frontiers in Immunology

REVIEW
published: 30 July 2018
doi: 10.3389/fimmu.2018.01770

Armed and Ready: Transcriptional Regulation of Tissue-Resident Memory CD8 T Cells

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A fundamental benefit of immunological memory is the ability to respond in an enhanced manner upon secondary encounter with the same pathogen. Tissue-resident memory CD8 T (T_{RM}) cells contribute to improved protection against reinfection through the generation of immediate effector responses at the site of pathogen entry. Key to the potential of T_{RM} cells to develop rapid recall responses is their location within the epithelia of the skin, lungs, and intestines at prime entry sites of pathogens. T_{RM} cells are among the first immune cells to respond to pathogens that have been previously encountered in an antigen-specific manner. Upon recognition of invading pathogens, T_{RM} cells release IFN-γ and other pro-inflammatory cytokines and chemokines. These effector molecules activate the surrounding epithelial tissue and recruit other immune cells including natural killer (NK) cells, B cells, and circulating memory CD8 T cells to the site of infection. The repertoire of T_{RM} effector functions also includes the direct lysis of infected cells through the release of cytotoxic molecules such as perforin and granzymes. The mechanisms enabling T_{RM} cells to respond in such a rapid manner are gradually being uncovered. In this review, we will address the signals that instruct T_{RM} generation and maintenance as well as the underlying transcriptional network that keeps T_{RM} cells in a deployment-ready modus. Furthermore, we will discuss how T_{RM} cells respond to reinfection of the tissue and how transcription factors may control immediate and proliferative T_{RM} responses.

Keywords: T cell diferentiation, tissue-resident memory T cells, transcription factors, homolog of Blimp-1 in T cells, BLIMP-1, Notch, RUNX3, secondary responses

OPEN ACCESS

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Specialty section:
This article was submitted to Immunological Memory, a section of the journal Frontiers in Immunology

Received: *08 June 2018*
Accepted: *17 July 2018*
Published: *30 July 2018*

Citation:
Behr FM, Chuwonpad A, Stark R and van Gisbergen KPJM (2018) Armed and Ready: Transcriptional Regulation of Tissue-Resident Memory CD8 T Cells. Front. Immunol. 9:1770. doi: 10.3389/fimmu.2018.01770

INTRODUCTION

CD8 T cell responses are an essential component of the adaptive immune system that serves to achieve sterile clearance after infection with intracellular pathogens as well as long-term protection against reinfection. To enable protective CD8 T cell responses against a wide spectrum of microbial threats, an extensive repertoire of naïve CD8 T cells is maintained. The diversity within the T cell repertoire is so large that, despite the millions of naïve CD8 T cells, each T cell specificity is only represented by a population in the order of 100–1,000 cells in mice (1–3). Strikingly, these few precursor cells are able to mount robust T cell responses that eliminate virally infected cells to completion within about 1–2 weeks. The efficiency of CD8 T cell responses depends on the highly effective recruitment of naïve CD8 T cells (4), their rapid proliferation resulting in a more than 1,000-fold expansion in about a week (5), and in the acquisition of effector functions

by the differentiation into effector CD8 T cells (6). Important effector functions of CD8 T cells include the production of the pro-inflammatory cytokine IFN-γ and the cytotoxic mediators perforin and granzyme B. These effector molecules assist in the activation and recruitment of other immune cells as well as in the elimination of infected cells, respectively. After resolution of infection, most of the effector CD8 T cells undergo apoptosis, resulting in contraction of the CD8 T cell response into an about 10-fold reduced population of memory cells (7, 8) that can be maintained for decades in men. Specific memory CD8 T cells are maintained at a higher frequency than naïve CD8 T cells, which enables them to establish secondary CD8 T cell responses with faster kinetics and of larger magnitude. In this manner, memory CD8 T cells can provide up to life-long protection against re-encounter with the same pathogen (6). Memory CD8 T cells do not only have a numerical advantage, they also display superior qualitative characteristics to provide improved protective immunity compared to naïve T cells (9).

