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Author/s:

Gebhardt, T;Palendira, U;Tscharke, DC;Bedoui, S

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DR THOMAS GEBHARDT (Orcid ID : 0000-0002-3768-9468)

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Tissue-resident memory T cells in tissue homeostasis, persistent infection and cancer surveillance

Thomas Gebhardt¹, Umaimainthan Palendria^{2,3}, David C Tschärke⁴ and Sammy Bedoui¹

¹Department of Microbiology and Immunology, The University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

²Centenary Institute, The University of Sydney, Sydney, NSW, Australia

³Sydney Medical School, The University of Sydney, Sydney, NSW, Australia

⁴The John Curtin School of Medical Research, The Australian National University, Canberra, Australia

Address correspondence to:

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Thomas Gebhardt (gebhardt@unimelb.edu.au), Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, 792 Elizabeth Street, 3000 Melbourne, Australia

Summary

A large proportion of memory T cells disseminated throughout the body are non-recirculating cells whose maintenance and function is regulated by tissue-specific environmental cues. These sessile cells are referred to as tissue-resident memory T (T_{RM}) cells and similar populations of non-recirculating cells also exist amongst unconventional T cells and innate lymphocyte cells. The pool of T_{RM} cells is highly diverse with respect to anatomical positioning, phenotype, molecular regulation and effector function. Nevertheless, certain transcriptional programs are shared and appear as important unifying features for the overall population of T_{RM} cells and tissue-resident lymphocytes. It is now widely appreciated that T_{RM} cells are a critical component of our immune defense by acting as peripheral sentinels capable of rapidly mobilizing protective tissue immunity upon pathogen recognition. This function is of particular importance in anatomical sites that are not effectively surveilled by blood-borne memory T cells in absence of inflammation, such as neuronal tissues or epithelial compartments in skin and mucosae. Focusing on the well-characterized subtype of $CD8^+ CD69^+ CD103^+ T_{RM}$ cells, we will review current concepts on the generation, persistence and function of T_{RM} cells and will summarize commonly used tools to study these cells. Furthermore, we will discuss accumulating data that emphasize localized T_{RM} responses as an important determinant of tissue homeostasis and immune defense in the context of microflora-immune interactions, persistent infections and cancer surveillance.

Running title

T_{RM} cells in peripheral immune surveillance

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Introduction

Barrier tissues such as skin and mucosa form the body's interfaces with the external environment and consequently, are exposed to a large number of diverse microbes. Most of these microbes have little potential for causing disease in immunocompetent individuals. To the contrary, their presence promotes tissue and immune homeostasis. Other microbes however are potentially pathogenic and can establish full-blown infection once they have invaded the body, which is often facilitated by a loss in barrier integrity or generalized immune suppression. Nevertheless, our immune defenses commonly succeed in keeping harmful pathogens at bay while avoiding overt tissue-damaging inflammation.

Chronic and persistent infections with pathogens that evade immune eradication pose another continuing threat. Effective immunity to such pathogens usually protects us from clinical disease, but fails to achieve complete pathogen elimination. This state of co-evolution between persisting pathogens and the host immune system is often referred to as a 'host-pathogen equilibrium' and reflects a dynamic balance between host and pathogen strategies aimed at survival, long-term persistence and transmission, respectively. Whereas

pathogens need at least intermittent replication to infect new individuals and so spread within the population, the host must establish a state of sufficient control without compromising vital organ functions as a result of excessive immunopathology.

These 'defense and preservation' strategies employed by the immune system in dealing with recurring and ongoing microbial attacks depend on immune cells that act in a rapid and highly localized manner at sites of pathogen persistence or recrudescence. Indeed, vast numbers of antigen-experienced T cells populate peripheral tissues. Work over the past 2 decades has progressively defined many of these as a distinct pool of tissue-resident memory T (T_{RM}) cells that intermix only infrequently with their counterparts in the blood (1-18). Indeed, the majority of $CD8^+$ memory T cells in extra-lymphoid tissues are now recognized as T_{RM} cells (18), which illustrates a major strategic advantage of seeding the body with sessile T cells specialized in local pathogen control and containment. Focusing on the well-characterized subset of $CD8^+ CD69^+ CD103^+ T_{RM}$ cells, we will review experimental approaches commonly used to study T_{RM} cells and will discuss evolving concepts on their generation, persistence and function as a first line of immune defense in peripheral tissues. Furthermore, we will discuss localized T_{RM} responses as an important determinant of tissue homeostasis in the context of microflora-immune interactions, persistent infections and cancer immune surveillance.

Different modes of 'tissue residency' and tools to study them

The term 'tissue residency' is interchangeably used to refer to the spatial positioning of T cells within a given tissue or their prolonged persistence in tissues with limited recirculation. There are several experimental approaches to study these key aspects of T_{RM} biology (**Table 1**). In the light of the emerging heterogeneity of cells that are now assigned to the T_{RM} category, it is important to consider specific advantages and limitations of these commonly used assays to identify T_{RM} cells.

Intravascular labelling

At its simplest, 'tissue residency' can refer to cells that have left the vasculature and entered the tissue parenchyma or stroma, where they are present or 'resident' at the time of analysis. *In vivo* labeling protocols that target cells in contact with the blood have been

instrumental in distinguishing intravascular cells from unlabeled extravasated tissue cells by microscopy and flow cytometry (19, 20). This technique is now widely applied and is of particular importance in studying T cell distribution in highly vascularized tissues such as lungs, as the vast majority of T cells in this organ appear confined to blood vessels and capillaries (20). It is important to note though, that this basic definition and approach to define 'tissue residency' falls short of embracing all types of T_{RM} cells. For instance, there are $CD8^+ T_{RM}$ cells residing in liver that dynamically patrol their environment from the luminal side of hepatic sinusoids and thus are in direct contact with the blood (21, 22). A similar mode of vascular or luminal surveillance has also been proposed for liver-resident NK cells and NKT cells in liver and lungs (23-26).

T cell dynamics revealed by *in vivo* migration assays

A more functional definition of the term 'tissue residency' is commonly used to describe prolonged tissue persistence of T cells with limited recirculation potential. There are a number of *in vivo* migration assays that have illustrated different modes of spatiotemporal T cell dynamics in peripheral tissues. For instance, rapidly migrating T cells enter peripheral tissues, but are present for only a short period of time before egressing via afferent lymphatics. Other T cells may be retained within tissues for prolonged periods of time, possibly weeks, but ultimately return to the blood circulation. Finally, there are truly non-migratory and sessile T cells that once formed, stay localized and do not rely on replenishment from the pool of circulating cells (**Figure 1**).

Parabiotic surgery has proven to be a key technique in tracking *in vivo* T cell dynamics in tissues and involves conjoining the blood circulation of two individual mice via a skin flap. Tissue analysis of parabiotic mice permits an assessment of peripheral migration by blood-borne recirculating memory T cells (T_{CIRC}) cells across the two parabionts. While T cells in blood usually equilibrate between the two partners within 2 weeks, populations of T cells in extra-lymphoid tissues can display varying degrees of equilibration with T_{CIRC} cells, including in some cases a prominent disequilibrium (5, 15, 18, 27, 28). Several parabiosis studies have shown that resting $CD8^+$ memory T cells circulating in blood do not efficiently migrate through peripheral organs in the steady state (5, 15, 18). Separate studies employing adoptive transfers of effector and memory T cells have confirmed that the ability

of T cells to access peripheral tissues is lost shortly after infection or activation (8, 11, 29). Despite this, almost all organs contain sizable CD8⁺ memory T cell populations (30-34) and separate *in situ* labelling experiments with fluorescent dyes have indicated their long-term local persistence (9, 35). Combined, such results imply that long-term maintenance of T_{RM} cells does not critically rely upon continuous replenishment from T_{CIRC} cells, although there is evidence for low-level protracted T_{RM} generation from T_{CIRC} cells in lungs (36) or in chronically infected salivary glands (35, 37). Regardless, experiments in which very late time points after parabiotic surgery were analyzed, have defined T_{RM} cells as a truly sessile and autonomous population of peripheral memory T cells.

Since parabiosis does not directly measure the process of tissue egress, cautious interpretation needs to consider the timing of tissue analysis and also the half-life of T_{CIRC} cells in blood. For instance, immune cells that rapidly home to their target tissues, such as neutrophils, dendritic cell precursors and potentially also some T cell subsets, do not equilibrate efficiently in blood of the two parabionts (38). This complicates any interpretation of the relationship between blood-borne and tissue cells, and in particular the residency status and longevity of the latter. Furthermore, tissue inflammation can result in long-term augmentation of chemokine networks and thereby more effectively recruit and retain blood-borne memory T cells (16, 27), adding a layer of complexity when comparing T_{RM} cells in tissues with a history of inflammation with those that were never inflamed. These considerations are particularly relevant in situations where cells from both partners contribute to the pool of peripheral T cells, albeit perhaps with a noticeable bias towards host-derived cells, as shown for CD4⁺ memory T cells in skin (27). Nonetheless, while there are potential limitations, parabiosis has been instrumental in demonstrating the functional disconnection between the pools of T_{CIRC} and T_{RM} cells, particularly in cases where near complete disequilibrium is maintained over several months (5, 15, 16, 18, 28).

Other widely used strategies similarly aim at disconnecting the populations of T_{RM} and T_{CIRC} cells to assess persistence of T_{RM} cells in the absence of input from their T_{CIRC} counterparts. This can be achieved, for instance, by transplanting tissues containing genetically marked T_{RM} cells onto animals that lack that particular mark on their T_{CIRC} cells (7, 39, 40). To the same effect, selective ablation of T_{CIRC} cells by antibody-mediated depletion,

selective T_{CIRC} rejection in sex-mismatched adoptive cell transfer systems or pharmacological blockade of T_{CIRC} recirculation have been used in different settings (10, 11, 15, 41, 42). As discussed before, for these assays also it is important to consider appropriate timing, as well as potential side-effects of administered antibodies or drugs. For instance, even though T_{RM} cells are largely spared from antibody-mediated depletion, depending on the dose administered, the antibodies may still bind to the T_{RM} cells and modulate their functional behavior. Likewise, drugs such as FTY720 reduce T cell egress not only from lymphoid organs, but also from extra-lymphoid tissues (43, 44), potentially complicating the assessment of T cell recirculation and persistence. Nevertheless, the combined results from carefully executed studies employing the techniques in **Table 1** uniformly support the notion that T_{RM} cells can exist as an autonomous, non-recirculating population of long-lived sessile cells. Of note, all these functional approaches, including parabiotic surgery, have also been instrumental in illuminating the protective potential of T_{RM} cells, as discussed further below.

Common transcriptional signatures shared by tissue-resident lymphocytes

The various experimental strategies have identified a growing number of different T_{RM} populations in lymphoid and extra-lymphoid tissues. These include CD8⁺ memory T cells and subsets of antigen-experienced CD4⁺ T cells, such as T_H1 cells, T_H2 cells, T_H17 cells, and regulatory T (T_{REG}) cells (5, 7, 8, 16, 28, 45-49). In addition, many unconventional T cells and innate lymphocyte cells (ILC) in peripheral tissues are now considered to be non-recirculating and sessile cells. These include $\gamma\delta$ T cells, intraepithelial CD8 $\alpha\alpha$ T cells, liver NKT cells, mucosa-associated innate T (MAIT) cells and various types of ILCs (50-52). Thus, there exists a considerable heterogeneity amongst the overall pool of T_{RM} cells and tissue-resident lymphocytes. Adding another layer of complexity, even T_{RM} cells of the same T cell lineage display marked phenotypic and functional differences depending on the tissue of residence and the nature of the infectious agent to which they respond.

