Genetic-tracing of CD8+ T cell fate decisions

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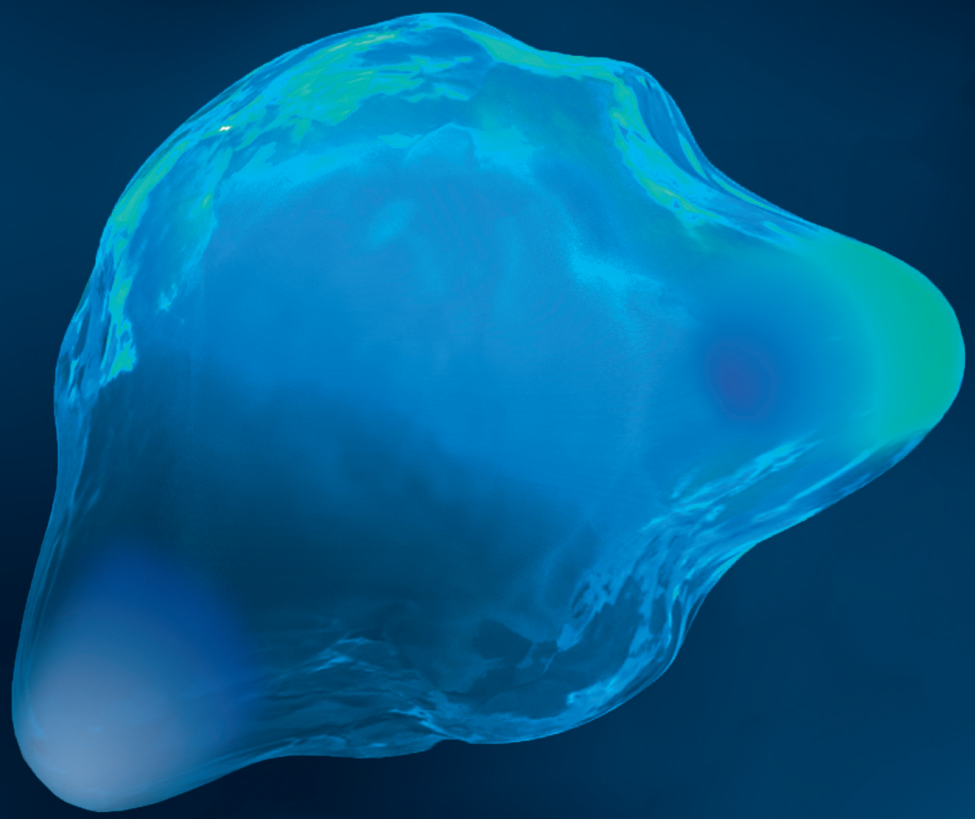

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The precursors of CD8$^+$ tissue-resident memory T cells: From lymphoid organs to infected tissues

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ABSTRACT

CD8$^+$ tissue resident memory T (T_{RM}) cells are essential for the immune defense against pathogens and malignancies, and the molecular processes that lead to T_{RM} cell formation are hence of substantial biomedical interest. Prior work has demonstrated that signals present in the inflamed tissue micro-environment can promote the differentiation of memory precursors cells into mature T_{RM} cells, and it was therefore long assumed that T_{RM} cell formation adheres to a "local divergence" model, in which T_{RM} cell lineage decisions are exclusively made within the tissue. However, a growing body of work provides evidence for a "systemic divergence" model, in which circulating T cells already become preconditioned to preferentially give rise to the T_{RM} cell lineage, resulting in the generation of a pool of T_{RM}-poised cells within the lymphoid compartment. Here, we review the emerging evidence that supports the existence of such a population of circulating T_{RM} cell progenitors, discuss current insights into their formation and highlight open questions in the field.

INTRODUCTION

A fundamental aspect of CD8$^+$ T cells is their ability to adapt to the type of pathogens encountered. First, through the process of clonal expansion upon antigen recognition, the T cell pool becomes biased to recognize pathogens that it has previously been exposed to[1]. Second, remodeling of the epigenetic landscape allows memory cells that are formed in this process to more rapidly exert effector functions[2]. Third, the distribution of the CD8$^+$ T cell memory compartment over different body sites maximizes the chance of early pathogen recognition upon renewed infection[3]. In line with the concept that the CD8$^+$ memory T cell pool can provide rapid effector functions and has the capacity for renewed clonal expansion, this cell pool is highly diverse at the epigenetic, transcriptional, and protein expression level. Specifically, within the circulation (i.e blood, lymph and secondary lymphoid organs [SLO]), two main subgroups of memory T cells can be distinguished, often referred to as central memory (T_{CM}) and effector memory T (T_{EM}) cells, which collectively form the circulating memory pool (here jointly referred to as T_{CIRCM} cells). T_{CM} cells can be distinguished by a high level expression of the lymphoid homing markers CD62L and CCR7. They are considered to be multipotent and at least a subset of this cell pool—often referred to as stem cell memory T (T_{SCM}) cells—displays a heightened expansion potential upon antigen re-encounter[4]. In contrast, T_{EM} cells possess limited expansion potential and lack the ability to enter lymph nodes from the blood, but are marked by expression of cytotoxicity-associated genes and can exert rapid effector functions upon renewed TCR signaling[5]. T_{EM} cells were long believed to be superior in penetrating and surveying peripheral tissues; however, this idea has come under scrutiny as recent work has suggested that T_{EM} and T_{EM}-like cells are mostly excluded from human and mouse non-lymphoid tissues (NLT)[6–9].

In addition to the systemic memory T cell pool, a pool of tissue resident memory T (T_{RM}) cells that permanently resides within NLT can be distinguished. Through a process of continuous migration and surveillance that is confined to distinct anatomic compartments, such as the stroma or the parenchyma of organs, T_{RM} cells patrol tissues to scan for foreign invaders[10,11]. Following antigen encounter, T_{RM} cells rapidly induce a local state of alarm, resulting in the recruitment of other immune cells and the local production of antimicrobial and antiviral proteins by epithelial cells[12,13]. In line with this 'pathogen alert' function, T_{RM} cells do not only produce cytotoxicity-associated molecules, such as granzyme B and perforin, but also cytokines such as IFNγ and TNF that can influence the behavior of neighboring cells[14–19]. Furthermore, the existence of T_{RM} cells that express minimal levels of cytolytic molecules, and may therefore mostly rely on this 'pathogen alert' function, has been reported in various human tissues[20–23]. Whereas T_{RM} cells share transcriptional features with both T_{CM} and T_{EM} cells, they are unique in their expression of a tissue residency-promoting transcriptional signature, which marks T_{RM} cells in a wide range of tissues. Besides this core tissue residency signature, T_{RM} cells also display transcriptional features that are specific to individual tissues and allow their survival and long-term retention at those different sites [24,25].