Subsets of Memory CD8 T Cells

Distinct subsets of memory CD8 T cells have been recognized that contribute to enhanced recall responses in different ways and at separate sites (10). Central memory CD8 T (T_{CM}) cells express lymph node (LN) homing molecules such as the CC-chemokine receptor 7 (CCR7) and adhesion molecules such as L-selectin (CD62L) that provide access to secondary lymphoid organs. Due to these properties, T_{CM} cells retain the capacity of naïve CD8 T cells to survey the secondary lymphoid organs for cognate antigens. In contrast, effector memory CD8 T (T_{EM}) cells express low levels of CCR7 and CD62L and gain access to the non-lymphoid tissues (11), which enables these memory CD8 T cells to directly patrol the peripheral tissues for immune surveillance. T_{CM} and T_{EM} cells continually recirculate through blood and lymph to survey LN and peripheral tissues, respectively. Recent evidence suggests further heterogeneity within the circulating memory CD8 T cell pool, where expression of the fractalkine receptor CX3CR1 identifies three subsets with distinct migratory properties (12). These include CX3CR1^{low} T_{CM} cells, CX3CR1^{int} peripheral memory T (T_{PM}) cells, which survey peripheral tissues, and CX3CR1^{high} T_{EM} cells, which are largely confined to the vasculature (12). Upon recognition of reinfection, T_{CM}, T_{PM}, and T_{EM} cells mount secondary responses, which involve proliferation and differentiation into secondary effector cells to target the re-invading pathogen.

Next to T_{CM}, T_{PM}, and T_{EM} cells, a fourth subset of memory CD8 T cells, tissue-resident memory CD8 T (T_{RM}) cells, has been identified. In contrast to the circulating memory populations, T_{RM} cells permanently reside within the peripheral tissues after infection without accessing the blood or the lymph (13, 14). The non-recirculating nature of T_{RM} cells has been experimentally demonstrated in different ways. Intravascular antibody injection does not label T_{RM} cells within skin, lungs, and small intestine in contrast to circulating memory CD8 T cells within the bloodstream (15, 16). However, intravascular labeling cannot distinguish circulating memory CD8 T cells transiently passing through the tissues from T_{RM} cells that permanently reside in these tissues. Another exception in this context are liver T_{RM} cells, which reside on the inside of the liver sinusoids in direct contact with the blood (17, 18). Further experiments employing parabiosis, in which the bloodstream of two mice is conjoined, demonstrated that, while circulating memory CD8 T cells rapidly establish equilibrium, T_{RM} cells are permanently retained in peripheral tissues within their host (14, 19–21). The inability of T_{RM} cells to exit donor tissue upon engraftment into recipients has also provided experimental evidence of tissue residency of memory CD8 T cells (13). Quantitative microscopy has shown that T_{RM} cells are more prevalent than circulating memory cells in the non-lymphoid tissues, suggesting that T_{RM} cells form a substantial fraction of the memory repertoire (21). T_{RM} cells do not contribute to systemic immune surveillance, but they establish residence at strategic locations, such as sites, where the primary infection has occurred, positioning them at the frontline of the antimicrobial defense. In this manner, T_{RM} cells are able to mediate border patrol for improved protection against reinfection within the peripheral tissues.

Phenotype of T_{RM} Cells

Tissue-resident memory CD8 T cells can be distinguished from their circulating counterparts through the expression of key cell surface molecules that include CD69 and the α_E integrin, CD103 (**Figure 1**). CD69 is ubiquitously expressed early after activation on T cells, but exclusively T_{RM} cells are able to constitutively maintain CD69 expression under steady state conditions. The majority of T_{RM} cells throughout different tissues express CD69, but parabiosis studies have demonstrated the existence of T_{RM} populations that lack CD69 expression (21, 22). CD69 contributes to the establishment of tissue residency by interfering with spingosine-1 phosphate receptor (S1PR1) function (23, 24). To maintain residency, T_{RM} cells limit expression of tissue exit receptors such as the S1PR1 (25, 26). S1PR1 responds to its ligand S1P that is released by endothelial cells in blood and lymph to attract circulating memory T cells from the tissues into the circulation. In T_{RM} cells, CD69 mediates the internalization and degradation of S1PR1, which results in removal of S1PR1 from the surface and limits the migratory capacities of these memory cells (**Figure 1**). T_{RM} cells do not form upon forced expression of S1PR1, demonstrating the incompatibility of this pathway with establishment of tissue residency in memory CD8 T cells (26). Expression of CD103 appears to be enriched in T_{RM} cells within mucosal compartments, including the skin, lungs, reproductive tract, salivary glands, and small intestine (25, 27–29). A large fraction of CD103$^+$ T_{RM} cells within these tissues locates near or within the epithelium. Epithelial cells express the adhesion molecule E-cadherin, and interaction between CD103 (as part of the αEβ7 integrin) and E-cadherin has been shown to mediate the adhesion between T lymphocytes and epithelial cells (30, 31), suggesting an important role in the retention of T_{RM} cells within epithelial tissues (**Figure 1**). T_{RM} cells are present outside of the epithelia within a wide array of tissues, including the lamina propria of the small intestine, parenchyma of internal organs, such as the brain, kidney, liver, and within the secondary lymphoid organs (32–34). T_{RM} cells within these tissues largely lack expression of CD103 and may employ other adhesion molecules for retention within the tissues. For instance, T_{RM} cells within the