Despite this heterogeneity, many T_{RM} types display overlapping transcriptional features that distinguish them from their counterparts in the circulation (53-56). For instance, murine CD8⁺ T_{RM} cells from skin, lung, gut and brain share a core transcriptional signature consisting of uniformly up- or down-regulated genes associated with cell

adhesion (e.g. *Itgae*, *Itga1*, *Cdh1*), migration (e.g. *S1pr1*, *S1pr5*, *Rgs1*, *Rgs2*, *Xcl1*, *Cxcr6*), immune regulation (e.g. *Cd244*, *Ctla4*, *Pdcd1*, *Icos*, *Tlr1*) and transcriptional activity (e.g. *Klf2*, *Hobit*, *Eomes*, *Nr4a1*, *Nr4a2*, *Litaf*, *Ahr*), as well as genes encoding enzymes with largely unknown functions in T cells (e.g. *Hpgds*, *Inpp4b*, *Qpct*, *Cmah*) (53-57). Many of these genes are similarly regulated in CD8⁺ T_{RM} cells from human tissues (58-62), meaning that certain features of T_{RM} transcriptional profiles are conserved between mice and humans. Furthermore, a recent study has described transcriptional commonalities between human CD4⁺ and CD8⁺ CD69⁺ T_{RM} cells and reported a significant enrichment of this broader human signature also in murine T_{RM} populations (53-55, 60).

The transcription factors Hobit and Runx3 have recently been identified as central regulators of 'tissue residency' transcriptional programs in mice and are involved in the signaling cascade of two most prominent T_{RM}-inducing cytokines, IL-15 and transforming growth factor (TGF)β, respectively (55, 63, 64). Combined genetic deficiency in expression of Hobit and its homologue Blimp1 almost completely abolishes CD8⁺ T_{RM} formation in skin, gut and liver and is accompanied by an absence of liver-resident innate lymphocytes, such as NK and NKT cells (55). The latter also share transcriptional signatures with Hobit-expressing CD8⁺ T_{RM} cells (55). Likewise, many CD8⁺ T_{RM} types share phenotypic and transcriptional features with ILC1 resident in salivary glands, which are largely driven by TGFβ signaling and presumably are dependent on Runx3 (54, 65, 66). In summary, there exist conserved transcriptional programs driven by molecular regulators, such as the Hobit-Blimp1 and Runx3 modules, that operate both in T_{RM} cells and in tissue-resident innate lymphocytes across different anatomical locations in mice. Nevertheless, the expression pattern of Hobit in human memory T cells appears to be more diverse, with high levels seen in cytomegalovirus (CMV)-specific T_{CIRC} cells and low levels reported in T_{RM} populations in spleen, lungs and liver (60, 67-69). Thus, it will be important to determine if these modules similarly underpin the transcriptional configuration of human T_{RM} cells.

Distribution of T_{RM} cells in mouse and human tissues

The most widely used 'residency markers' defined by parabiosis and other *in vivo* migration assays are CD69 and CD103 (7, 9-11, 15), although several T_{RM} types actually lack expression of CD103 (16, 18, 21, 70). Other surface molecules variously expressed by subtypes of T_{RM}

cells and often used to distinguish them from their T_{CIRC} counterparts include CD49a, CD11a, CXCR6, CXCR3 and CD101 (7, 12, 21, 55). It is important to note though that neither of these markers, including CD69 and CD103, unequivocally denotes permanent tissue residence. Conversely, their absence does not necessarily indicate that the cells are rapidly recirculating (18, 27, 47, 71, 72). This means that care is required when interpreting studies where surface phenotype is the primary tool for identification of T_{RM} cells.

T_{RM} cells identified by these surface phenotypes have now been described in almost every organ in mice and humans, including in gut, lungs, skin, brain, salivary glands, liver, pancreas, female reproductive tract, kidney, bladder and heart (4, 7-11, 14-16, 18, 32-34, 73). Large numbers of T_{RM} cells also populate lymphoid organs, including thymus, lymph nodes, tonsils, bone marrow and spleen (32-34, 74-79). Although in human studies long-term tissue residency of memory T cells is often inferred by CD69 and CD103 expression, there are some notable examples where the migratory behavior of human CD69⁺ CD103^{+/-} T_{RM} cells has been analyzed in patients. For instance, treatment of patients with cutaneous T cell lymphoma using low doses of anti-CD52 antibody (alemtuzumab) has been shown to effectively deplete T_{CIRC} cells in the blood while leaving intact mixed populations of CD4⁺ and CD8⁺ T_{RM} cells in skin (14). Importantly, long-term persistence of these T_{RM} cells is associated with efficient protection of T_{CIRC}-ablated patients from renewed or reactivating infections (14). In a separate study, epidermal CD69⁺ CD103⁺ T_{RM} cells of donor origin have been shown to persist in allogeneic face transplants for up to two years (17). Intriguingly, these T_{RM} cells are often found in close association with injured graft cells, implying an important contribution of donor T_{RM} cells to the chronic rejection response (17). Such results unequivocally demonstrate the presence of non-recirculating T_{RM} cells in humans and provide fascinating examples for their participation in localized immune reactions in human skin.

Interestingly, CD4⁺ and CD8⁺ T_{RM} cells often co-localize in distinct micro-anatomical clusters that also contain antigen presenting cells (APCs), such as dendritic cells and macrophages (16, 27, 70, 80). For instance, virus-specific T_{RM} cells in the brain can be scattered within the parenchyma but are also concentrated in certain cerebral regions, presumably corresponding to the site of previous encounter with infectious virus (1, 9).

Cluster formation is also seen for T_{RM} cells responding to protracted bacterial infection in the intestinal mucosa (70) or in peri-bronchiolar foci in the lungs (81, 82). Likewise, $CD4^+$ T_{RM} cells generated after herpes simplex virus (HSV) infection in the vaginal mucosa form aggregates that are established by chemokine networks in response to ongoing $IFN\gamma$ production (16). In skin, similar accumulations of HSV-specific $CD4^+$ memory T cells and APCs are found in the dermis around the isthmus region of hair follicles and are equally dependent on ongoing chemokine signaling (27, 83). Human genital skin intermittently exposed to reactivating HSV-2 also contains distinct clusters of $CD4^+$ and $CD8^+$ T_{RM} cells (84). By contrast, $CD69^+$ $CD103^+$ $CD8^+$ T_{RM} cells in skin epidermis and intestinal epithelium are more evenly scattered (10, 13, 70). Epidermal T_{RM} cells in mouse skin further display a slow crawling behavior and extend long dendritic protrusions into the extracellular space in between their neighboring cells (10, 13, 85). This type of movement enables the T_{RM} cells to dynamically scan a large proportion of their microenvironment for the presence of antigen (13, 85).

Strong systemic infections, lymphopenia-driven proliferation or repeated immunizations all result in a broad anatomical dissemination of T_{RM} cells (8, 15, 73, 86, 87), but the highest T_{RM} frequencies are commonly found in regions with a history of resolved infection or inflammation (7, 29, 81, 86). This is explained by the chemokine-driven recruitment of large numbers of effector T cells with T_{RM} precursor potential specifically to sites of acute infection, as well as an abundance of T_{RM} -inducing factors such as $TGF\beta$ in regenerating tissues. In addition, tissue retention of some T_{RM} types is facilitated by profound changes in the post-inflammatory tissue environment. These changes include an increase in the numbers of dendritic cells and macrophages and sustained production of T cell-targeting chemokines, such as CCL1, CCL5, CCL8, CXCL9, CXCL10 and others (16, 27, 80, 83). Thus, due to resulting variations in T_{RM} densities, there can be dramatic differences in the level of T_{RM} -mediated regional immunity throughout the body, even within a given organ. The latter is best documented for skin, where immune response in areas of resolved infection or inflammation can be readily compared with control regions, for instance on the contralateral side of the body. The demonstration of site-specific immunity in such studies (7, 15, 29, 88, 89) has sparked a strong interest in identifying the correlates of tissue-specific

immunity and contributed to our current appreciation of the role of T_{RM} cells in peripheral immune protection.

Intraepithelial CD8⁺ CD69⁺ CD103⁺ T_{RM} cells

The combined expression of CD69 and CD103, often together with CD49a (the α-chain of very late antigen-1 [VLA-1]) and other markers, identifies a distinct population amongst peripheral CD8⁺ memory T cells in mice and humans. These CD8⁺ CD69⁺ CD103⁺ VLA-1⁺ T_{RM} cells, like their CD69⁺ CD103⁺ VLA-1⁺ ILC1 counterparts, display a notable tropism for epithelial compartments within peripheral organs (7, 10, 37, 61, 65, 78, 90, 91). As such, they are spatially segregated from blood capillaries and lymphatic vessels, which localize below the basement membrane and are absent from epithelium. CD8⁺ CD69⁺ CD103^{+/-} also form in neuronal tissues, such as brain and sensory ganglia, where they are separated from the circulation by the blood-brain-barrier (9, 29). Importantly, the nature of epithelial CD8⁺ CD69⁺ CD103⁺ T_{RM} cells as non-recirculating and permanently sessile cells has invariably been demonstrated with all experimental approaches listed in **Table 1** (7-11, 15, 66). Therefore, these cells are arguably the most extensively studied and best characterized T_{RM} subtype and for the remainder of this article, we will focus much of the discussion on these CD8⁺ CD69⁺ CD103⁺ T_{RM} cells.

Key checkpoints in formation of CD8⁺ CD69⁺ CD103⁺ T_{RM} cells

The formation of epithelial CD8⁺ CD69⁺ CD103⁺ T_{RM} cells following an acute infection in peripheral tissues involves several critical steps. These include (1) T cell activation by dendritic cells in lymphoid tissues, (2) infiltration of infected tissues, (3) retention by blockade of tissue egress, (4) epithelial migration, (5) in situ acquisition of the T_{RM}-specific transcriptional program and (6) long-term local persistence. Various extrinsic factors are at play in regulating these individual checkpoints, including cytokines, chemokines, adhesion molecules and the availability of bioenergetic fuels. However, individual contributions of these factors can differ quite substantially depending on the target organ and the nature of the infectious pathogen or immunization modality. In the following sections, we will outline the differentiation pathway of CD8⁺ CD69⁺ CD103⁺ T_{RM} cells and by way of example, will focus a large part of this discussion on a localized virus infection of skin (**Figure 2**).

T cell activation and infiltration of peripheral tissues

CD8⁺ CD69⁺ CD103⁺ T_{RM} cells derive from naïve T cells that become activated in lymphoid tissues during acute infection or immunization. Upon appropriate stimulation by antigen-presenting dendritic cells, activated T cells leave the lymphoid organs to infiltrate peripheral sites of infection using migration receptors, such as E- and P-selectin ligands, as well as CCR4, CCR8 and CCR10 for skin homing (4, 15, 92-94), or $\alpha 4\beta 7$ integrin and CCR9 for gut homing (8, 92, 95). In addition, inflammatory cues in infected tissues drive expression of endothelial adhesion molecules, such as ICAM-1 and VCAM-1, which bind to T cell-expressed lymphocyte function-associated (LFA)-1 and VLA-4, respectively, thereby greatly augmenting T cell infiltration and subsequent T_{RM} formation (92). Nevertheless, strong systemic infection or repeated immunization can result in substantial effector T cell infiltration and T_{RM} generation in resting tissues not directly involved in infection (8, 15, 86). Remarkably, even lymphopenia-driven activation in absence of antigen stimulation is sufficient to induce broad T cell dissemination and T_{RM} generation across several organs, as shown after adoptive transfer of naïve CD8⁺ T cells into T and B cell-deficient *Rag*^{-/-} mice (73).

