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 cells (T_{RM} cells) are essential for immune defence against pathogens and malignancies, and the molecular processes that lead to T_{RM} cell formation are therefore of substantial biomedical interest. Prior work has demonstrated that signals present in the inflamed tissue micro-environment can promote the differentiation of memory precursor 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} cell-poised T 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.

CD8+ central memory T cells ($CD8^+$ T_{CM} cells). $CD8^+$ 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 found in the spleen, blood and lymph nodes.

CD8+ effector memory T cells ($CD8^+$ T_{EM} cells). $CD8^+$ 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 found in the spleen and blood.

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https://doi.org/10.1038/s41577-021-00590-3

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, remodelling 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 levels. Specifically, within the circulation (that is, blood, lymph and secondary lymphoid organs) two main subgroups of $CD8^+$ memory T cells can be distinguished, often referred to as $CD8^+$ central memory T cells (T_{CM} cells) and $CD8^+$ effector memory T cells (T_{EM} cells), which collectively form the pool of $CD8^+$ circulating memory T cells (here jointly referred to as T_{CIRCM} cells). T_{CM} cells can be distinguished by 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 display a heightened expansion potential upon antigen re-encounter[4]. By 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 signalling[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} cells and T_{EM} cell-like cells are mostly excluded from human and mouse non-lymphoid tissues (NLTs)[6–9].

In addition to the systemic $CD8^+$ memory T cell pool, a pool of $CD8^+$ tissue resident memory T cells (T_{RM} cells) that permanently reside within NLTs 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 not only produce cytotoxicity-associated molecules, such as granzyme B and perforin, but also cytokines such as IFNγ and TNF that can influence the behaviour of neighbouring 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} cells and T_{EM} cells, they are unique in their expression of a tissue residency-promoting

CD8⁺ circulating memory T cells
($CD8^+$ T_{CIRCM} cells). A collective term for all of the $CD8^+$ memory T cells that 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 $CD8^+$ central memory T cell (T_{CM} cell) and the $CD8^+$ effector memory T cell (T_{EM} cell) lineages.

CD8⁺ tissue resident memory T cells
($CD8^+$ T_{RM} cells). $CD8^+$ memory T cells that, under steady-state conditions, are consistently excluded from the circulation and reside in tissues; T_{RM} cells in mucosal tissue, such as the lung, gut and skin, are typically identified as $CD103^+CD69^+$.

CD8⁺ effector-stage T cells
($CD8^+$ T_{EFF} cells). All activated $CD8^+$ T cells present around the peak of the expansion phase elicited by infection or vaccination, regardless of phenotype or function.

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 NLTs, 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 the case of T_{RM} cells localized within epithelial tissues, the E-cadherin binding αE integrin (CD103, encoded by *Itgae*), which both promote tissue retention (for a comprehensive review of the molecular pathways that control tissue retention, please see REF.[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 of expression does not exclude the potential to leave NLTs[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, T_{RM} cells have been shown to both prevent and exacerbate pathologies. For instance, T_{RM} cells are not only superior to 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, tumour infiltrating lymphocytes that strongly 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 signalling events that occur within the lymphoid compartment.

T_{RM} cell precursors within NLTs

At an early stage of an antigen-specific $CD8^+$ T cell response, infected tissues are seeded by $CD8^+$ effector-stage T cells (T_{EFF} cells); that is, 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 signalling, 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 has 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 BLIMP1 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 IL-7Rα, ID3 and TCF1 (REF.[50]). A similar dichotomy in phenotype and fate has been documented for the pool of T_{EFF} cells within NLTs[51–53]. Furthermore, T cells in NLTs that resemble circulating terminal effector T 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 NLTs that resemble memory precursor cells express CD103 and CD69, indicative of their potential to persist long term within the NLTs[51,52]. Interestingly, at very early stages of the immune response, before the appearance of cells with the terminally differentiated ($KLRG1^+IL\text{-}7R\alpha^-$) 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 IL-2Rα and EZH2, an epigenetic regulator known to modulate early effector T cell fate decisions[54,55], are prone to give rise to $KLRG1^+$ terminal effector T cell-like cells, in contrast to their $EZH2^{low}IL\text{-}2R\alpha^{LO}{}_{low}$ counterparts that are superior in the generation of $CD103^+CD69^+$ T_{RM} cells[48].