The residency signature that marks T_{RM} cells in multiple tissues is characterized both by a reduced expression of proteins that promote tissue egress and a heightened expression of proteins that promote tissue retention. For instance, T_{RM} cells show reduced expression of the cell-surface molecules S1PR1 and CCR7 that promote T cells to leave NLT, an observation that is explained by a lowered expression of the transcription factor KLF2, which drives *S1PR1* and *CCR7* transcription[26]. On the other hand, T_{RM} cells express CD69 and, in case of T_{RM} cells localized within epithelial tissues, the E-Cadherin binding integrin alpha-E (CD103, encoded by *Itgae*), that both promote tissue retention (for a comprehensive review of the molecular pathways that control tissue retention please see[27]). The expression of CD69 and CD103 should be considered imperfect markers to infer tissue residency, as absence of their expression does not rule out long-term tissue retention, and presence does not exclude the potential to leave NLT [28–32]. Nevertheless, much of our current understanding of T_{RM} cells is based on analyses of $CD69^+CD103^+$ T_{RM} cells in epithelial tissues.

In line with their role as local sentinels, $CD8^+$ T_{RM} cells have been shown to both prevent and exacerbate pathologies. For instance, T_{RM} cells are not only superior over T_{CIRCM} cells in conferring protection to recurring local pathogens [33,34], but these cells can also provide protection against the development of skin malignancies[35–37]. Moreover, tumor infiltrating lymphocytes that highly resemble conventional T_{RM} cells have been associated with improved disease prognosis[38,39]. At the same time, T_{RM} cells may drive immunotherapy-induced colitis[40], the skin autoimmune disorders vitiligo[41] and psoriasis[42,43] and also other autoimmune and allergic diseases[44], and may play a central role in allograft rejection[45]. The involvement of T_{RM} cells in a range of human diseases makes the design of therapeutic strategies that can modulate either their production or their activity an attractive goal, and to realize this goal, it is critical to understand how the formation of this cell pool is regulated[46]. In this review, we discuss the processes that drive the formation of the $CD103^+$ epithelial T_{RM} cell lineage, with a strong focus on signaling events that occur within the lymphoid compartment.

T_{RM} cell precursors within non-lymphoid tissues

At an early stage of an antigen-specific $CD8^+$ T cell response, infected tissues are seeded by effector-stage T cells (T_{EFF} cells, i.e. activated T cells that can be observed around the peak of the expansion phase, regardless of their phenotype and function)[47]. T_{EFF} cells forming the first wave of T cells that can be detected at inflamed sites already show transcriptional differences relative to circulating T cells that are specific for the same antigen[14,48]. Differentially expressed genes are associated with a wide range of cellular functions, including cell adhesion, cytokine and chemokine signaling, co-stimulation and co-inhibition, and transcriptional regulation[14,48]. Interestingly, early T_{EFF} cells present at the tissue site display increased expression of core T_{RM} cell genes, and at the peak of the T cell response the T cell population present at the tissue site already expresses more than 90% of the gene signature that differentiates T_{RM} cells from T_{CIRCM} cells[49]. This

illustrates that the initiation of a T_{RM} cell differentiation process already occurs during early stages of the immune response.

Although the T_{EFF} cells at tissue sites show a rapid transcriptional and phenotypic divergence from their circulating counterparts, these T_{EFF} cells nevertheless do display the same diversity in cell states that have previously been described for circulatory T_{EFF} cells. Specifically, within the circulating T_{EFF} cell compartment, two cell states are commonly distinguished: the relatively short-lived terminal effector cells that express high levels of KLRG1, T-BET and BLIMP-1 and show high cytotoxic potential, and the memory precursor cells that give rise to stable circulating memory T cell populations and are generally defined by an elevated expression of IL7Rα, ID3, and TCF1[50]. A similar dichotomy in phenotype and fate has been documented for the pool T_{EFF} cells within NLT [51–53]. Furthermore, T cells in NLTs that resemble circulating TE cells fail to express the T_{RM}-associated markers CD103 and CD69, and gradually perish over time [51,52]. On the contrary, T cells within NLT that resemble memory precursor cells express CD103 and CD69, indicative of their potential to persist long term within the NLT [51,52]. Interestingly, at very early stages of the immune response, before the appearance of cells with the terminally differentiated ($KLRG1^+IL7R\alpha^-$) phenotype, two transcriptionally disparate subgroups of T_{EFF} cells that differ in their differentiation potential can already be distinguished in the epithelium of the small intestine. Specifically, early effector T cells that are marked by high expression of IL2Rα and EZH2, an epigenetic regulator known to modulate early effector T cell fate decisions[54,55], are prone to give rise to $KLRG1^+$ TE-like cells, in contrast to their $EZH2^{LO}IL2R\alpha^{LO}$ counterparts that are superior in the generation of $CD103^+CD69^+$ T_{RM} cells[48].

A number of signals that promote the differentiation of T_{RM} cells within the tissue micro-environment have been described, and these signals presumably contribute significantly to the emergence of cells with T_{RM}-cell-like properties at the tissue site early during the immune response. For example, the presence of antigen[56–60], IL-7[61], IL-15[41,52,61–63], and TGF-β[64,65] within the non-lymphoid microenvironment promote T_{RM} cell differentiation in tissues such as skin and lung. In particular TGF-β is considered a central mediator of epithelial T_{RM} cell differentiation, as it can modulate the expression of many molecules that specifically mark T_{RM} cells[26,62,66,67]. In line with this, T cells that are insensitive to TGF-β signaling lack the capacity to develop into $CD103^+CD69^+$ T_{RM} cell precursors and T_{RM} cells in many epithelial tissues [51,57,66,68]. Other T cell extrinsic factors that can influence T_{RM} cell formation are TNF and IL-33 [26,66,69], which can induce CD69 and CD103 expression and suppress KLF2 expression, and IL-21, which has recently been identified to boost the formation of $CD103^+$ brain T_{RM} cells[70]. However, a critical issue that has not been fully settled is whether these various signals primarily modulate T_{RM} cell fate at the inflamed tissue site, or may also play a role in lineage instruction in the lymphoid compartment prior to tissue entry.