Blockade of tissue exit and early T cell retention

Many tissue-infiltrating effector T cells may ultimately die *in situ* or exit the tissue via afferent lymphatics to rejoin the blood circulation (43, 95, 96). T cell access to lymphatics is guided by a gradient between lymph and tissues of the signaling sphingolipid, sphingosine-1-phosphate, which is sensed by T cells using the sphingosine-1-phosphate receptor 1 (S1P1) (44, 97). Some effector T cells however, may respond to environmental cues by upregulating the expression of CD69, a type II C-lectin receptor that interferes with the function of S1P1 and thereby promotes tissue retention (43, 98). The function of CD69 in limiting tissue exit appears most relevant at early stages after infiltration prior to the transcriptional shut-down of S1P1 expression during subsequent T_{RM} differentiation (43, 54, 81). In line with this, even though CD8⁺ effector T cells genetically deficient in CD69 expression generate normal effector responses in lymphoid tissues and are capable of infiltrating infected skin and lungs, their early accumulation in tissues is greatly impaired and fewer T_{RM} cells are formed (43, 54, 81, 99). CD69 expression has also been shown to promote persistence of CD4⁺ T_{RM} cells in the bone marrow, however the precise function of CD69 in this case is not known (100). In

addition to its blocking interaction with S1P1, CD69 also binds to galectin-1 expressed by neighboring cells and CD69 downstream signaling may further contribute to T_{RM} generation by modulating cellular metabolism and TGFβ production (101-103), although this remains to be tested.

The factors that induce CD69 expression in T cells are diverse and include combinations of inflammatory cytokines such as type I interferons, IL-33, TNFα and TGFβ (35, 43, 73, 97, 104). In addition, TCR stimulation by antigen results in rapid CD69 expression and concomitant down-regulation of Krüppel-like factor-2 (KLF2, encoded by *Klf2*) and its targets, S1P1 and CCR7 (105, 106). Although initially described as an important lymphoid-homing receptor (107), CCR7 also acts to promote T cell egress from peripheral tissues (108, 109), and its down-regulation therefore supports tissue retention (54, 96). In line with this, local antigen encounter greatly augments T_{RM} generation and in doing so, can shape the composition of TCR specificities amongst the developing T_{RM} pool (110, 111). Nevertheless, many tissues, including skin, gut or female reproductive tract, intrinsically support T_{RM} differentiation even in absence of antigen (29, 73, 112). Yet in other organs, such as lungs and neuronal tissues, CD8⁺ CD103⁺ T_{RM} development is strictly dependent on peripheral antigen re-encounter (9, 29, 99). However, this requirement for antigen is not well understood and likely involves molecular pathways other than simple interference with tissue egress.

Epithelial infiltration by T_{RM} precursor cells

Epithelial infiltration is another critical checkpoint in T_{RM} development and is largely dependent on signaling through G-protein-coupled receptors, including CXCR3. CXCL9 and CXCL10 are epithelial chemokines that are highly expressed in inflamed tissues and signal to T cells via CXCR3 to promote tissue infiltration, epithelial entry and migration towards infected cells (54, 70, 113-118). Nevertheless, CD8⁺ T cells genetically deficient in CXCR3 expression are not fully excluded from epithelia, meaning that additional chemokines and adhesion factors are operative, likely also in a tissue-specific manner. Efficient epithelial localization by effector CD8⁺ T cells in lungs and vaginal mucosa further depends on the presence of IFNγ-producing CD4⁺ T cells to augment chemokine production (116, 119). Yet,

this requirement does not apply to all tissues since virus-specific T_{RM} cells in skin and brain develop and persist normally in absence of CD4⁺ helper T cells (15, 53).

Interestingly, early epithelial-infiltrating effector CD8⁺ T cells in skin, small intestine and lungs are highly enriched for CD127^{int/+} cells that lack expression of the killer cell lectin-like receptor G1 (KLRG1) (54, 95, 110, 120). As such, they resemble the memory precursor effector cells (MPECs) that give rise to long-lived memory cells in lymphoid tissues and the circulation (121-123). KLRG1⁻ effector CD8⁺ T cells express higher levels of CXCR3 than their KLRG1⁺ counterparts and display enhanced migration towards CXCL9 and CXCL10 (54, 124), which in part, could explain their preferential epithelial localization. To the same effect, epithelial-derived TGFβ may down-modulate KLRG1 expression in CD8⁺ effector T cells *in situ* (125). Importantly, a large fraction of these KLRG1⁻ CD8⁺ effector T cells also contribute to pathogen control by producing pro-inflammatory cytokines such as IFNγ upon direct recognition of virus-infected epithelial cells (126).

While the precise relationship between epithelial KLRG1⁻ effector T cells and MPECs in lymphoid organs remains unclear, adoptively transferred KLRG1⁻ MPECs isolated from spleen generate both T_{RM} and T_{CIRC} cells in infection-challenged new hosts. By contrast, the memory precursor potential of KLRG1⁺ cells appear to be dramatically reduced (54, 63). Furthermore, single TCR-identical clones of naïve CD8⁺ T cells equally give rise to T_{RM} cells in skin and T_{CIRC} cells in lymphoid tissues (88), suggesting that T_{RM} and T_{CIRC} cells are derived from similar, if not identical memory cell precursors. Regardless, it appears remarkable that, already from the earliest stages of T cell infiltration, the epithelial layer is almost exclusively seeded by T cells with a MPEC phenotype and presumably, T_{RM} precursor potential. This is consistent with the observation that the pool of CD8⁺ CD69⁺ CD103⁺ T_{RM} cells usually establishes very early during resolution of infection or inflammation (7, 8, 10, 35).

In situ maturation and long-term persistence of T_{RM} cells

The transition of KLRG1⁻ effector T cells into long-lived sessile T_{RM} cells is marked by an early upregulation of pro-survival molecules, such as Bcl-2 (9, 54, 70), and a step-wise acquisition of the T_{RM}-defining transcriptional profiles and surface phenotypes discussed earlier (53, 54, 56, 97). In skin, this process can be abolished by pharmacological blockade of T cell access to

the epithelium, illustrating that CD8⁺ CD69⁺ CD103⁺ T_{RM} maturation in this organ is largely confined to the epidermis and hair follicle epithelium (54). Furthermore, skin CD8⁺ T_{RM} cells gradually upregulate the fatty acid binding proteins (FAB), FAB4 and FAB5, which facilitate uptake of skin lipids and their utilization as bioenergetic fuels through mitochondrial oxidative metabolism (56). Accordingly, T_{RM} cells genetically deficient in FAB4 and FAB5 expression display impaired skin persistence and protective function (56). Thus, developing T_{RM} cells progressively establish molecular pathways that promote long-term survival and persistence within the epithelial niche. While T_{RM} differentiation usually coincides with pathogen control or elimination, this process may be further accelerated in resting tissues not directly involved in infection (8, 54, 63, 127).

A key feature of the residency-associated transcriptional networks in T_{RM} cells and innate lymphocytes is a sustained shut-down of pathways essential for T cell egress from peripheral tissues, including S1P1 and CCR7 and their transcriptional regulator KLF2. The critical importance of this process is evident from experiments in which forced expression of S1P1 in CD8⁺ T cells resulted in broadly impaired generation of T_{RM} cells (97). The transcriptional regulators Hobit, Blimp1 and Runx3 are essential for the efficient down-regulation of the KLF2-S1P1-CCR7 pathway in CD8⁺ T_{RM} cells in many tissues and in addition, regulate a large number of other T_{RM}-defining genes (55, 63). Other T_{RM}-specific transcriptional elements echo the need for T_{RM} cell tethering to their cellular and extracellular microenvironments, for instance via surface expression of adhesion molecules, including CD103, E-cadherin, CD49a, CXCR6 and LFA-1.

The widely used T_{RM} marker CD103, the α -chain of the $\alpha_E\beta_7$ integrin, is a binding partner for E-cadherin, the latter of which is expressed by epithelial cells and interestingly, also by the T_{RM} cells themselves (11, 128). Genetic approaches have implicated CD103 in regulating numerous key aspects of T_{RM} biology, including epithelial positioning and/or persistence (9, 35, 37, 54, 73, 95, 99, 129-131), intraepithelial movement (93, 132), as well as cytotoxic granule release (133). However, the degree to which CD103 deficiency impacts on those T_{RM} functions appears to depend on anatomical location and infection status within the tissues, with only marginal effects in some systems (35, 134). Interestingly, CD8⁺ T cell-intrinsic deficiency in E-cadherin expression similarly results in diminished T_{RM}

accumulation in salivary glands (11), most likely caused by a lack of homodimeric adhesive interactions with E-cadherin on neighboring epithelial cells. Furthermore, given that CD103 and E-cadherin are co-expressed in the T_{RM} cells, it appears possible that they may form intracellular heterodimers and thereby regulate each other's surface expression or other intracellular functions.

Surface expression of CD49a, the α -chain of the $\alpha_1\beta_1$ integrin (VLA-1), is another hallmark of many T_{RM} cells and innate lymphocytes, although a proportion of T_{CIRC} cells in lymphoid tissues also express this integrin (3, 7, 26, 61, 65, 72, 135-137). VLA-1 is a receptor for the extracellular matrix proteins collagen type I and IV (138). Type IV collagen is a major structural component of basement membranes. Accordingly, epidermal $CD8^+$ T_{RM} cells in human and mouse skin localize in close proximity to the basement membrane (61, 85, 90, 139, 140) and a similar juxtaposition with collagen networks is also seen in other organs (3, 73, 136). Interestingly, VLA-1 binding to collagen IV has been shown to promote cytokine production by $CD8^+$ $CD69^+$ $CD103^+$ T_{RM} cells from human skin (61). Furthermore, *in situ* VLA-1 expression denotes a heightened potential for IFN γ production and cytotoxic activity amongst T_{RM} cells in human skin (61). However, genetic deficiency in CD49a expression or antibody-mediated blockade of VLA-1 function in animal models have yielded inconsistent results with regards to its role in T_{RM} accumulation and maintenance, possibly related to a functional redundancy of multiple adhesion factors expressed by T_{RM} cells (3, 135). For instance, the CXCL16 receptor CXCR6 is also highly expressed by many types of resident lymphocytes and is essential for early accumulation and long-term persistence of $CD8^+$ T_{RM} cells in skin and liver, as well as for maintenance of liver-resident NK cells (21, 93, 141). Likewise, liver persistence by $CD8^+$ $CD103^-$ T_{RM} cells and NKT cells is promoted by the $\alpha_L\beta_2$ integrin LFA-1, consisting of CD11a (α -chain) and CD18 (β -chain). Its adhesive interactions with endothelial-expressed intercellular adhesion molecule (ICAM)-1 allows liver-resident lymphocytes to patrol the luminal side of sinusoids and to interact with hepatocytes through specialized endothelial fenestrations (22).