Numerous 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 (REF.[61]), IL-15 (REFS[41,52,61–63]) and TGFβ[64,65] within the non-lymphoid micro-environment promote T_{RM} cell differentiation in tissues such as the skin and lung. In particular, TGFβ 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 TGFβ signalling 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 (REFS[26,66,69]), which can induce CD69 and CD103 expression and suppress KLF2 expression, and IL-21,

Lymphoid tissues
A collective term for the thymus, bone marrow, lymph nodes and spleen; in this Review, this term predominantly refers to spleen and lymph nodes.

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 tissue types. For instance, abrogation of T cell intrinsic TGFβ signalling 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 signalling 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, but is not 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 NLTs 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} cells and T_{CIRCM} cells is compatible with two models for T_{RM} cell generation. In the 'local divergence' model, the circulating memory precursor pool is proposed to consist of cells that are equal in their potential to contribute to both the T_{RM} cell pool and the T_{CIRCM} cell pool. Only upon stochastic tissue entry and subsequent encounter of local micro-environmental factors, such as TGFβ 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 NLTs 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 numerous tissue-derived factors that can support T_{RM} cell formation, and based on these observations it was generally assumed that the tissue micro-environment autonomously instructs T_{RM} cell lineage decisions in uncommitted infiltrating memory precursor cells. However, numerous studies have subsequently identified factors within lymphoid tissues that are essential for the formation of the T_{RM} cell lineage, but not the

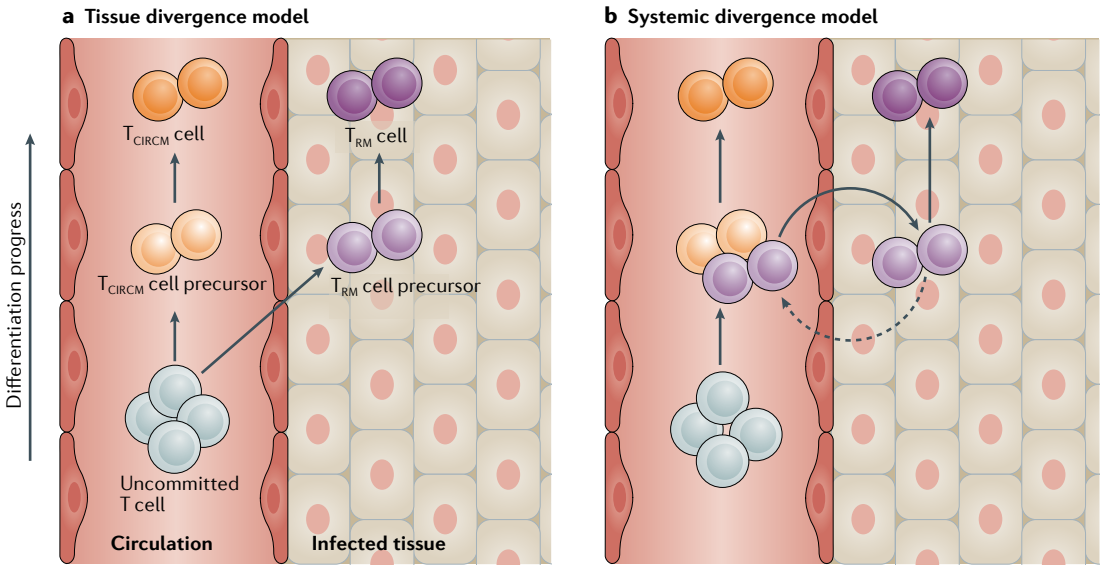

Fig. 1 | **Models of T_{RM} cell lineage divergence.** Branching of the CD8$^+$ tissue resident memory T cell (T_{RM} cell) lineage from the circulating T cell lineages can be explained by two models. **a** | The tissue divergence model postulates that memory precursors within the circulation are equal in their potential to give rise to CD8$^+$ circulating memory T cells (T_{CIRCM} cells) and T_{RM} cells. Only upon reaching the tissue do 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 T_{CIRCM} cell 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 rejoin the circulation.