It is important to note that the signals driving the formation of T_{RM} cells differ between epithelial tissues types. For instance, abrogation of T cell intrinsic TGF-β signaling results in impaired production of T_{RM} cells in the lung, whereas the formation of T_{RM} cells in the nasal cavity is unaffected[71]. Similarly, IL-15 signaling is required for T_{RM} cell formation in some, but not all, tissues[72]. The idea that different routes to tissue residency exist is also supported by the observation that the transcription factor HOBIT promotes T_{RM} cell development in the skin and small intestine, while not being required for lung T_{RM} cell formation[73,74]. Collectively, these results strengthen the idea that the processes that yield T_{RM} cells show a level of redundancy, and that environmental conditions can change the requirements for T cells to develop into T_{RM} cells.

Models of T_{RM} cell lineage divergence

Based on the studies discussed above, it is apparent that the potential for T_{RM} cell differentiation is already present in part of the T_{EFF} cell population that is located within NLT early during infection. However, these findings do not address whether this potential is induced only after tissue entry or is already present before that stage. An analysis of T_{RM} cell-forming potential within the pool of activated circulating T cells has shown that cells with a memory precursor phenotype possess a superior potential to yield T_{RM} cells, but this cell pool is also well equipped to yield T_{CIRCM} cells [49,52,75]. The hypothesis that the circulating memory precursor cell pool can sprout both T_{RM} and T_{CIRCM} cells is compatible with two models for T_{RM} cell generation. In the "local divergence" model, the circulating MP pool is proposed to consist of cells that are equal in their potential to contribute to both the T_{RM} or T_{CIRCM} pool. Only upon stochastic tissue entry and subsequent encounter of local micro-environmental factors, such as TGF-β and IL-15, by a selection of memory precursor cells, would these cells commit to the T_{RM} cell lineage and adopt tissue residency (**Fig. 1A**). In other words, in this model, signals within the NLT dictate T_{RM} cell lineage commitment. In the alternative "systemic divergence" model, events that occur prior to tissue entry, within the lymphoid tissue or in blood, already steer some memory precursor cells to their subsequent fate as T_{RM} cells. In this model, a dichotomy in memory-forming potential would already be present within the circulating memory precursor cell pool, providing part of that pool with an enhanced capacity to migrate into inflamed tissue and/or respond to inflamed tissue-derived environmental factors that support T_{RM} cell formation (**Fig. 1B**).

As described above, earlier work has identified a number of tissue-derived factors that can support T_{RM} cell formation, and based on these observations it was generally assumed that the tissue microenvironment autonomously instructs T_{RM} cell lineage decisions in uncommitted infiltrating memory precursor cells. However, a number of studies have subsequently identified factors within lymphoid tissues that are essential for the formation of the T_{RM} cell lineage, but not the T_{CRICM} cell lineage. Furthermore, a combination of single cell transcriptome analysis and lineage-tracing allowed to identify the existence of a circulating effector T cell population that preferentially gives rise to

T_{RM} cells and transcriptionally resembles mature T_{RM} cells[76]. These observations argue for a "systemic divergence" model of T_{RM} cell formation, in which the capacity to develop into T_{RM} cells is at least partially driven by lymphoid-derived signals.

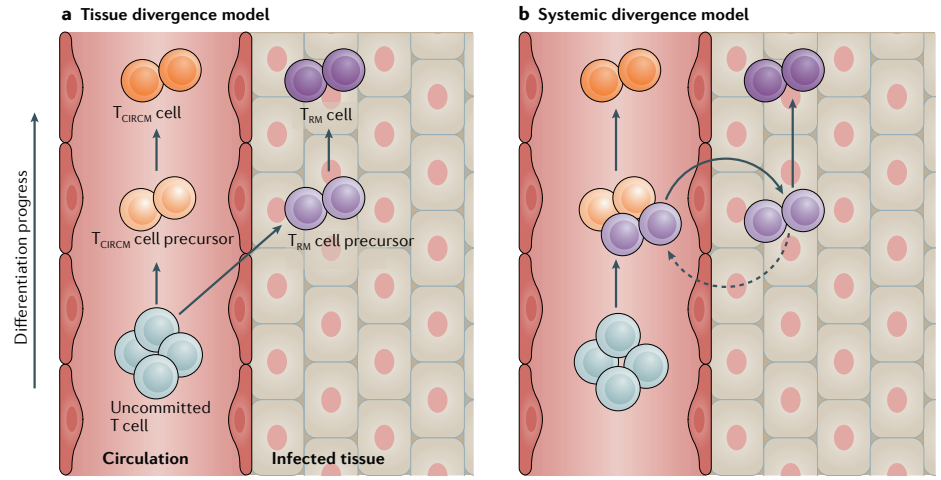

Figure 1. Models of T_{RM} cell lineage divergence
The branching of the T_{RM} cell lineage from the circulating T cell lineages can be explained by two models. (**A**) The tissue divergence model postulates that memory precursors within the circulation are equal in their potential to give rise to T_{CIRCM} and T_{RM} cells. Only upon reaching the tissue, cells undergo changes that skew them towards the T_{RM} cell lineage, whereas those memory precursor T cells that remain in circulation start to differentiate into the circulating memory lineages (**B**) The systemic divergence model postulates the existence of memory precursors within the circulating T cell pool that are poised to produce the T_{RM} cell lineage and these cells are superior in giving rise to T_{RM} cells relative to other circulating memory precursors. Note that these models do not address whether a fraction of cells with reduced T_{RM} cell-forming potential enter the tissue and later on rejoin the circulation.

Skewed T_{RM} cell production by naïve T cells

A "systemic divergence" model of T_{RM} cell differentiation proposes that the propensity to give rise to this lineage of memory cells is at least partially imprinted prior to tissue entry. As T_{RM} precursors can already be detected in tissues at an early stage of the T cell response, any systemic imprinting of T_{RM} lineage decisions should therefore also occur prior to, or within the first few days following, T cell activation. Importantly, direct evidence that T cells undergo T_{RM} fate conditioning prior to substantial antigen-driven expansion has been obtained. Specifically, two studies have shown that naïve T cells, either expressing variable[77] or identical[76] TCRs, show diversity in their ability to yield T_{RM} and T_{CIRCM} cells. This observed skewing of the progeny of individual T cells to either the T_{RM} or T_{CIRCM} cell lineage can conceptually be explained by: (1) differential exposure to signals that allow T_{RM} cell formation by early progeny, or (2) a gentle 'nudge' towards the production of T_{RM} cells that is already received at the naïve T cell stage, prior to TCR triggering. Notably, evidence in favor of imprinting both during T cell priming