The environmental cues that promote T_{RM} maturation are determined by anatomical location and the type of infection or immunization. In skin and salivary glands, formation and survival of $CD8^+$ $CD69^+$ $CD103^+$ T_{RM} cells critically depends on the pleiotropic cytokine IL-

15, whereas their counterparts in intestinal epithelium do not rely on this cytokine (54, 87). Genetic deletion of IL-15 or its receptor components also results in a profound defect in the establishment and maintenance of other tissue-resident lymphocytes, including epidermal $\gamma\delta$ T cells, intraepithelial CD8 $\alpha\alpha$ T cells and liver-resident NK cells (137, 142-144). In addition, IL-15 supports survival and homeostatic turnover of CD8⁺ T_{CIRC} cells and NK cells (142, 144). Within epithelial tissues, IL-15 is trans-presented by dendritic cells and epithelial cells, such as keratinocytes in skin, in a complex consisting of the cytokine itself bound to a component of its receptor, the α -chain, while the T cells express the corresponding β - (CD122) and γ - (CD132) receptor chains (145, 146). IL-15 signaling in CD8⁺ T cells induces expression of Hobit in a manner dependent on the presence of the transcription factor T-bet (55). Elevated expression of Hobit is seen in murine T_{RM} cells from skin, gut and liver and in liver-resident innate lymphocytes, where Hobit cooperates with its homologue Blimp1 to repress transcription of *Klf2* and consequently, *S1pr1* and *Ccr7* (55, 78). In addition, IL-15 promotes other key steps in the generation of T_{RM} cells, including migration and peripheral accumulation of effector T cells (147-150), as well as induction and maintenance of pro-survival molecules such as Bcl2 and Bcl6 in developing T_{RM} cells (54, 151).

Another widely required signal for T_{RM} formation is delivered by TGF β . This cytokine is constitutively expressed in many tissues, including epithelial compartments, and its production and activation is further upregulated during the regenerative resolution and wound healing phases following infection. TGF β is produced in a latent form bound to a latency-associated protein and requires activation by proteolytic cleavage or conformational change through exposure to proteases, such as matrix metalloproteases and chymase, reactive oxygen species or the epithelial cell-expressed integrin $\alpha_v\beta_6$ (152). Active TGF β , in combination with IL-15, induces expression of CD103, E-cadherin and CD49a in T cells and innate lymphocytes in a manner independent of canonical Smad4-containing TGF signal transducer complexes, (69, 73, 78, 153-155). CD103 induction by TGF β in lymphocytes and other cell types is further dependent on the transcription factor Runx3 (156). Accordingly, genetic deficiency in TGF β receptor and Runx3 expression abolishes CD103 induction in lymphocytes in a wide range of models (37, 54, 63, 73, 95, 99, 130, 156, 157). IL-15 and TGF β further down-modulate and fine tune the expression of the T-box transcription factors Eomesodermin (Eomes) and T-bet, respectively, and shut down the KLF2–S1P1–CCR7-

dependent tissue exit pathway (66, 78, 119, 155, 158). Low level expression of T-bet together with profound suppression of Eomes also facilitates sustained surface expression of the receptors for IL-15 and TGF β , which ensures continued responsiveness of T_{RM} cells towards the two cytokines (66). This is important, since persistence of fully matured CD8⁺ T_{RM} cells can rely on continuous signaling from both IL-15 and TGF β , as shown by pharmacological blockade of IL-15 or conditional deletion of the TGF β -activating integrin, $\alpha_V\beta_6$, in the epidermis (66, 159). Together, such results emphasize a key function of niche-specific IL-15 and TGF β activity in promoting CD8⁺ CD69⁺ CD103⁺ T_{RM} populations and innate lymphocytes in epithelial tissues. Nevertheless, the extent to which continuous exposure to these cytokines is required to sustain the transcriptional profile in CD8⁺ CD69⁺ CD103⁺ T_{RM} cells, as opposed to a more stable epigenetically imprinted T_{RM} identity, remains to be determined. Relevant to this, there is evidence that CD8⁺ CD69⁺ CD103⁺ T_{RM} cells generated in intestinal epithelium after systemic infection with lymphocytic choriomeningitis virus (LCMV) can display a degree of plasticity in that they can form T_{CIRC} cells after isolation and reinfusion into newly infected hosts (6), although this feature is not readily apparent in other models (11, 53).

Of note, not all types of CD8⁺ T_{RM} cells rely on the molecular pathways described above. For instance, there are CD103⁺ and CD103⁻ CD8⁺ T_{RM} cells that are independent of IL-15 and/or TGF β signaling, including in intestinal mucosa, female reproductive tract and lymph nodes (70, 76, 87). Accordingly, many other cytokines have been implicated in promoting the T_{RM} phenotype, including type I interferons, IL-12, IL-33 and TNF α (35, 73, 97, 160). Furthermore, several other transcription factors and molecules have been shown to regulate survival and functionality of T_{RM} cells in specific organs, including the Notch pathway (58), the aryl hydrocarbon receptor (85, 161, 162), the nuclear receptor subfamily 4 group A1, or nuclear hormone receptor Nur77 (163), as well as ATP-binding cassette transporters (163).

In summary, there are two major waves of activation events that instruct the formation of T_{RM} cells from their naïve precursors. This first one occurs in lymphoid tissues where appropriate stimulation by antigen-presenting dendritic cells drives clonal T cell expansion and acquisition of trafficking capabilities required for seeding of T_{RM}-permissive

anatomical niches. The second wave occurs within peripheral tissues, where micro-environmental cues, which may include antigen reencounter and exposure to niche-specific cytokines, drive further maturation towards the T_{RM} phenotype. This final checkpoint of tissue-specific adaptation explains the considerable heterogeneity amongst T_{RM} cells in different anatomical locations. Thus, there exist various types of T_{RM} cells and innate lymphocytes that can establish residency programs by employing a range of distinct or interconnected transcriptional circuitries tailored to distinct cytokine and nutrient environments in individual organs. A detailed understanding of the molecular adaptations by different T_{RM} types will be integral for any attempt to manipulate T_{RM} cells at defined target sites with future immunotherapies.

Limitations in peripheral immune surveillance by recirculating memory T cells

Owing to their ability to rapidly recall cytokine production and cytolytic activity, memory T cells provide efficient protection from infection with previously encountered pathogens. This is best documented in bacterial and viral models of systemic infection, where pathogen control may be focused on lymphoid filter organs that contain large numbers of memory T cells, including lymph nodes and spleen (164-169). However, mucosal barriers are the major portals of entry for most pathogens and, prior to the description of sessile T_{RM} cells, immune surveillance in these locations has predominantly been discussed in the context of continuous T cell re-circulation (170-174). Accordingly, blood-borne T_{CIRC} cells transiting through peripheral tissues as part of their wider migration pattern have long been regarded as key determinants of protective immunity (174, 175).

These peripheral-migrating T_{CIRC} cells have widely been assumed to belong to the 'effector memory' T (T_{EM}) cell subset initially described in human blood and aptly coined according to their heightened capacity to exert effector functions (176). Their 'central memory' (T_{CM}) counterparts, on the other hand, are characterized by expression of the lymphoid-targeting receptors, CCR7 and CD62L, and as such, are thought to traffic through lymphoid tissues (176). This delineation into peripheral- and lymphoid-homing subsets has since been a central dogma in the field and broadly reflects the migratory behavior of $CD4^+$ memory T cells seen in animal models. Accordingly, skin infection imprints stable expression

of peripheral-targeting molecules, such as E- and P-selectin ligands, in a subset of recirculating CD4⁺ CD62L⁻ T_{EM} cells (10). CD4⁺ memory T cells represent a large fraction of the overall T cell pool in many extra-lymphoid tissues (4, 91, 177) and several *in vivo* migration assays, including lymph cannulation and parabiosis, have demonstrated that at least a proportion of these cells are constitutively recirculating between tissues and the blood via the lymphatic system (27, 170, 178). Nevertheless, these peripheral-migrating CD4⁺ memory T cells at least transiently express CCR7, reflecting its critical function in tissue exit (108, 109), as well as variable levels of CD62L, so they do not fit the classical definition of CCR7⁻ CD62L⁻ T_{EM} cells (47, 71, 91).

It has also become apparent that the migratory pattern of memory CD8⁺ T cell subsets, as defined by CCR7 and CD62L expression, is more complex than initially anticipated (179). For instance, CX3CR1^{int} memory cells distinct from CX3CR1^{hi} T_{EM} and CX3CR1^{low} T_{CM} cells have recently been identified as the main migratory CD8⁺ T cell subset patrolling peripheral tissues (180). Furthermore, circulatory surveillance by CD8⁺ memory T cells in the steady state may wane over time to a point where the majority of peripheral CD8⁺ T cells are actually non-recirculating T_{RM} cells (18). This decay however is accelerated under certain experimental conditions, for instance in animals housed under specific-pathogen free conditions (181, 182). By contrast, when mice are exposed to dirtier environments or sequential infections, chronic T cell activation and low-level inflammatory conditions driven by type I interferon activity support more sustained trafficking of CD8⁺ T cells around the body, as well as tissue retention and formation of T_{RM} cells (181, 182). Thus, the latter situation more faithfully models real-world immune system function. Contrary to the initial predictions, it has also been shown that CD62L⁺ CD8⁺ T_{CM} cells make up a large, and in some cases the predominating, fraction of memory T cells recruited to acutely inflamed tissues (10, 18, 148). These observations are consistent with stable expression of E- and P-selectin ligands by a subset of CD8⁺ T_{CM} but not T_{EM} cells, as well as a distinct ability of CD8⁺ T_{CM} cells to further up-regulate these molecules in response to IL-15 stimulation (10, 147, 148).

The above discussion highlights that recirculating CD4⁺ and CD8⁺ memory T cells can contribute to infection control in extra-lymphoid tissues, including skin and mucosa, in

addition to providing robust protection from systemic infection (10, 117, 147, 183). Nevertheless, there are limitations with respect to the densities of antigen-specific memory T cells that can be maintained in peripheral tissues through continuous migration in the steady state, particularly at late time points after infection or immunization when T_{CIRC} cells with appropriate homing molecule expression exist at relatively low frequencies. This numerical attrition of memory cells is more pronounced for $CD4^+$ T cells in many systems (10, 184-186) and is linked to functional polarization such that Th1-polarized cells can be more stable than their Th17 counterparts (185). Further, the process of site-directed recruitment of blood-borne T_{CIRC} cells or secondary effector cells can result in a decisive delay in T cell accumulation and protective activity within tissues. This lag before recruited T_{CIRC} cells can fully engage infected tissues provides a window of opportunity for rapidly replicating pathogens to establish robust infection and potentially, seed persisting reservoirs throughout the body (187-189). As a consequence, the protective potential of T_{CIRC} cells in dealing with infections in extra-lymphoid tissues is somewhat limited and can rapidly wane in absence of continued antigen stimulation (15, 29, 164, 165, 190).

These limitations represent major obstacles for vaccine development and likely explain why immunization approaches focused on the generation of antibodies and T_{CIRC} cells by transient activation have largely proven ineffective in generating long-lived protection from pathogens that target peripheral tissues, such as human immunodeficiency virus (HIV) and HSV (191-194). By contrast, studies employing CMV-based immunization to protect nonhuman primates from mucosal SIV infection have emphasized that ongoing T cell activation by persisting vaccine vectors is needed for effective peripheral immune surveillance by memory T cells (195). While this requirement for vector persistence may pose a major barrier for translation to human vaccine trials, such results nevertheless underscore the importance of T cell responses directly within the target tissues. Of note, such peripheral responses are rarely analyzed in immunogenicity studies due to ethical, technical and logistical hurdles inherent to repeated tissue sampling, as opposed to standard analysis of blood samples.