Fate 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.

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

T_{CIRCM} cell lineage. Furthermore, a combination of single-cell transcriptome analysis and lineage tracing allowed identification of 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.

Skewed T_{RM} cell production by naive 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} cell precursors can already be detected in tissues at an early stage of the T cell response, any systemic imprinting of T_{RM} cell 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} cell fate conditioning/poising prior to substantial antigen-driven expansion has been obtained. Specifically, two studies have shown that naive T cells, either expressing variable[77] or identical[76] TCRs, show diversity in their ability to yield T_{RM} cells and T_{CIRCM} cells. This observed skewing of the progeny of individual T cells to either the T_{RM} cell or T_{CIRCM} cell lineage can conceptually be explained by differential exposure to signals that allow T_{RM} cell formation by early progeny, or by a gentle 'nudge' towards the production of T_{RM} cells that is already received at the naive T cell stage, prior to TCR triggering. Notably, evidence in favour of imprinting both during T cell priming and at the naive 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 subtypes that interact with T cells within lymphoid tissues can help steer early T_{RM} cell differentiation. For instance, priming of human T cells by $CD1c^+CD163^+$ dendritic cells may preferentially induce T_{RM} cell fate, as suggested by the observation that in vitro activation of naive T cells by $CD1c^+CD163^+$ dendritic cells, but not other dendritic cell 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 $BATF3^+$ dendritic cells, a subgroup of antigen-presenting cells (APCs) that are 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 $BATF3^+$ dendritic cells favours the production of terminal effector T cells and T_{EM} cells over T_{CM} cells, whereas $CD11b^{hi}$ dendritic cells, a subset that is poor at promoting T_{RM} cell differentiation[80], favoured T_{CM} cell differentiation[81]. Although the above data indicate that $BATF3^+$ dendritic cells can skew naive T cells towards both the T_{RM} cell lineage and the T_{EM} cell lineage, lineage-tracing data indicate that T_{RM} cells and T_{EM} cells are largely derived from distinct naive T cells[76]. This apparent contradiction may potentially be explained by an unappreciated diversity in T_{RM}/T_{EM} cell priming abilities within the $BATF3^+$ dendritic cell lineage, or by naive 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 dendritic cell encounters in the absence of antigen recognition has also been obtained. Specifically, migratory dendritic cells within lymph nodes have been reported to epigenetically reprogram naive T cells in the absence of inflammation, leading to a T_{RM} cell-poised state that licenses naive T cells to preferentially give rise to skin T_{RM} cells in response to local inflammation[82].

The relative output of naive T cells towards either the T_{RM} cell or T_{CIRCM} cell pool after skin inflammation has been shown to be linked to the production of circulating T_{EFF} cells with a T_{RM} cell-like transcriptional signature by the progeny of individual cells[76]. It is plausible that encounter of the above-mentioned T_{RM} cell-biasing dendritic cell subtypes prior to, and during, priming drives the creation of this specialized group of T_{EFF} cells. However, a contribution of signals within NLTs in this process cannot be formally excluded. Specifically, late memory precursor cells that exist in skin 14 days after viral skin infection have been reported to locally receive TGFβ-induced signalling, 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 whether, and to what extent, signals within NLTs can contribute to the production of the circulating T_{RM} cell-poised T cell pool.

Molecular signals that induce a T_{RM} cell-poised state. Signals provided by the dendritic cell subtypes described above may imprint an enhanced T_{RM} cell-forming propensity in T cells by promoting two different biological properties. First, dendritic cell-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 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]. Second, signals provided by dendritic cells may also promote T_{RM} cell lineage decisions by shaping an epigenetic and transcriptional landscape that makes cells commit more readily to the T_{RM} cell lineage upon encounter of signals within the tissue micro-environment (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 TGFβ can either induce the expression of CD103 or induce apoptosis in some T_{EFF} cells[66,86].