and at the naïve T cell stage has been obtained. With respect to the imprinting of T_{RM} cell differentiation capacity during T cell priming, it is becoming increasingly evident that the specific dendritic cell (DC) subtypes that interact with T cells within lymphoid tissues can help to steer early T_{RM} cell differentiation. For instance, priming of human T cells by $CD1c^+CD163^+$ DCs may preferentially induce T_{RM} cell fate, as suggested by the observation that in vitro activation of naïve T cells by $CD1c^+CD163^+$ DCs, but not other DC subsets, induces the expression of a wide range of T_{RM} cell-associated genes in human T cells, and endows cells with enhanced capacity to accumulate in human epithelial grafts in mice[78,79]. Furthermore, data obtained in mouse models have demonstrated that only priming by $BATF3^+$ DCs, a subgroup of antigen presenting cells (APC) that is efficient in antigen cross-presentation, allowed the formation of T_{RM} cells in skin and lung tissue[80]. Interestingly, another study, comparing terminal effector T cell versus T_{CIRCM} cell differentiation in mice, demonstrated that priming mediated by $BATF3^+$ DCs favors the production of terminal effector T cells and T_{EM} cells over T_{CM} cells, whereas $CD11b^{HI}$ DCs, a subset that is poor at promoting T_{RM} cell differentiation[80], favored T_{CM} cell differentiation[81]. Although the above data indicate that $BATF3^+$ DCs can skew naïve T cells towards both the T_{RM} and T_{EM} cell lineage, lineage-tracing data indicate that T_{RM} and T_{EM} cells are largely derived from distinct naïve T cells[76]. This apparent contradiction may potentially be explained by an unappreciated diversity in T_{RM} / T_{EM} cell priming abilities within the $BATF3^+$ DC lineage, or by naïve T cell intrinsic variation in T_{RM} cell-forming potential. The above data provide solid evidence that the nature of the APCs that induce T cell priming can influence their capacity to differentiate into T_{RM} cells. In addition, evidence for such a 'sculpting effect'of DC encounters in the absence of antigen recognition has also been obtained. Specifically, migratory DCs within lymph nodes have been reported to epigenetically re-program naïve T cells in the absence of inflammation, leading to a T_{RM} cell-poised state that licenses naïve T cells to preferentially give rise to skin T_{RM} cells in response to local inflammation[82].

The relative output of naïve T cells towards either the T_{RM} or T_{CIRCM} cell pool after skin inflammation has been shown to be linked to the production of circulating T_{EFF} cells with an T_{RM} cell -like transcriptional signature by the progeny of individual cells[76]. It is plausible that encounter of above-mentioned T_{RM} cell - biasing DC subtypes prior to, and during, priming drives the creation of this specialized group of T_{EFF} cells. However, a contribution of signals within NLT in this process cannot be formally excluded. Specifically, late MP cells that exist in skin 14 days after viral skin infection have been reported to locally receive TGF-β induced signaling, after which these cells are able to rejoin the circulation[64]. It is presently unknown at what rate T cells egress from inflamed tissues at early stages of the immune response, and it will be of interest to determine if, and to what extent, signals within NLT can contribute to the production of the circulating T_{RM}-poised T cell pool.

Molecular signals that induce a T_{RM} cell-poised state

Signals provided by the DC subtypes described above may imprint an enhanced T_{RM} cell-forming propensity in T cells by promoting two different biological properties. 1) DC-derived signals may prime T cells for T_{RM} cell fate by enhancing the ability of T cells to accumulate in tissues through either increased tissue entry or tissue retention (**Fig. 2A**); for instance by driving a heightened expression of relevant chemokine receptors[83,84], integrins and other adhesion molecules[27]. Related to this, the observation that enhancement of tissue entry or inhibition of tissue egress increases the T_{RM} cell pool size[52,85] implies that migration and retention do represent bottlenecks in T_{RM} cell generation. In addition, heightened expression of the chemokine receptors CCR8, CCR10 and CXCR6 by circulating T_{EFF} cell clones responding to skin inflammation is associated with heightened T_{RM} cell formation in the skin[76]. 2) Signals provided by DCs may also promote T_{RM} cell lineage decisions by shaping an epigenetic and transcriptional landscape that makes cells more readily commit to the T_{RM} cell lineage upon encounter of signals within the tissue microenvironment (**Fig. 2B**). Such variable responsiveness to T_{RM} cell-inducing signals within the pool of T_{EFF} cells is exemplified by the observation that exposure to TGF-β can either induce the expression of CD103 or induce apoptosis in some T_{EFF} cells[66,86].

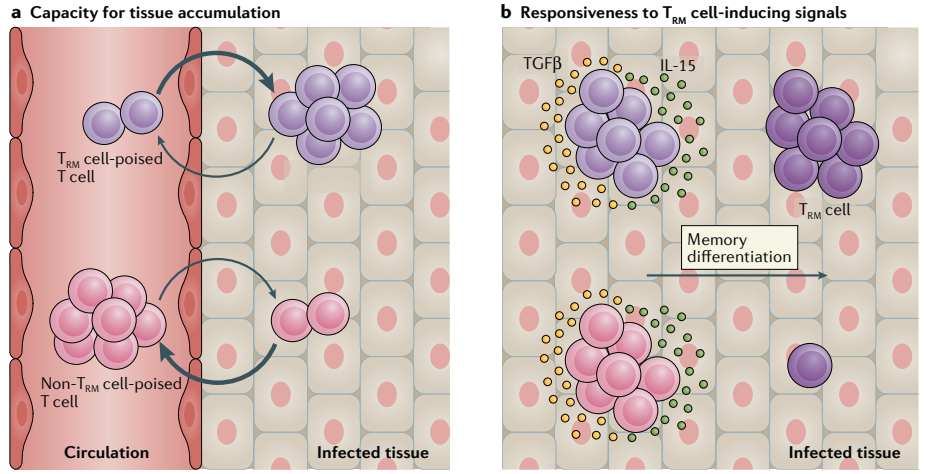

Figure 2. Properties of T_{RM}-poised cells

Two properties that endow T_{RM}-poised T cells with an enhanced capacity to form T_{RM} cells. (**A**) T_{RM}-poised memory precursor cells are more prone to enter non-lymphoid tissues and are well equipped to persist within this tissue, as compared to other T cells. (**B**) T_{RM}-poised memory precursor cells are more sensitive to signals, such as IL-15 and TGF-β, that drive T_{RM} cell differentiation within inflamed tissues, and thus more readily give rise to mature T_{RM} cells than other T cells that reach the tissue microenvironment.

