non-lymphoid tissues require the synergistic effects of IL-7 and IL-15 for survival and homeostatic proliferation, memory cells in the intestinal mucosa may use alternative growth signals.

Another molecule that is differentially expressed by intestinal memory T cells is CD69 [29,41,63]. Experiments using parabiotic mice demonstrate that the small number of memory cells capable of entering the intestinal mucosa upregulate CD69 upon entry [41]. Further, our laboratory and others have shown that almost all resident lymphocytes in the intestinal mucosa express CD69 [41,63]. While very few circulating lymphocytes express CD69, its expression is inducible following activation through the TCR or other activation-induced stimuli such as cytokines [92–94]. However, expression of CD69 is rapidly lost after withdrawal of stimulus. While it is possible that low-level persistent Ag may maintain mucosal lymphocytes in a partially activated state, our laboratory has failed to detect Ag in the intestinal mucosa after viral clearance, and it seems unlikely that antigen would persist in only this tissue after a variety of infections (unpublished data). To date, it remains unclear what role CD69 plays in maintenance of homeostasis at this site. CD69 is a type II membrane protein and a C-type lectin [95]. Although the CD69 molecule was described >10 years ago, its ligand and specific signaling properties remain largely unknown [96]. However, overexpression of CD69 or blockade of CD69 affects thymocyte development [97,98] in an interesting fashion. Modulation of CD69 expression results in an apparent blockade in emigration of mature T cells from the thymus, perhaps due to retention of cells in the thymic medulla [97,98]. Whether this effect is due to aberrant CD69 signaling triggered through antibody treatment or by overexpression is not known, and T cell development in the CD69 null mouse has not been characterized in detail [99]. Nevertheless, we hypothesize that upregulation of CD69 expression simultaneous with entry into the intestinal mucosa is involved in retention of memory cells at this site and may also regu-

late their functional capabilities. We further suggest that, in general, entry of circulating memory cells into non-lymphoid tissues will have functional consequences for memory cells including modulation of migration.

## 5. Concluding remarks

While substantial research has deciphered the homeostatic signals required for memory cell survival and division, for the most part these studies have not extended beyond the realm of lymphoid tissues. At present, memory CD8 T cells, and we believe memory CD4 T cells as well, can be subdivided into at least three distinct, but partially overlapping pools based on migratory properties. Since following immunization or infection a majority of the memory cells are found in non-lymphoid sites, future research will focus on understanding the relationship between the memory cell pools and the homeostatic factors regulating each population. The integration of these memory T cell pools into the immune system and into the organism as a whole emphasizes the goal of the immune system to prevent subsequent infections and increase survival. Therefore, understanding and manipulating the homeostatic signals required to maintain each of these pools will have profound effects on current vaccine development where enhancement of a particular memory T cell subpopulation is warranted.

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