T_{RM} cells orchestrate immediate local immunity

In the light of these limitations in peripheral immune surveillance by T_{CIRC} cells, it is increasingly appreciated that memory T cells already pre-existing in peripheral tissues at the time of pathogen re-exposure are critical to rapid infection control (195-198). We now know that a large proportion of these are non-recirculating T_{RM} cells and work over the last 10 years has firmly established their contribution to local immunity in various experimental models. Accordingly, there is a conspicuous correlation between the local densities of $CD4^+$ and $CD8^+ T_{RM}$ cells and the level of site-specific protection. For instance, regions of skin containing large numbers of antigen-specific T_{RM} cells after a primary HSV-1 infection are effectively protected from renewed infection, with infectious virus being suppressed below detection limits as early as two days after challenge infection (7). This is a remarkable level of immediate control and contrasts with delayed virus clearance in remote areas of skin not involved with primary infection and hence, containing considerably fewer T_{RM} cells (7).

While $CD4^+$ and $CD8^+ T_{RM}$ cells cooperate locally to provide optimal immunity (7), epidermal $CD8^+ CD103^+ T_{RM}$ cells alone can afford a level of protection from HSV-1 infection that is far superior than what is achieved by $CD8^+ T_{CIRC}$ cells (10, 29, 66). Similarly, $CD4^+ CD103^- T_{RM}$ cells generated after a mucosal infection with HSV-2 confer protection from lethal re-challenge infection with a related HSV-2 strain (16). Epidermal $CD8^+ CD103^+ T_{RM}$ cells also drive greatly accelerated control of skin infection with vaccinia virus (VACV) (15, 199) and epithelial $CD8^+ CD69^+ CD103^+ T_{RM}$ cells in salivary glands rapidly clear infectious virus after intra-glandular infection with LCMV or murine cytomegalovirus (MCMV) (11, 37). Along the same lines, both $CD8^+ CD69^+ CD103^+$ and $CD4^+ CD69^+ CD11^+ T_{RM}$ cells are now recognized as essential components of cross-protection against pulmonary infection with related influenza viruses (IAV) (2, 3, 12, 200-203), although as discussed earlier, T_{RM} cells in the lower airways are not as long-lived as in other organs and hence, heterosubtypic immunity wanes over time (36, 190, 201, 204). Finally, brain-resident $CD8^+ CD69^+ CD103^+ T_{RM}$ cells have been shown to prevent fatal infection after intracranial inoculation with LCMV (205).

The experimental demonstration of T_{RM} -mediated local immunity is not restricted to viral models but is also evident in bacterial, fungal and parasitic infections. For instance, $CD8^+ CD69^+ CD103^+ T_{RM}$ cells residing within the intestinal epithelium are essential for

efficient control of oral infection with *Listeria monocytogenes* (95). Likewise, CD4⁺ T_{RM} cells drive protective immunity against mucosal infection with *Chlamydia trachomatis* (206). Furthermore, CD4⁺ T_{RM} in skin mediate control of cutaneous infection with the parasite *Leishmania major* (39, 207), as well as the fungus *Candida albicans* (47). Finally, CD8⁺ CD103⁺ T_{RM} cells generated in liver following immunization with attenuated sporozoites of the malaria parasite *Plasmodium berghei* mediate potent protection from a subsequent infection with live sporozoites (21).

T_{RM} cells as potential targets for future vaccines

Given this compelling evidence for T_{RM} cells as central players in protective immune responses to infectious pathogens, there is a rapidly emerging interest in provoking this type of peripheral immune surveillance with novel immunization approaches. Importantly, T_{RM}-mediated immunity in many tissues does not depend on continued presence of antigen and appears to be very long-lived, with examples of local donor T_{RM} persistence in skin grafts for up to 2 years (17). As such, T_{RM} cells appear to meet some of the key expectations of future vaccine targets to deliver potent cellular immunity.

A key component of many strategies to generate T_{RM} cells in vaccine settings is the direct manipulation of the target tissues. This is done, for instance, by topical delivery of vaccine vectors, which results in T cell activation by dendritic cells in lymphoid tissues draining the site of application, followed by T cell migration to the mildly inflamed target tissue and subsequent formation of CD8⁺ CD103⁺ T_{RM} cells. Examples such approaches include cervico-vaginal immunization with papilloma virus vectors encoding model antigens, such as M/M2 from respiratory syncytial virus (RSV) (208) or glycoproteins B and D from HSV (209), as well as combined intranasal and intravaginal immunization with IAV vectors expressing HIV p24 (210). Protective T_{RM} cells in genital mucosa have also been generated by combined topical application of inactivated *Chlamydia trachomatis* elementary bodies with charge-switched synthetic adjuvant particles (206). To the same effect, T_{RM} cells in lungs and airways have been elicited by direct intranasal or intratracheal administration of vaccine vectors, such as adenovirus encoding antigens from *Mycobacterium tuberculosis* (211, 212), plasmid DNA encoding HIV gp120 formulated with polymer polyethyleneimine

(213), live attenuated IAV vaccine (214) and *Mycobacterium bovis* Bacille Calmette-Guérin (215).

Other approaches have emphasized the requirement for administration of inflammatory adjuvants directly to the target tissues in order to efficiently recruit T_{RM} precursors generated in remote lymphoid tissues not necessarily connected to the target organs. Such strategies are referred to as “prime and pull” or “prime and trap” approaches (21, 112). For instance, activation of virus-specific CD8⁺ T cells in combination with non-specific inflammation induced by a skin irritant, or treatment of vaginal mucosa with a mildly inflammatory spermicide, is sufficient to induce epithelial CD8⁺ CD103⁺ T_{RM} cells that mediate protection from *de novo* infection with HSV-1 (29). To the same effect, local application to the vaginal mucosa of the chemokines, CXCL9 and CXCL10, during the effector phase after subcutaneous immunization with attenuated HSV-2 facilitates the formation of mucosal HSV-specific T_{RM} cells and these cells can reduce disease severity and mortality after challenge with a more virulent HSV-2 strain (112). Likewise, transient adenovirus infection of the liver has been used to “trap” effector CD8⁺ T cells in this location (21). Finally, reflecting the requirement for local antigen in facilitating T_{RM} formation in the lungs, intranasal instillation of antigen either in a complexed form or coupled to antibodies targeting pulmonary dendritic cells is sufficient to recruit effector CD8⁺ T cells from the circulation and drive their local conversion into CD103⁺ T_{RM} cells (211, 216).

In summary, these promising results provide a proof-of-principle that protective T_{RM} cells can be generated in vaccine settings. A major goal of vaccinating against viruses such as HIV and HSV is to prevent the establishment of latent or persisting reservoirs. This will require close to sterilizing immunity, although this level of protection may be impossible to attain in experimental settings with high-dose challenge infections in animals. Nevertheless, T_{RM}-mediated protection, particularly in conjunction with strong CD4⁺ and CD8⁺ T_{CIRC} responses, may be potent enough to achieve profound protection against challenge with low doses of infectious pathogens, as expected to occur in most cases during human transmission. Separately, boosting the numbers of already existing T_{RM} cells appears as an attractive possibility to prevent clinical symptoms caused by periodic virus recrudescence

during latent-reactivating infections such as HSV (140). It remains to be tested how effective such T_{RM}-targeting vaccine approaches will be in clinical settings.

Protective effector functions of T_{RM} cells

The dominant role of T_{RM} cells in immune defense in the aforementioned examples arises from their strategic positioning within tissues targeted by the pathogens under study, including skin and mucosal barriers, as well as brain and liver. Accordingly, without the need for site-specific recruitment, T_{RM} cells can immediately recognize infected cells or pathogen-derived antigen presented by dendritic cells and rapidly recall canonical T cell effector functions such as cytokine secretion or perforin- and granzyme-mediated target cell killing.

Like innate lymphocytes (217), resting T_{RM} cells contain elevated levels of transcripts encoding a number of pro-inflammatory cytokines, meaning that they are poised for rapid cytokine production upon appropriate stimulation (9, 56, 58, 61, 140, 218). In line with this, CD8⁺ CD69⁺ CD103⁺ T_{RM} cells isolated from various mouse and human tissues exhibit enhanced production of cytokines, such as IFN γ , TNF α , IL-2 and IL-17, as well as target cell killing, upon re-stimulation *ex vivo* (1, 9, 11, 15, 21, 30, 45, 57, 58, 61, 77, 91, 200, 219). Rapid cytokine production by T_{RM} cells has also been confirmed *in vivo* (27, 42). Likewise, there is evidence that brain T_{RM} cells can readily kill peptide-pulsed target cells *in situ* (9), which is consistent with elevated levels of preformed granzyme B in T_{RM} cells. Whether T_{RM} cells can also employ the FAS/FAS-ligand pathway for target cell elimination is not known. Heightened expression of granzymes is also seen in T_{RM} cells in other organs, including intestinal mucosa and skin (73, 140, 220, 221), although the opposite, that is poor *ex vivo* cytolytic activity and lack of preformed granzyme and perforin, has been described for IAV- and RSV-specific T_{RM} cells in lungs (119, 136, 222, 223). Hence, the extent to which T_{RM} cells utilize cytolytic activity in infection control is likely to depend on tissue location and the nature of the infectious agents (200, 222). Regardless, genetic models and pharmacological blockade of cytokine activity have established that, in principle, T_{RM} cells can utilize both cytolytic and non-cytolytic effector functions to control infection with a broad variety of pathogens (41, 200, 205, 224, 225).

The “sensing and alarm” function of T_{RM} cells

Importantly, through the production of pro-inflammatory cytokines, T_{RM} cells can promote a loco-regional state of broad infection resistance (41, 42, 224). This mode of protection has a distinct advantage over direct target cell killing in that it does not require direct contact between T_{RM} cells and most of the responding tissue cells. Thus, even low densities of T_{RM} cells can induce effective immunity upon recognition of relatively few infected tissue cells. Remarkably, activation by infectious virus or topically applied cognate antigen of $CD8^+$ T_{RM} cells in female reproductive tract and skin, respectively, is sufficient to protect even against antigenically unrelated pathogens (41, 224). Such cross-protective responses are strictly cytokine-dependent and have been shown to be potent enough to confer near sterilizing immunity against mucosal VACV challenge infection (224).

T_{RM} -derived cytokines have pleiotropic functions in local immune protection. For instance, in addition to directly suppressing virus propagation within infected cells, T_{RM} -derived $IFN\gamma$ triggers the expression of a wide array of innate effector molecules, including the interferon-induced transmembrane protein, $IFITM3$ (41). Interestingly, some $CD8^+$ $CD69^+$ $CD103^+$ T_{RM} cells themselves constitutively express $IFITM3$, which has been shown to support survival of lung T_{RM} cells during re-challenge infection with influenza virus (226). Importantly, regional induction of antimicrobial genes within hours after T_{RM} activation confers a level of heightened infection resistance in surrounding tissue, which is important in curtailing dissemination of infection. Furthermore, the combined actions of T_{RM} -derived $IFN\gamma$, $TNF\alpha$ and $IL-2$ can swiftly activate local NK cells and dendritic cells and also initiate the recruitment of T_{CIRC} cells, B cells and inflammatory monocytes via the induction of chemokines, such as $CXCL9$, $CXCL10$, $CCL2$, and the endothelial cell-expressed vascular cell adhesion molecule, $VCAM-1$ (42, 207, 224). In addition, the T_{RM} cells themselves are a source of pro-inflammatory chemokines, including $CCL3$, $CCL4$ and $CCL5$ (27, 224).