A number of signals within lymphoid tissues have been identified that help to skew T cells towards the T_{RM} cell lineage through either of the above-mentioned mechanisms. TGFβ, an immune modulator that promotes T_{RM} cell formation by acting locally at the tissue site[64,65], can also steer T_{RM} cell differentiation within lymphoid tissues, both in the absence and presence of infection. In the absence of foreign antigen, TGFβ activation by migratory DCs in lymph nodes has been shown to induce epigenetic reprogramming of naïve T cells, resulting in enhanced accessibility of signature T_{RM} genes, such as Itgae and Ccr8, and to modulate the accessibility of target genes of transcription factors that are involved in T_{RM} cell differentiation[82]. Such TGFβ-mediated conditioning of naïve T cells was found to be essential for the differentiation of their progeny into T_{RM} cells upon skin infection, but was dispensable for T_{CIRCM} cell formation[82]. Notably, this TGFβ-dependent poising of naïve T cells towards the T_{RM} cell-fate is reversible, implying that naïve T cells require periodic TGFβ signaling to maintain their ability to differentiate into T_{RM} cells. This suggests that naïve T cells may vary in their T_{RM} cell-poised state, depending on the level or frequency of prior TGFβ encounter, potentially explaining the clonal variation in T_{RM} cell-forming capacity that has been observed[76,77]. Emerging tools that allow for the parallel determination of the epigenetic state of cells at a particular point in time and assessment of their ultimate fate at a later stage could be of major value to link epigenetic heterogeneity in the naïve T cell pool to T_{RM} cell differentiation potential[87].

In the presence of foreign antigen, TGFβ has also been shown to promote the induction of a T_{RM} cell-poised state. Upon TCR mediated activation, T cells rapidly downregulate TGFβ receptor expression—perhaps to reduce the immunosuppressive effects of TGFβ—but regain expression around 24h later[88,89]. Borges da Silva and colleagues have shown that such TGFβ receptor re-expression by T_{EFF} cells in lymphoid tissues of mice is induced by P2RX7, an extracellular receptor that senses ATP. Interestingly, as a result of their insensitivity to TGFβ, $P2rx7^{-/-}$ early effector T cells in the spleen display diminished Itgae and elevated Eomes expression[89], two characteristics that are negatively correlated with a T_{RM} cell-poised state[14,76], in line with the diminished T_{RM} cell-forming capacities of these cells. It should be noted that lack of P2rx7 does not affect TGFβ receptor expression on naïve T cells, suggesting that the TGFβ-mediated T_{RM} cell-fate conditioning that occurs prior to antigen encounter remains unaffected. Although the authors demonstrated that the lack of P2rx7 also negatively influenced T_{RM} pool size within the small intestine[89,90], an effect of P2rx7 deficiency on T_{RM} cell-forming capacity of T cells within the same tissue was not observed by Stark and co-workers[91]. As TGFβ signaling is vital for T_{RM} cell differentiation in the gut[51,68], mechanisms independent of the ATP-P2RX7 axis may exist that ensure TGFβ receptor re-expression.

A role for TGFβ in stimulating T_{RM} cell differentiation during priming has also been described for human T cells. Specifically, the preferential induction of a T_{RM} cell-like transcriptome by human $CD11c^+$ DCs marked by CD1c and CD163 expression has been explained by their ability to provide active TGFβ during T cell priming[78,79]. It is noted

though that an inability of murine $CD11c^+$ DCs to activate TGFβ during T cell priming does not impair murine skin T_{RM} cell development[82], suggesting that the TGFβ signal that prepares cells for T_{RM} cell fate during priming in mice is provided by another cell source.

The cytokines IL-15 and IL-12, and the co-stimulatory molecule CD24—three signals provided by $BATF3^+$ DCs during T cell priming—have been shown to be essential for the differentiation of mouse skin and lung T_{RM} cells, whereas these signals are dispensable for T_{CIRCM} cell formation[80]. However, how these signals promote T_{RM} cell programming is less well understood. Similar to TGFβ, IL-12 drives the expression of CD49a (Itga1), a T_{RM} cell-associated integrin that shows heterogeneous expression in circulating T_{EFF} cells[92], and of which elevated transcript levels mark T_{EFF} cell clones with heightened capacity to form T_{RM} cells[76]. Although CD49a is not required for the initial establishment of a T_{RM} cell pool in the skin, the expression of this integrin is vital for long-term T_{RM} cell persistence and locomotion[92,93]. Whether early stage CD49a expression induced by lymphoid-derived TGFβ and IL-12 signaling affects the ability of mature T_{RM} cells to persist in tissues is unclear. Both IL-12 and IL-15 have been shown to drive the activation of the mTORC1 protein complex[94,95]. This observation may explain the effect of these cytokines on T_{RM} cell formation, as inhibition of mTORC1 activity during T cell priming reduces T_{RM} cell formation due to a reduced ability of T_{EFF} cells to migrate to the gut epithelium and to express CD103, while enhancing their ability to form T_{CIRCM} cells[95–97]. Directly following T cell priming, T cells show variable levels mTORC1 activity[98], and it may be proposed that the level of mTORC1 activity may be used to identify T cells biased towards either the T_{RM} or T_{CIRCM} cell lineage. Although the exact mechanisms through which mTORC1 steers T_{RM} cell fate decisions are unknown, it is plausible that mTORC1 and other downstream signaling molecules induced by IL-15, IL-12, and CD24 signals mediate T_{RM} cell formation through the induction of molecular networks that also drive terminal effector and T_{EM} cell lineage commitment. Specifically, studies focusing on the formation of circulating T cell subsets have shown that IL-12[99] and CD24[81], provided by priming DCs, and elevated T cell intrinsic mTORC1 activity[98] strongly favor terminal effector and T_{EM} cell differentiation over T_{CM} cell differentiation, suggesting substantial parallels between the creation of the T_{RM} cells and the terminally differentiated T cell lineages. Nevertheless, T_{RM} cells display a significant level of multipotency[32], highlighting that these cells cannot be considered terminally differentiated. T_{CM} cell precursors are protected from terminal differentiation by the anti-inflammatory cytokine IL-10, which reduces their sensitivity and exposure to inflammatory stimuli[100]. By analogy, it may be speculated that periodic TGFβ signaling in lymphoid tissues could 'rescue' T_{RM}-poised T_{EFF} cells from terminal differentiation. In such a model, T_{RM} cell-forming potential is coupled to the prevention of terminal differentiation of cells that would otherwise contribute to the T_{EM} and terminal effector cell pools (**Fig. 3**).