Numerous signals within lymphoid tissues have been identified that help skew T cells towards the T_{RM} cell

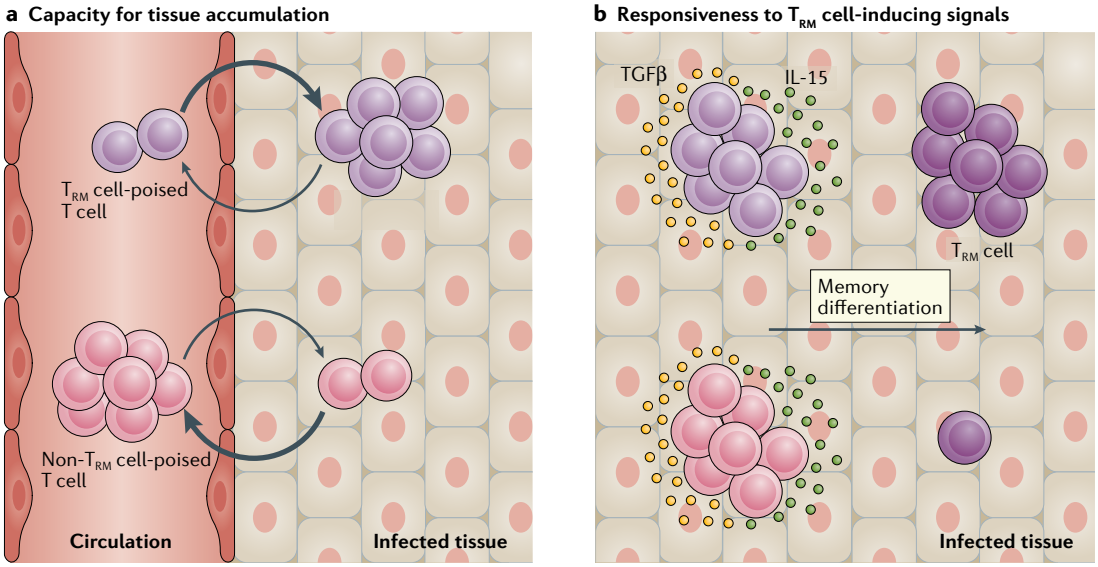

Fig. 2 | **Properties of T_{RM} cell-poised T cells.** Two properties endow $CD8^+$ tissue resident memory T cell (T_{RM} cell)-poised T cells with an enhanced capacity to form T_{RM} cells. **a** | T_{RM} cell-poised memory precursor cells are more prone to enter non-lymphoid tissues (NLTs) and are well equipped to persist within this tissue, compared with other T cells. **b** | T_{RM} cell-poised memory precursor cells are more sensitive to signals, such as IL-15 and TGFβ, 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 micro-environment.

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 the presence of infection. In the absence of foreign antigen, TGFβ activation by migratory dendritic cells in lymph nodes has been shown to induce epigenetic reprogramming of naive T cells, resulting in enhanced accessibility of signature T_{RM} cell 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 naive 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 naive T cells towards the T_{RM} cell fate is reversible, implying that naive T cells require periodic TGFβ signalling to maintain their ability to differentiate into T_{RM} cells. This suggests that naive 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 naive 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 24 h later[88,89]. Borges da Silva et al. 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 naive 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 the T_{RM} cell pool size within the small intestine[89,90], Stark et al. did not observe an effect of *P2rx7* deficiency on T_{RM} cell-forming capacity of T cells within the same tissue.[91]. As TGFβ signalling 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 $CD11c^+$ dendritic cells 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, however, that an inability of mouse $CD11c^+$ dendritic cells to activate TGFβ during T cell priming does not impair mouse 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 $BATF3^+$ dendritic cells 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 signalling 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 of 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} cell 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 signalling 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 (REF.[99]) and CD24 (REF.[81]), provided by priming dendritic cells, and elevated T cell intrinsic mTORC1 activity[98] strongly favour terminal effector and T_{EM} cell differentiation over T_{CM} cell differentiation, suggesting substantial parallels between 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 signalling in lymphoid tissues could 'rescue' T_{RM} cell-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} cell and terminal effector cell pools (FIG. 3).