In summary, T_{RM} cells act as peripheral immune sentinels that rapidly sense invading pathogens and subsequently trigger a cascade of antimicrobial innate and adaptive effector mechanisms culminating in rapid infection control (**Figure 3**). This type of immune surveillance is now widely referred to as the 'sensing and alarm' function of T_{RM} cells (42). As part of this response, T_{RM} cells promote the recruitment of various types of immune cells from the circulation, including T_{CIRC} cells, which can go on to form new T_{RM} cells upon

resolution of infection (227, 228). At the same time, pre-existing T_{RM} cells that become activated by cognate antigen can undergo bouts of local proliferation (40, 208, 228, 229). Thus, corresponding to the severity and duration of the renewed infection challenge, repeated antigen encounter can result in a readjustment of the size and composition of the pool of T_{RM} cells.

T_{RM} responses to commensal microbiota and their relevance to tissue homeostasis

$CD8^+$ and $CD4^+ T_{RM}$ cell responses are not only elicited after challenge with infectious pathogens, but also following colonization of mouse skin with *Staphylococcus epidermidis* or *Candida albicans*, respectively (45, 47). These microorganisms are part of the normal microflora in humans, nevertheless they can cause clinically relevant diseases when immune control at barrier tissues is compromised. T_{RM} responses to these commensals are characterized by functional polarization towards a IL-17 producing phenotype and, importantly, IL-17-producing $CD8^+ T_{RM}$ cells and *Candida*-specific $CD4^+ T_{RM}$ cells are readily detected also in human skin (45, 47). Furthermore, intestinal infection with *Toxoplasma gondii* or chemically-induced colitis can trigger the generation of long-lived memory $CD4^+ T$ cell responses to commensals such as *Clostridium* subspecies (230). *Clostridium*-specific T cell responses are dominated by IFN γ -producing $CD4^+ T$ cells and persist in the intestinal mucosa for at least several months after induction, meaning that they are likely to represent long-lived populations of gut-resident $CD4^+ T_{RM}$ cells (230).

Alongside epithelial cells, APCs, $CD4^+ T_{REG}$ cells and innate lymphocytes, microflora-specific T_{RM} cells are likely an important cellular component of the well-documented and highly beneficial interactions between the host immune system and the microflora. While most of the times commensal microorganisms may be physically segregated from immune effectors, for instance by tight junctions between epithelial cells or the mucus covering intestinal barriers, intermittent exposure may be rapidly sensed by T_{RM} cells to prevent invasion and establishment of clinically apparent infection. In addition to keeping specific microorganisms at bay, T_{RM} activation in the context of microflora recognition can trigger a regional state of alert and cross-protection against unrelated microorganisms. For instance, secretion of IL-17 by $CD8^+ CD69^+ CD103^+ T_{RM}$ cells in response to *Staphylococcus epidermidis* colonization drives keratinocyte production of antimicrobial molecules, such as S100A8 and

S100A9, which are capable of restricting infection with *Candida albicans* (45) (**Figure 4a**). Furthermore, it is tempting to speculate that T_{RM} cells could act as an 'adaptive sensor' of structural barrier integrity. In case of physical injury, close association with otherwise anatomically segregated members of the microflora may result in rapid antigen-specific activation of T_{RM} cells by local dendritic cells or macrophages. The rapidity and strength of the ensuing 'sensing and alarm' reaction elicited by T_{RM} cells may be critical in defending wounded tissues, which are temporarily vulnerable to pathogen invasion, and in facilitating tissue regeneration. Thus, via versatile interactions with commensal microorganisms, T_{RM} cells can contribute to tissue homeostasis by promoting a level of ongoing immune activation resulting in broad infection resistance.

Human T_{RM} cells guard sites of recurring and persisting infection

Large numbers of CD69⁺ CD103^{+/-} T_{RM} cells populate human lymphoid and extra-lymphoid tissues (4, 32-34, 60, 77, 78, 231, 232). By contrast, tissues from newborns and infants display lower T_{RM} frequencies, consistent with a progressive accumulation of T_{RM} cells throughout life (233). Therefore, the overall composition of the T_{RM} pool within a given tissue is likely an accurate reflection of ongoing and previous exposures to pathogens and commensal microbes, as well as to dietary and other environmental antigens. As such, the buildup of specific T_{RM} cells reveals a site-specific and dynamic adaptation of a form of tissue-embedded immune memory to local infection and antigen challenge.

Human CD69⁺ CD103^{+/-} T_{RM} cells specifically recognize a number of prevalent viruses. These include viruses that are frequently re-encountered through environmental exposure, such as IAV (136, 231) and RSV (223), as well as viruses that cause latent-reactivating or chronically active infections, such as HSV-1 and HSV-2 (139, 140, 234), Epstein Barr virus (EBV) (74, 77, 78), CMV (235) and hepatitis B virus (69), and possibly many more. Thus, the corresponding T_{RM} populations are established and continuously shaped in response to repeated bouts of virus emergence. Importantly, presence of virus-specific T_{RM} cells in humans correlates with enhanced protection and immune control, as shown for pulmonary infection with RSV (223), chronic HBV infection in liver (69) and HSV-2 infection in genital skin (140). Furthermore, selective depletion of T_{CIRC} cells by treatment with anti-CD52 antibodies does not result in increased infection susceptibility or clinical manifestation of

reactivating infections in patients with cutaneous T cell lymphoma, which has been attributed to the protective function of peripheral T_{RM} cells (14).

T_{RM} cells in chronic and persisting virus infections

Chronic and persisting virus infections are likely to be major drivers of T_{RM} dynamics in many human tissues. Such infections are exceedingly widespread with an estimated prevalence of several billion cases in the human population and multiple co-infections established within a given individual (236). In addition, there are various bacteria and parasites that cause protracted or persisting infections. Although these pathogens establish reservoirs that escape complete eradication, they are nevertheless constantly kept in check by the immune system in a dynamic state of 'host-pathogen equilibrium'. As a consequence, many of these infections are clinically silent and cause overt symptoms and disease mainly only in immunocompromised individuals. As part of their multifaceted evasion strategies, persistent pathogens often target specific anatomical niches, including the central nervous system, the gastrointestinal tract and the liver. Many persistent viruses further require intermittent productive replication within epithelial tissues to facilitate transmission. Importantly, T_{RM} cells take up residency in all these tissues and display appropriate specificities for the viruses that periodically reemerge in these locations (69, 74, 77, 78, 84, 136, 139, 140, 223, 231).

T_{RM} cells and herpesvirus infections

The family of *Herpesviridae* includes highly prevalent latent-reactivating human viruses, such as HSV, varicella-zoster virus, CMV, EBV and others, that have co-evolved with their hosts for millions of years. It stands to reason that, over time, our immune system has developed optimal strategies to deal with the lifelong presence of herpesviruses in many parts of the body. The host's ultimate goal is to ensure survival by avoiding excessive inflammation and immunopathology and host survival is equally important for pathogen persistence. A number of landmark studies on HSV and EBV infection have established the concept that CD8⁺ T_{RM} cells are an integral component of these defense strategies.

Primary infection with HSV in skin and mucosal tissues results in the establishment of a lifelong latent infection confined to sensory ganglia that innervate the site of infection.

Contrary to the initial assumption that HSV latency represents a form of largely quiescent virus persistence, there is molecular evidence for ongoing transcriptional activity of lytic viral genes in a large proportion of infected neurons, as well as clinical evidence for frequent asymptomatic virus shedding in human genital and orolabial skin (237-239). Remarkably, most of these episodes of reactivation and shedding are very short-lived and therefore remain clinically inapparent, with clearance observed within hours after detection (140, 238, 239). These findings strongly imply rapid virus containment by immune cells resident within the affected tissues.

Indeed, HSV-specific CD8⁺ T_{RM} cells in mice and humans occupy latently infected sensory ganglia (234, 240, 241). In mice, ganglionic CD8⁺ T_{RM} cells can curtail virus replication during reactivation by producing IFN γ (242) and importantly, they can undergo repeated rounds of activation without losing proliferative capacity or effector functions (219). Intriguingly, T_{RM} cells can also prevent HSV reactivation by directly interfering with viral gene expression in neurons. This function is dependent on granzyme B-mediated degradation of the HSV-1 immediate early protein, ICP4, a major transcriptional activator required for progression to full reactivation (243). Of note, this mechanism does not induce neuronal apoptosis and therefore represents an example of a non-cytolytic function of granzyme B. Consistent with these functions, augmenting the pool of ganglionic CD8⁺ T cells has been shown to reduce disease in a UV-induced reactivation model of ocular HSV-1 infection (244).

HSV-specific CD8⁺ T_{RM} cells also reside at the dermal-epidermal junction in skin, where they are contiguous to sensory nerve endings that connect the latently infected ganglia with the skin and genital mucosa (84, 139, 140). Upon asymptomatic HSV-2 shedding, these T_{RM} cells rapidly express elevated levels of perforin and pro-inflammatory cytokines and form clusters with virally infected epithelial cells, which also coincides with recruitment of CD8⁺ T cells from the underlying dermis. Such immediate T_{RM} responses have the potential to extinguish the earliest rounds of peripheral virus replication, and thus, are likely to be critical for the prevention of genital herpes lesions (140). Conversely, it is possible that absence or impaired functionality of HSV-specific CD8⁺ T_{RM} cells are associated

with full-blown lesion development and recurrent herpetic disease upon reactivation in some patients.

A similar role of epithelial CD8⁺ T_{RM} cells has been proposed for the control of EBV infection. EBV establishes a latent infection in memory B cells that recirculate around the body, including the organized lymphoid tissues in the oropharynx. Sporadic reactivation of EBV from B cells can result in productive infection in tonsillar epithelium, resulting in virus shedding into the throat. Much like genital HSV-2 infection, intermittent low-level virus shedding is observed in many asymptomatic virus carriers. Thus, the oropharyngeal epithelium represents an important site for successful transmission and spread of EBV within the human population. Interestingly, human tonsils harbor a mixture of EBV-specific CD8⁺ memory T cells, including CD69⁻ CD103⁻ and CD69⁺ CD103^{+/-} cells that recognize antigens produced during both the lytic and latent life cycles of EBV. Of those, CD8⁺ CD69⁺ CD103⁺ T_{RM} cells preferentially localize to the tonsillar epithelium (74, 77, 78). Thus, it is tempting to speculate that these cells are responsible for limiting viral shedding in the throat, thereby preventing overt clinical symptoms such as recurring tonsillitis or EBV-associated hairy leukoplakia. At the same time, even though human tonsils also harbor small populations of CD8⁺ memory T cells specific for another herpesvirus, CMV, these cells largely display a CD69⁻ CD103⁻ phenotype (78).

In summary, a picture emerges where CD8⁺ CD69⁺ CD103⁺ T_{RM} cells with appropriate specificities take up residence in and guard anatomical regions that come under periodic attack by recurring, persisting or reactivating viruses. Being strategically positioned at these sites enables them to respond immediately upon virus re-emergence, which can result in rapid extinction of local virus replication in a clinically asymptomatic manner. Thus, in case of highly prevalent chronic and persistent infections, T_{RM} afford virus containment, but not elimination, and therefore are likely to have an essential contribution to the equilibrium or stalemate established between the host and pathogens.