In addition to cytokines and co-stimulatory signals, metabolites that are synthesized in processes mediated by DCs also play a major role in promoting T_{RM} cell formation,

by driving the expression of tissue homing molecules. Specifically, work over the past years has demonstrated that the expression of certain homing markers on T cells is influenced by the route of pathogen entry into the body[64,101–103] and that this effect is, at least partly, due to a variation in availability of molecular compounds that can be processed by DCs at different lymphoid tissue sites. For example, DCs can metabolize Vitamin D3—a compound that is abundantly present in the skin—into its active form, and this metabolite suppresses the gut-homing program in T cells, at the same time as inducing the expression of the chemokine receptor CCR10 that allows skin homing[104]. Vice versa, DCs located in gut-associated lymphoid tissue can convert Vitamin A into retinoic acid, thereby driving T cell expression of the gut-homing molecules CCR9 and α4β7[105,106]. Collectively, these data illustrate that the differential encounter of cytokines, co-stimulatory molecules and metabolites within lymphoid tissues can induce a bias with regards to the T_{RM} cell-forming potential within the T_{EFF} cell pool (**Fig. 3**). In addition, the idea that the molecular signals present at various priming sites can differentially affect the nature of T_{RM}-poised cells indicates that recently activated T cells are not primed as a "universal" T_{RM} cell precursor, but are primed to form T_{RM} cells at specific anatomical sites.

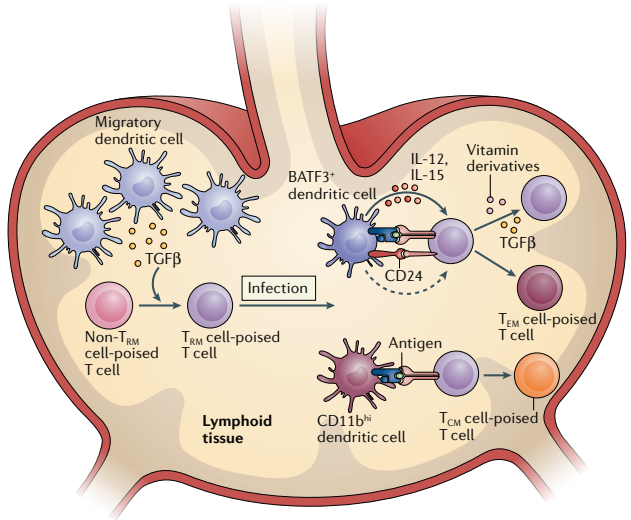

Figure 3. Signals within lymphoid tissues that poise T cells towards T_{RM} cell development
Overview of the signals within lymphoid tissues that affect the ability of T cells to form T_{RM} cells in mouse models Prior to antigen encounter, naïve T cells require periodic TGF-β signalling to adopt and retain a T_{RM} cell-poised state. Upon infection, priming by BATF3$^+$ dendritic cells (DCs), which provide IL-15, IL-12 and CD24 signalling, biases T cells to form T_{RM} cells. The presence of tissue-derived factors, such as derivatives of vitamin A and D, during priming can stimulate the expression of tissue-specific homing molecules, thereby guiding T_{RM} cell-poised cells to the relevant affected tissues. The presence of TGF-β during priming further maintains the T_{RM} cell-poised state, and it may be proposed that in the absence of TGF-β, T cells primed by BATF3$^+$ DCs are prone to give rise to the T_{EM} and TE cell lineages.

Transcriptional regulation

Although it is clear that T cells can undergo conditioning that increases their potency to develop into T_{RM} cells at very early stages of the immune response, and while still located in lymphoid tissues[80,82,96], the transcriptional program(s) that underpin this heightened potential have not been identified._Notably, multiple transcription factors have been described that coordinate the development of T_{RM} cells, and to better understand how T_{RM} cell lineage conditioning is regulated within lymphoid tissues, it is useful to examine whether the transcription factors that are known to affect T_{RM} cell development could be regulating T_{RM} cell differentiation already prior to tissue infiltration.

T-bet (encoded by *Tbx21*), EOMES (Eomesodermin, encoded by *Eomes*) and TCF1 (encoded by *Tcf7*) are transcription factors that are abundantly expressed by subsets of circulating T cells, but that are not or only minimally expressed by T_{RM} cells in NLT [17,52,62,107]. Early poising towards T_{RM} cell fate is associated with the expression of T-bet[80], and mature T_{RM} cells also require low-level T-bet expression to allow IL-15 receptor cell surface expression[62,108]. However, higher levels of T-bet negatively affect TGFβ receptor expression and hence the ability of T cells to form CD103$^+$ T_{RM} cells[62,109,110]. Similarly, EOMES is essential for T_{CIRCM} cell formation[108,111] but also counteracts the generation of T_{RM} cells by reducing the expression of the TGFβ receptor[62]. TCF1 is a transcriptional regulator that coordinates early fate decisions both in response to acute[112] and chronic infections [113,114] and can block TGFβ-induced CD103 expression through direct interaction with the *Itgae* locus, and ablation of this transcription factor enhances the formation of lung T_{RM} cells in mouse models[115]. The observation that circulating T_{EFF} cell clones poised for T_{RM} cell-fate display diminished expression of these three transcription factors[76] suggests that the levels of T-bet, EOMES and TCF1 may control early-stage T_{RM} cell lineage decisions within the lymphoid compartment. As a side note, TGFβ signaling suppresses the expression of these three transcription factors [62,115], and IL-12 signaling can induce transcriptional repression of both *Eomes* and *Tcf-7* [94,116,117]. In humans, evidence for the existence of a circulating pool of T_{RM}-poised T_{EFF} cells, marked by diminished expression of the aforementioned transcription factors—as observed in mice[76]—is currently lacking. However, datasets describing single cell gene- or protein-expression of large numbers of CD8$^+$ T cells in blood of recently infected or vaccinated human subjects could serve as valuable resources to study their presence [118–120]. Mathew et al. described a pool of cycling EOMES^{low}TBET^{low}TCF1^{low} T cells that were enriched in SARS-CoV-2 infected individuals, compared to healthy individuals or individuals who have recovered from COVID-19[119]. To test whether this CD8$^+$ T cell population harbors heightened T_{RM} cell-forming capacity in humans, it would be interesting to match the TCR repertoire of this cell pool to that of other blood-derived T_{EFF} cell subsets and to the TCR repertoire of mature T_{RM} cells derived from tissue biopsies.

In addition to the transcription factors that repress T_{RM} cell differentiation, a number of transcriptional regulators, including RUNX3, BLIMP1 and its analogue HOBIT,

BHLHE40, and NR4A1 have been shown to positively influence T_{RM} cell formation. Although RUNX3 has also been shown to promote T_{CIRCM} cell generation, ablation of RUNX3 affects the T_{RM} than the T_{CIRCM} pool more severely[49,121]. Additional evidence for a dominant role of RUNX3 in the generation of the T_{RM} cell subset over the T_{CIRCM} cell pool comes from the observations that T_{EFF} cells in tissues display increased expression of RUNX3 compared to circulating T_{EFF} cells[48], and that forced expression of RUNX3 in activated T cells results in increased expression of core T_{RM} cell signature genes and decreased expression of T_{CIRCM} cell related genes[49]. As RUNX3 has been shown to influence gene expression in recently primed T cells[121], it is plausible that RUNX3 already aids T_{RM} cell formation at a very early stage of the immune response, prior to tissue entry.