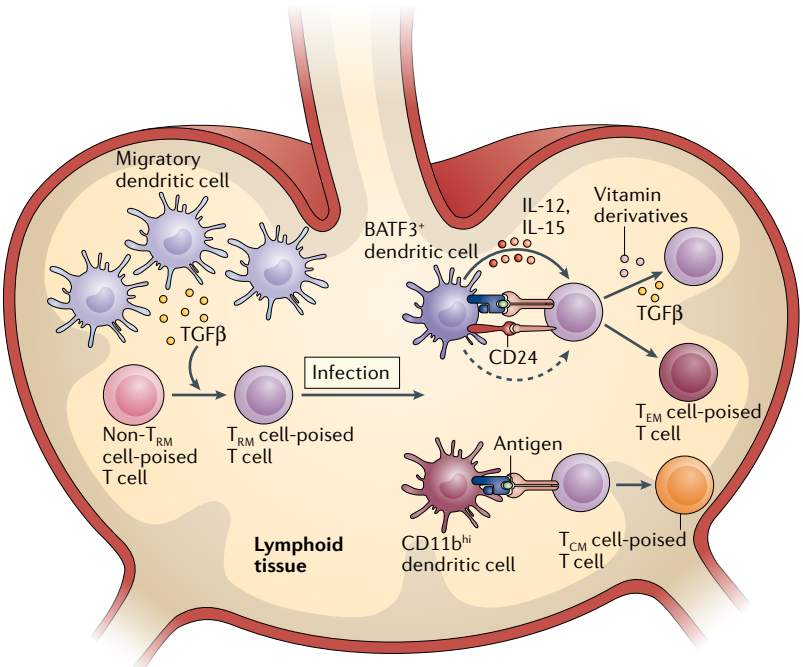

Fig. 3 | **Signals within lymphoid tissues that poise T cells towards T_{RM} cell development.** Overview of signals within lymphoid tissues that affect the ability of T cells to form CD8$^+$ tissue resident memory T cells (T_{RM} cells) in mouse models. Prior to antigen encounter, naive T cells require periodic TGFβ signalling to adopt and retain a T_{RM} cell-poised state. Upon infection, priming by BATF3$^+$ dendritic cells, which provide IL-15, IL-12 and CD24 signalling, biases T cells to form T_{RM} cells. Presence of tissue-derived factors, such as derivatives of vitamin A and vitamin D, during priming can stimulate the expression of tissue-specific homing molecules, thereby guiding T_{RM} cell-poised T cells to the relevant affected tissues. The presence of TGFβ during priming further maintains the T_{RM} cell-poised state, and it may be proposed that in the absence of TGFβ, T cells primed by BATF3$^+$ dendritic cells are prone to give rise to the CD8$^+$ effector memory T cell (T_{EM} cell) and terminal effector T cell lineages. T_{CM} cell, CD8$^+$ central memory T cell.

In addition to cytokines and co-stimulatory signals, metabolites that are synthesized in processes mediated by dendritic cells 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 dendritic cells at different lymphoid tissue sites. For example, dendritic cells can metabolize vitamin D3 — a compound that is abundantly present in the skin — into its active form, and this metabolite suppresses the gut-homing programme in T cells, at the same time as inducing the expression of the chemokine receptor CCR10 that allows skin homing[104]. Vice versa, dendritic cells 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 (REFS[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} cell-poised T cells implies 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.

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, while still located in lymphoid tissues[80,82,96], the transcriptional