Expression of checkpoint inhibitory receptors by T_{RM} cells

It is of critical importance for the host to avoid excessive tissue damage as a consequence of the continuing battle with persisting infections. Therefore, viral immunity needs to be

carefully regulated and adjusted, which involves the induction of inhibitory molecules in T cells, including PD-1, LAG-3, TIM-3, 2B4 and many more, the generation of T_{REG} cells, as well as the action of immuno-regulatory cytokines, such as IL-10 and TGFβ (236, 245). Strong systemic and chronically active infections, such as experimental LCMV clone 13 infection in mice, can greatly dampen CD8⁺ T cell cytokine production and cytolytic activity. This is mediated through the ligation of PD-1, LAG-3 and other inhibitory receptors whose expression is driven by chronic antigen stimulation and exposure to pro-inflammatory cytokines. The resulting functional impairment is widely referred to as 'T cell exhaustion' (245). Nevertheless, these functionally restrained T cells still contribute to virus control and promote the stalemate between host and pathogen (246, 247). Accordingly, their functional status has been proposed to reflect an adaptation to avoid immunopathology, rather than defective differentiation (247, 248).

While CD8⁺ T_{RM} cells in mice can be subject to regulation by CD4⁺ T_{REG} cells (249, 250), they also constitutively express mRNAs encoding for the checkpoint inhibitory receptors commonly used to identify 'exhausted' T cells, including PD-1, CTLA-4, LAG-3, TIM-3, 2B4, CD160, CD101 and others (53, 54, 56). Transcriptional activity of these genes in T_{RM} cells is evident across many organs, including skin, small intestine, lungs and brain, although curiously, protein expression is only seen in some, but not all models. For instance, while CD8⁺ T_{RM} cells generated in intestinal mucosa after acute infection with LCMV lack surface expression of PD-1, elevated expression is seen during chronic infection (73, 127). Furthermore, PD-1 protein expression is detected on CD8⁺ T_{RM} cells in skin following HSV-1 infection, but not after acute infection with VACV (15, 228). Likewise, in T_{RM} cells residing in brain, PD-1 expression is readily detected during infection with murine CMV and polyomavirus (251, 252), but not after infection with vesicular stomatitis virus (9). These discrepancies appear to be related to differences in anatomical location and course of infection. It should be noted though that PD-1 induction in T_{RM} cells occurs independently of local antigen recognition (228, 251), but instead can be driven by a combination of the T_{RM}-inducing cytokines IL-15 and TGFβ (69). Importantly, protein expression of checkpoint inhibitory receptors is uniformly seen in human CD8⁺ T_{RM} cells in lungs, tonsils and liver (58, 69, 78).

Curiously, despite constitutive expression of these inhibitory molecules, T_{RM} cells rapidly exert potent effector function upon appropriate activation *in vivo* and *ex vivo*. This may be due to only low-level expression of the corresponding ligands in peripheral tissues in absence of inflammation, whereas higher expression in immunologically active tissues may indeed serve to fine-tune T_{RM} responses. Separately, it is tempting to speculate that T_{RM} -derived pro-inflammatory cytokines rapidly induce ligands, such as PD-L1, B7 and MHC class II molecules, in surrounding tissue cells and thereby limit the extent of T_{RM} activation in a negative feedback loop. Restricting the duration of T_{RM} activation by such a mechanism may be critical to limit inflammation and preserve the integrity of barrier tissues containing large numbers of T_{RM} cells in close proximity to potentially activating microbes, although this hypothesis remains to be addressed experimentally. Finally, it has recently been shown that PD-1 expression facilitates the generation and persistence of $CD8^+$ T_{RM} cells in the brain (252), presumably by preventing terminal effector differentiation of MPECs (253). The rapid emergence of novel immunotherapies targeting such receptors in cancer patients warrants a better understanding of the functional significance of checkpoint receptor expression specifically in T_{RM} cells.

Role of T_{RM} cells in cancer immune surveillance

Akin to their role in containing recurring and persisting viruses that target epithelial cells for replication, T_{RM} cells may have a similar function in controlling cancer cells arising within epithelial niches. Of note, the vast majority of cancers originate within epithelial tissues, including by definition all carcinomas and melanoma. $CD8^+$ T cells can suppress tumor progression and reoccurrence, and experimental and clinical data argue that cancer cells can persist as occult tumors in absence of clinical disease for prolonged periods of time, possibly over decades in humans (254, 255). In analogy to the 'host-pathogen equilibrium' in the context of persisting infections, this mode of cancer suppression by the immune system is widely referred to as a 'cancer-immune equilibrium' (256).

Tumor-associated human $CD8^+$ T cells with a $CD69^+$ $CD103^+$ T_{RM} phenotype have been described in patients suffering from a broad variety of cancers, including urothelial, colorectal, ovarian, endometrial, cervical and lung cancer, as well as glioblastoma and melanoma (59, 131, 257-266). Often these cells are confined to epithelial compartments

within or around tumors (259, 262, 265, 266) and their transcriptional profile resembles that of T_{RM} cells in murine tissues, meaning that tumor-associated CD8⁺ CD69⁺ CD103⁺ T cells are likely bona fide T_{RM} cells (59, 260, 264). Intriguingly, strong accumulation of these T_{RM} cells or their transcriptional marks, respectively, correlates with prolonged survival in a spectrum of patients and has been shown to be a better prognostic marker than total CD8⁺ T cell counts in some cancers (259-261, 263-265). Elevated T_{RM} frequencies in the analyzed tumors are likely to reflect the patient's ability to generate cancer-specific T_{RM} cells not only in the surgically resected material, but also in distant sites within the body that may harbor micro-metastasis or dormant cells from the same cancer. Therefore, efficient T_{RM}-mediated control of subclinical metastases in these sites could be a major contributor to the observed survival advantage. Indeed, there is recent data from transplantable tumor models indicating that TGFβ-dependent CD8⁺ CD69⁺ CD103⁺ T_{RM} cells, either alone or in cooperation with T_{CIRC} cells, can protect from head and neck cancer or melanoma challenge by delaying or preventing tumor growth (227, 263, 267, 268). However, their precise mechanisms of action remain to be defined.

Therapeutic blockade of immune checkpoint receptors, such as PD-1 and CTLA-4, has emerged as a highly promising treatment modality for a spectrum of cancer patients (269-271). The rationale behind this approach is to reinvigorate chronically stimulated and functionally impaired or exhausted T cells to unleash their tumor-destructive potential. Although exhausted T cells are often identified by their expression of checkpoint receptors, human T_{RM} cells in healthy tissue constitutively express several of these molecules, as discussed before (58, 69, 78). Importantly, tumor-associated T_{RM} cells also express checkpoint receptors, including PD-1, LAG-3 and TIM-3 (59, 259, 260, 262). However, whether this expression is a consequence of chronic stimulation by tumor-associated antigens, as seen in exhausted CD8⁺ T cells, or alternatively, imprinted by exposure to T_{RM}-inducing cytokines in the tumor microenvironment remains unclear. Regardless, in some cases, this expression is of functional significance since blockade of PD-1 or PD-L1 has been shown to greatly enhance *ex vivo* killing of autologous lung cancer cells by T_{RM} cells (260). Such data suggest that T_{RM} cells may be cellular targets of checkpoint blockade therapy and clearly warrant further investigations into the role T_{RM} cells play in protective responses, as well as adverse immune reactions, during cancer immunotherapies.

Conclusions

The growing appreciation that non-recirculating T_{RM} cells are central players in tissue immunity has refined current concepts on peripheral immune surveillance and the defense mechanisms involved. As an important implication of this, it has become increasingly apparent that the blood as a diagnostic window to assess T cell immunity has limited prognostic value in predicting the level of immune reactivity in peripheral tissues. The generation of T_{RM} cells alongside other resident lymphocytes can be interpreted as the establishment of a form of long-lived tissue-embedded immune memory that is directly adapted to ongoing local challenges and a regional history of infection and inflammation. As such, immune cells in extra-lymphoid tissues may regularly function as largely autonomous units, whereas activation and recruitment of recirculating effectors could mainly be triggered in cases of delayed pathogen clearance or extensive tissue injury. Importantly, as part of the latter process, the composition of the local memory pool is dynamically shaped with regards to antigen specificities and population size. Most remarkably, although large parts of our body are under ongoing microbial attack, localized tissue responses orchestrated by T_{RM} cells regularly afford effective immunity in a clinically silent manner. As such, T_{RM} cells make an important contribution to the maintenance of tissue homeostasis and to the establishment of a dynamic 'host-microbe equilibrium' in response to commensal microflora and recurring or persisting pathogens. Likewise, T_{RM} cells are now emerging as critical components of cancer immune surveillance, particularly in epithelial and neuronal tissues.

As a consequence, therapeutic or prophylactic manipulations of T_{RM} cells appear as promising strategies for future vaccines and immunotherapies against infection, cancer or chronic inflammation. Checkpoint blockade therapies in cancer patients may inadvertently represent one such example. Given that T_{RM} cells constitutively express checkpoint inhibitory receptors, it appears likely they contribute to tumor regression and long-term control during successful therapy. At the same time however, they may also trigger adverse side effects, for instance in cases where a T_{RM} -mediated 'host-microbe equilibrium' could break down as a consequence of T_{RM} disinhibition to result in excessive inflammation and tissue damage. Future experimental and clinical studies will have to clarify the role of T_{RM}

cells in cancer immunotherapy. Furthermore, animal studies employing localized immunizations or 'prime and pull' and 'prime and trap' strategies have demonstrated the feasibility of generating pathogen-specific T_{RM} cells in desired anatomical locations. Separately, tissue-specific targeting of already established T_{RM} populations represents another possible therapy option. Such an approach could be used to boost T_{RM} numbers and functionality at sites of periodic pathogen recurrence, or to diminish T_{RM} populations at sites of chronic inflammation. Developing these targeted approaches however, will require a detailed understanding of phenotype, function and location-specific regulation of the various T_{RM} cell subsets in mice and humans. A combination of future experimental and clinical studies will be needed to explore and demonstrate the clinical potential of harnessing T_{RM} cell biology in human disease.

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Conflict of interest

The authors declare no conflicts of interest.

Figure legends

Figure 1. Spectrum of peripheral T cell dynamics. T cells within peripheral tissues can be (i) in rapid transit, (ii) retained for prolonged periods of time or (iii) retained indefinitely. The latter are permanently tissue-resident T cells that can persist long-term without replenishment from blood-borne cells. Well-characterized examples of these include epithelial CD8⁺ CD69⁺ CD103⁺ T_{RM} cells.

Figure 2. Checkpoints in the generation of CD8⁺ CD69⁺ CD103⁺ T_{RM} cells after skin infection. Following activation by antigen-presenting dendritic cells, short-lived effector T cells (yellow) and memory precursor effector T cells (MPEC, orange) infiltrate infected skin, where MPECs gain access to the epithelial layer and further differentiate *in situ* into long-lived T_{RM} cells. The rates of MPEC survival, retention and tissue egress within infected skin are important determinants of the size of the developing T_{RM} cell pool. The highlighted basic steps in T_{RM} generation are likely involved in most epithelial tissues, whereas the examples of genes and molecules operating at the various stages refer to the generation of T_{RM} cells in skin.