BLIMP1 promotes T_{RM} cell formation in various tissues, in part by directly suppressing the expression of *Tcf7*, as well as by suppressing *Klf2*, *Ccr7* and *S1pr1*[74], genes that encode proteins that promote tissue egress, and thereby inhibiting the formation of the T_{CM} cell lineage[73]. Although genetic deletion *of Blimp1* diminishes T_{RM} cell formation in lung, it does not affect the number of T_{RM} cells in gut and skin, potentially due to activity of the BLIMP1 homologue HOBIT, which shares the ability to suppress the expression of tissue egress-promoting genes[74]. However, gut T_{RM} cells that do form in the absence of BLIMP1 are defective in granzyme B production[122], highlighting that BLIMP1 is important to support the acquisition of some aspects of T_{RM} cell function. With respect to a potential role of BLIMP1 in determining T_{RM} cell fate in the circulating T cell pool, we note that circulating T_{EFF} cell clones with enhanced T_{RM} cell-forming capacity are marked by elevated transcript levels of *Gzmb,* which encodes granzyme B, relative to other MP cells[76]. As BLIMP1 is an essential driver of *Gzmb* expression within the circulating T_{EFF} cell pool[122,123], this relationship may conceivably reflect a molding of the circulating T_{EFF} cell population into a T_{RM} cell-poised state by BLIMP1. In support of this hypothesis, DC-derived IL-15 and IL-12 within lymphoid tissues are required to induce a T_{RM} cell-poised state[80] and these signals are also known drivers of BLIMP1 expression in early effector T cells [95,123]. Within the mouse $CD8^+$ T cell lineage, *Hobit* is highly expressed by T_{RM} cells, but not or only minimally by T_{CM} and T_{EM} cells [74,124]. Whether circulating mouse effector T cells at any stage express HOBIT has not been reported. On the other hand, abundant expression of HOBIT has been described in circulating human effector-like T cells[17,125–127], but unlike in mouse T_{RM} cells, HOBIT does not prominently mark human T_{RM} cells[17,128]. Thus, whether HOBIT plays a role in both mouse and human T_{RM} cell formation prior to tissue entry remains undefined.

Marked expression of the transcriptional regulators BHLHE40 and NR4A1 has been observed in T_{EFF} cells in tissues and in mature T_{RM} cells in both mice and humans, and genetic deletion of these factors selectively hinders the formation of T_{RM} cells in mice[49,129,130]. In addition, NR4A1 expression has been reported within the circulating pool of $CD8^+$ T_{EFF} cells, where it functions as a suppressor of cell division and effector differentiation[131–133]. BHLHE40 expression has been observed within the pool of effector

$CD4^+$ T cells[134,135]; but BHLHE40 expression by circulating $CD8^+$ T_{EFF} cells is less well described. More research is required to investigate whether BHLHE40 and NR4A1 are involved in T_{RM} cell lineage decisions within lymphoid tissues, or selectively act once T cells have seeded affected tissues.

Although differential expression of transcription factors, such as T-bet and RUNX3, is likely to form a major driver of T_{RM} cell lineage divergence, target gene accessibility is thought to represent a second layer of control. T_{RM} cells are characterized by a distinct epigenetic state as compared to T_{EM} and T_{CM} cells[25,31,32,38] and differences in the epigenetic landscape are already apparent at the T_{RM} precursor cell stage. For instance, a distinct set of RUNX3 target genes are accessible in T_{EFF} cells localized in the gut epithelium and in the spleen of LCMV infected mice[49]. Notably, an enhanced RUNX3 target gene accessibility has been described in T_{RM} cell-poised naïve T cells, coinciding with a reduced accessibility of T-box target genes[82]. Furthermore, RUNX3 has been reported to induce global chromatin changes shortly after T cell activation, enhancing accessibility of BLIMP1 target sites, while also inducing BLIMP1 expression[121]. Together, these data suggest a mechanism for early T_{RM} cell lineage poising that both relies on the expression of certain transcriptional regulators and the increased accessibility of their target genes.

T_{RM} precursors during reinfection

The data described above document the existence of circulating T cells that are poised to give rise to T_{RM} cells after a primary infection. Remarkably, recent studies have uncovered that a similar population can also be detected upon recurring infection, however, these cells have a different origin. Upon local reinfection, T_{RM} cells can proliferate, and whereas part of the offspring remains at the tissue site[136,137], some of these cells may leave the tissue site. In addition to the observation that such 'ex-NLT' T_{RM} cell offspring can take up permanent residence in tissue draining lymph nodes[30,138], a recent study revealed that skin-T_{RM} cell-derived T_{EFF} cells that are marked by the T_{RM} cell associated proteins CD103 and CD49a can be detected within the circulation[32]. Furthermore, it was shown that the offspring of intravenously transferred T_{RM} cells possesses a high propensity to home to the tissue of origin and to again differentiate into resident memory cells upon infection. Combined, these observations suggest that T_{RM}- cell-derived offspring that naturally egresses from tissues may also be primed to again form T_{RM} cells[32]. Evidence that T_{RM} cells can produce circulating offspring that possesses a heightened potential to again form T_{RM} cells has also been obtained in two other studies. Work by Behr et al demonstrated that gut-derived T_{RM} cells that were engrafted into liver tissue produced circulating effector T cells upon LCMV infection, which preferentially formed T_{RM} cells in gut tissue [124]. Furthermore, Klicznik et al demonstrated that $CD4^+$ T_{RM} cells derived from human skin xenografts can egress from the tissue, form $CD103^+$ circulating T cells, and subsequently form T_{RM} cells at distant skin tissue sites[139]. It has not been resolved which factors drive the re-entry of a selection of T_{RM}- cell-derived offspring into the circulation. Conceivably, the type or activation state of the APC encountered locally

could play a critical role in this process, as distinct types of APCs can differentially affect the gene expression profile of activated T_{RM} cells, including genes involved in tissue-egress[140].