programmes 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 are not or are only minimally expressed by T_{RM} cells in NLTs[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 in response to both acute[112] and chronic[113,114] infections. It 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 signalling suppresses the expression of these three transcription factors[62,115], and IL-12 signalling can induce transcriptional repression of both *Eomes* and *Tcf-7* (REFS[94,116,117]). In humans, evidence for the existence of a circulating pool of T_{RM} cell-poised T_{EFF} cells, marked by diminished expression of the aforementioned transcription factors as observed in mice[76], is currently lacking. However, data sets describing single-cell gene or protein expression of large numbers of $CD8^+$ T cells in the blood of human subjects who have been recently infected or vaccinated 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 individuals infected with SARS-CoV-2, compared with healthy individuals or individuals who have recovered from COVID-19 (REF.[119]). To test whether this $CD8^+$ T cell population harbours 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, numerous transcriptional regulators including RUNX3, BLIMP1 and its homologue 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} cell pool more severely than the T_{CIRCM} cell pool[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 with 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, thereby inhibiting the formation of the T_{CM} cell lineage[73]. Although genetic deletion *of Blimp1* diminishes T_{RM} cell formation in the lung, it does not affect the number of T_{RM} cells in the 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 memory precursor 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 moulding of the circulating T_{EFF} cell population into a T_{RM} cell-poised state by BLIMP1. In support of this hypothesis, dendritic cell-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} cells 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 with T_{EM} cells 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 lymphocytic choriomeningitis virus (LCMV)-infected mice[49]. Notably, an enhanced RUNX3 target gene accessibility has been described in T_{RM} cell-poised naive 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 and also inducing BLIMP1 expression[121]. Together, these data suggest a mechanism for early T_{RM} cell lineage poising that relies on both the expression of certain transcriptional regulators and the increased accessibility of their target genes.

T_{RM} cell 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 remain 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 egress from tissues may also be primed to again form T_{RM} cells[32]. Evidence that T_{RM} cells can produce circulating offspring that possess 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 T_{EFF} cells upon LCMV infection, which preferentially formed T_{RM} cells in gut tissue[124]. Furthermore, Klicznik et al. demonstrated that CD4$^+$ tissue resident memory T cells derived from human skin xenografts can egress from the tissue, form CD103$^+$ circulating T cells and, subsequently, form tissue resident memory T 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 APCs 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].

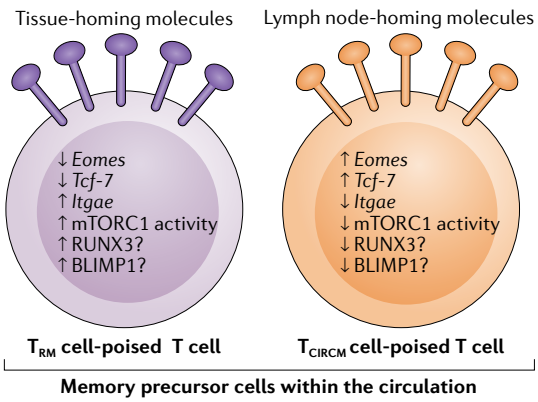

Fig. 4 | **Distinguishing characteristics of T_{RM} cell-poised memory precursor cells within the circulation.** Circulating CD8$^+$ tissue resident memory T cell (T_{RM} cell)-poised and CD8$^+$ circulating memory T cell (T_{CIRCM} cell)-poised memory precursor T cells share the classical memory precursor phenotype IL-7Rα^{hi}KLRG1^{low}. However, numerous 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 BLIMP1 and RUNX3 are indirect.

Concluding remarks

It has become increasingly clear that cues within lymphoid tissues can condition T cells, at the level of both naive and early effector T cells, to preferentially develop into T_{RM} cells, and the presence of T_{RM} cell-poised T 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 NLTs is not restricted to the CD8$^+$ T cell compartment. Specifically, recent work has revealed the existence of CD4$^+$ regulatory T cells within lymphoid tissues that epigenetically and transcriptionally resemble regulatory T cells within NLTs, and such cells are fated to traffic to NLTs 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 programmes 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 NLTs (for example, CD103$^+$ migratory dendritic cells, vitamin D, vitamin A)[80,82,144]. The importance of such crosstalk 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, however, that T_{RM} cells do form in various NLTs following systemic LCMV infection, indicating that although tissue-derived signals present at the priming site may promote T_{RM} cell formation, such signals are not 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 signalling events and subsequent changes in epigenetic and transcriptional states is unlikely to become feasible in the coming years, numerous 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 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 the epigenetic state of early effector T cells to the memory T cell state they assume later on.

Published online 3 September 2021

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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
Nature Reviews Immunology thanks Benjamin A. Youngblood and the other, anonymous, reviewers for their contribution to the peer review of this work.

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