Figure 3. The ‘sensing and alarm’ function of T_{RM} cells. Owing to their strategic positioning at sites of pathogen recurrence or reactivation, T_{RM} cells rapidly sense pathogens and trigger a cascade of innate and adaptive effector functions that together induce a tissue-wide state of alert and protection. This ‘sensing and alarm’ function is dependent on T_{RM}-derived cytokines IFN γ , TNF α and IL-2. These cytokines can have (i) direct antimicrobial effects, (ii) induce expression in surrounding epithelium of an array of innate antimicrobial molecules such as IFITM3, (iii) promote the recruitment of T_{CIRC} cells, B cells and monocytes from blood and (iv) activate local innate cells such as NK cells and dendritic cells. The extent to which these individual functions are triggered may depend on the severity and duration of the microbial challenge.

Figure 4. Interactions of T_{RM} cells with commensal microbes in skin. (A) While on most occasions T_{RM} cells and commensal microbes may be micro-anatomically separated, intermittent exposure involving antigen presentation by local dendritic cells triggers production of IL-17 by commensal-specific T_{RM} cells. IL-17 in turn induces expression of

antimicrobial molecules, such as S100A8 and S100A9, in keratinocytes. These molecules confer a broad infection resistance that also extends to unrelated microbes and potential pathogens. (B) In wounded tissue, extensive exposure of T_{RM} cells to microflora-derived antigens may trigger production of additional cytokines, including IFN γ , TNF α and IL-2, which drive recruitment and activation of blood-borne and local immune cells, respectively. In this scenario, T_{RM} cells may act as 'adaptive sensors' of structural barrier integrity, promoting wound defense and regeneration.

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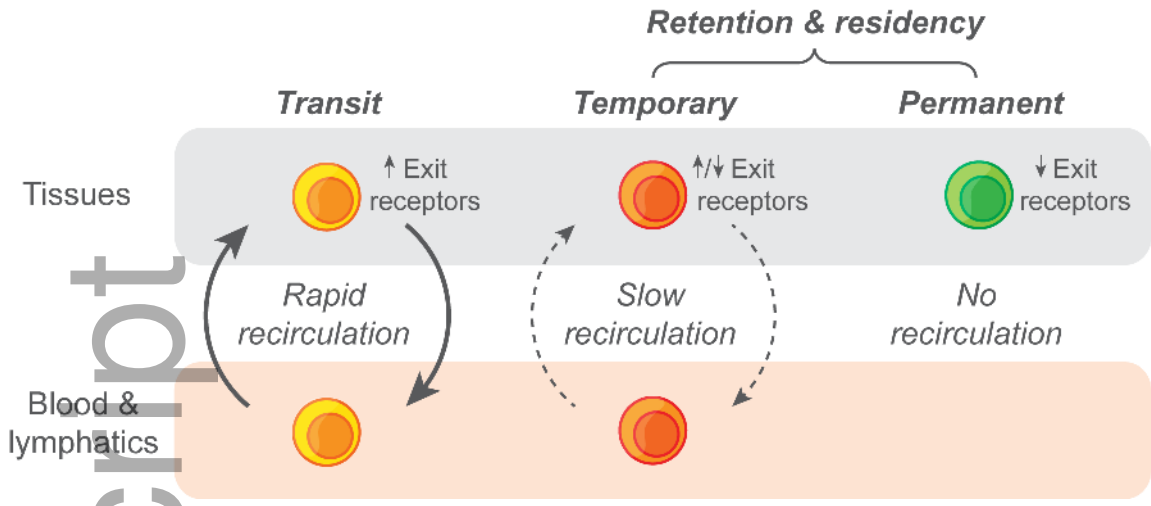
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Table 1. Examples of experimental approaches commonly used to identify and characterize tissue-resident lymphocytes

Approach	Aims and conclusions	Examples
Phenotyping	Measures expression of putative residency markers, e.g. CD69, CD103 and CD49a → <i>Infers residency, but markers are imperfect</i>	(32, 33, 78, 231)
Intravascular labeling	Identifies extravasated cells in tissues (label-negative) → <i>Demonstrates positioning within tissue parenchyma or stroma</i>	(19, 20)
Parabiosis	Measures contribution of blood-borne T _{CIRC} cells to the T _{RM} pool → <i>Evidence for T_{RM} – T_{CIRC} disequilibrium</i>	(5, 12, 15, 18, 27)
Tissue transplantation	<ul style="list-style-type: none"> • Graft with genetically marked T_{RM} cells: Measures T_{RM} persistence in absence of T_{CIRC} cells • Graft without T_{RM} cells: Measures contribution of blood-borne T_{CIRC} to T_{RM} pool → <i>Evidence for T_{RM} persistence or T_{RM} – T_{CIRC} disequilibrium</i>	(7, 8, 17, 39)
In situ labeling	Identifies non-migrating cells in tissues (label-positive) and indirectly, tissue access by T _{CIRC} cells (label-negative) → <i>Evidence for T_{RM} persistence</i>	(9, 35, 47, 49)
Blocking T_{CIRC} migration	Excludes T _{CIRC} cells from peripheral tissues to measure T _{RM} persistence without replenishment from T _{CIRC} cells → <i>Evidence for T_{RM} persistence</i>	(11, 15, 81, 201)
Selective T_{CIRC} ablation	Measures T _{RM} persistence in absence of T _{CIRC} cells → <i>Evidence for T_{RM} persistence</i>	(10, 14, 42)

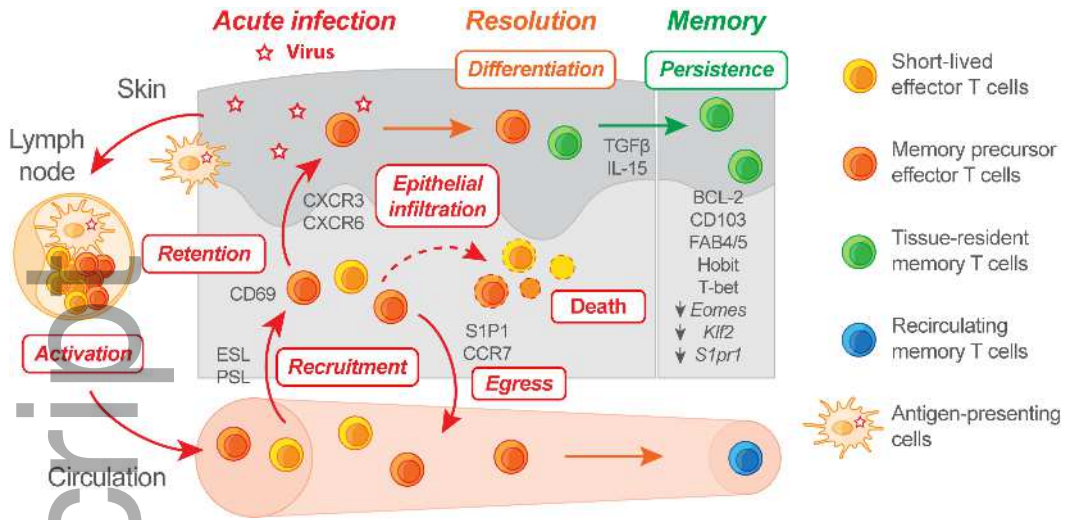


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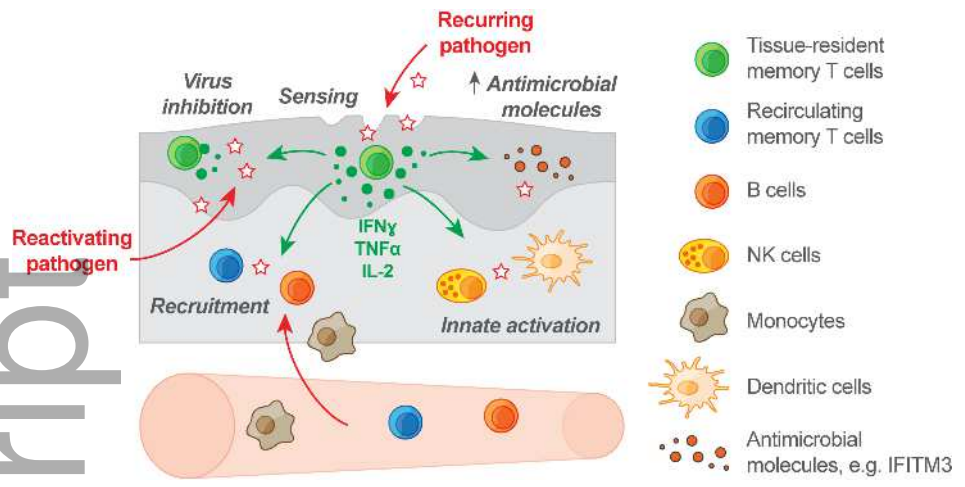
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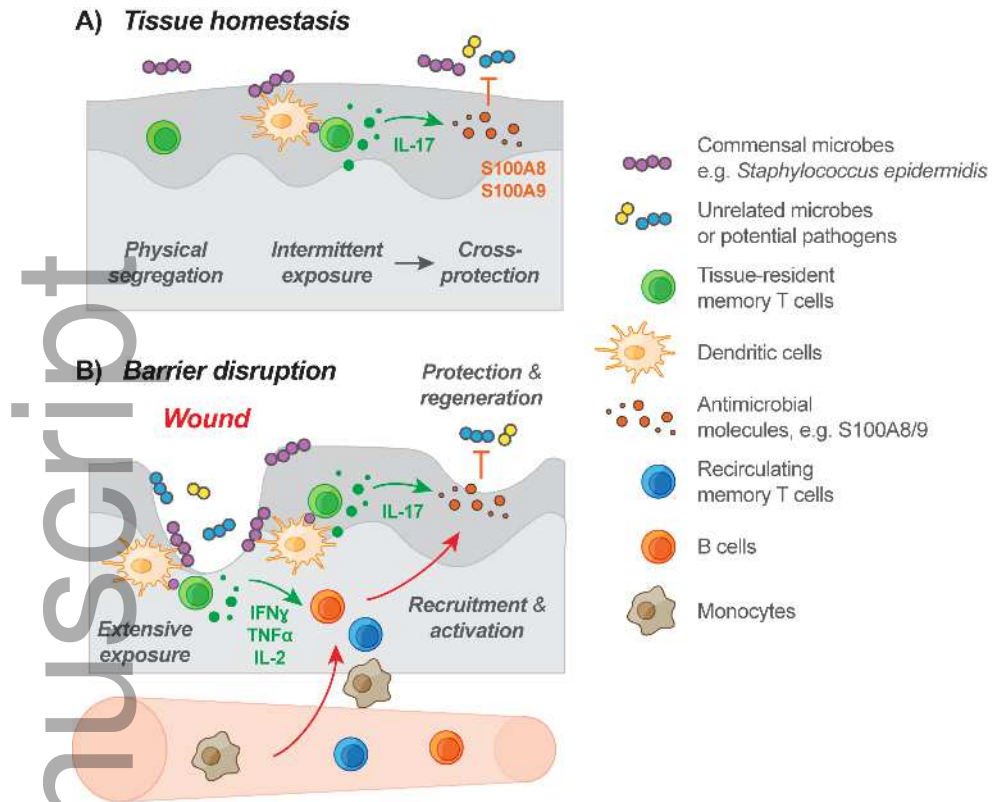


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