Concluding remarks

It has become increasingly clear that cues within lymphoid tissues can condition T cells, both at the level of naïve and early effector T cells, to preferentially develop into T_{RM} cells, and the presence of T_{RM}-poised cells within the circulating memory precursor cell pool reflects the result of these processes (**Fig. 4**). It is of interest to note that evidence supporting the existence of a circulating precursor population in lymphoid organs that has an increased propensity to take up residence in NLT is not restricted to the $CD8^+$ T cell compartment. Specifically, recent work has revealed the existence of $CD4^+$ regulatory T (T_{REG}) cells within lymphoid tissues that epigenetically and transcriptionally resemble T_{REG} cells within NLT, and such cells are fated to traffic to NLT and subsequently take up residency in peripheral tissue[141–143]. As the molecular mechanisms used to instruct a 'tissue fate' in different subsets of circulating leukocytes are likely to share common themes, the parallel study of residency promoting and inhibiting programs in different cell subsets may be attractive.

The processes that are involved in the creation of the circulating T_{RM} cell-poised T cell pool will, at least partly, differ depending on the route of infection. Specifically, a number of the molecular and cellular cues that induce a T_{RM} cell-poised state within lymphoid tissues find their origin in the associated NLT (e.g $CD103^+$ migratory DCs, vitamin D, vitamin A)[80,82,144]. The importance of such cross-talk may also be reflected by the fact that no T cells that transcriptionally mimic T_{RM} cells were detectable within the circulating T_{EFF} cell pool after systemic LCMV infection[48]. It should be noted though that T_{RM} cells do form in various NLT following systemic LCMV infection, indicating that while tissue-derived signals present at the priming site may promote T_{RM} cell formation, such signals aren't always essential. In future work, it will be valuable to compare the formation of, and properties of, circulating T_{RM} cell precursors in response to local infections at different tissue sites, to better understand the role of different tissue cues in the creation of this cell pool.

As a final area of future research, our current understanding of T_{RM} cell fate conditioning within lymphoid tissues is predominantly based on work that tests the contribution of individual signals at a particular point in time, through the use of mouse models that are deficient in such signals. It will be attractive to complement this type of perturbation studies with studies that record the signals that cells receive, to test which signals are most predictive of future cell fate. Although a comprehensive monitoring of signaling events and subsequent changes in epigenetic and transcriptional states is unlikely to become feasible in the coming years, a number of previously established or recently developed tools will be valuable for this purpose. Specifically, methods that record—

preferably quantitatively—the historic exposure to external signals, such as CRISPR-based approaches that induce genomic modifications upon the reception of a signal of interest[145], are likely to serve as a useful approach to monitor the relationship between early signals and subsequent T_{RM} cell formation. Similarly, the use of reporter systems in which the expression of genes of interest leads to a stable genetic or protein marks[75,124] could provide insights into the gene-expression profile that marks T_{RM} cell precursors before they reach the tissue site. Finally, a recently developed transposon-based tool that 'immortalizes' the pattern of historic interactions of transcription factors with available DNA target sites[87] may be of significant value to also the epigenetic state of early effector T cells to the memory T cell state they assume later on.

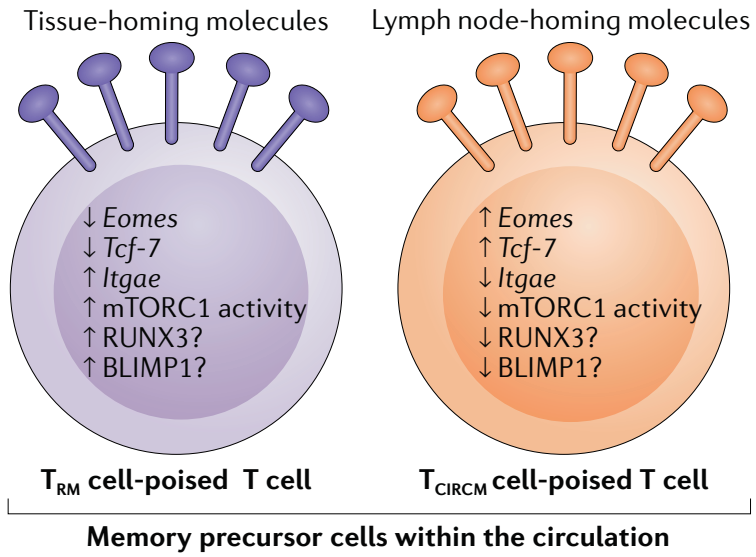

Figure 4. Distinguishing characteristics of T_{RM} cell-poised memory precursor cells within the circulation
Circulating T_{RM} cell-poised and T_{CIRCM} cell-poised memory precursor T cells share the classical memory precursor phenotype $IL7R^{HI}KLRG1^{LO}$. However, a number of other properties could be used to distinguish the two groups of memory precursor cells[76,82,89,96]. Arrows depict relative level of activity or expression. Data on BLIMP-1 and RUNX3 are indirect.

AUTHOR CONTRIBUTIONS

L.K. researched data for the article. L.K. and T.N.M. discussed content and wrote the initial concept. L.K., D.M., and T.N.M. reviewed and edited the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

PEER REVIEW INFORMATION

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GLOSSARY TERMS

Lymphoid tissues
Collective term for thymus, bone marrow, lymph nodes and spleen; in this review this term predominantly refers to spleen and lymph nodes.

Tissue resident memory T cell (T_{RM} cell)
Memory T cells which, under steady state conditions, are consistently excluded from the circulation and reside in tissues; T_{RM} cells in mucosal tissue, such as lung, gut and skin, are typically identified as $CD103^+CD69^+$.

Effector–stage T cell (T_{EFF} cell)
All activated T cells present around the peak of the expansion phase elicited by infection or vaccination, regardless of phenotype or function.

Central memory T cell (T_{CM} cell)
Memory T cells with a high degree of proliferative potential upon reactivation, commonly identified by the expression of lymphoid homing marker CD62L, and that can be abundantly be found in the spleen, blood and lymph nodes

Effector memory T cell (T_{EM} cell)
Memory T cells with a high degree of cytotoxicity upon reactivation, which are commonly identified by the lack of CD62L expression, and that can be abundantly be found in the spleen and blood.

Circulating memory T cells (T_{CIRCM} cell)
Collective term for all the memory T cells which can circulate through the body and that are predominantly found in the blood, spleen and lymph nodes; the T_{CIRCM} cell population encompasses both the T_{CM} and T_{EM} cell lineage.

T_{RM} cell-poised state
A state that skews the differentiation potential of T cells towards the T_{RM} cell lineage.

Conditioning / poising
Enhancing the intrinsic capacity of a cell to give rise to a particular cell lineage through the induction of epigenetic and/ or transcriptional changes.

Tissue retention
The ability of cells to persist in tissues after entry

Cell fate
The ultimate identity that a cell or its offspring assumes

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