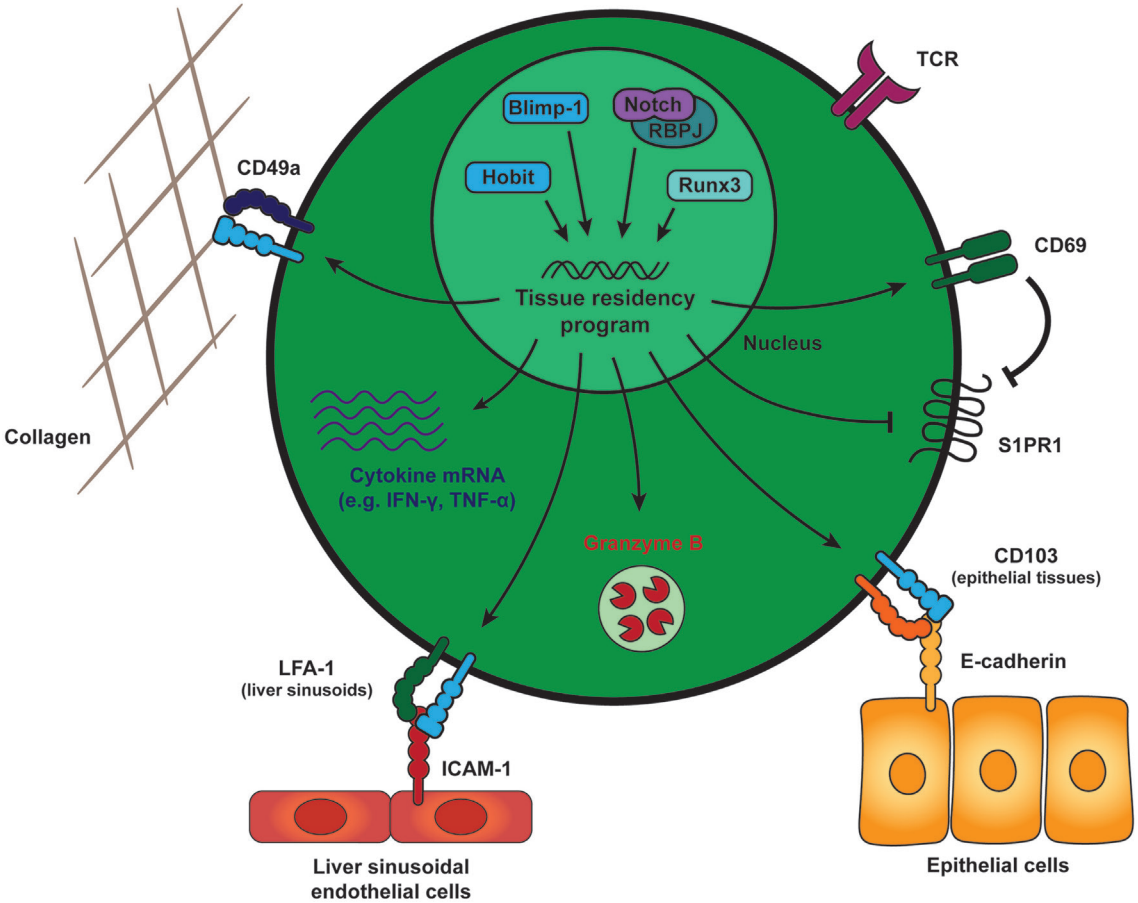

FIGURE 1 | General features of tissue-resident memory CD8 T (T_{RM}) cells. Generation and maintenance of T_{RM} cells is regulated by a distinct set of transcription factors, including Runx3, Blimp-1, and its homolog of Blimp-1 in T cells as well as the transcriptional activator Notch. These transcription factors instruct a tissue-residency program that allows for the long-term retention and maintenance of T_{RM} cells within peripheral tissues. T_{RM} cells across tissues maintain expression of CD69, which promotes tissue residency by interfering with spingosine-1 phosphate receptor (S1PR1) function. S1PR1 mediates egress of T cells into the circulation and its downregulation is a core characteristic of T_{RM} cells. In many tissues, T_{RM} cells also express high levels of CD49a, an adhesion molecule binding to collagen (in complex with β1 integrin) to establish tissue residency. The α_E integrin CD103 is expressed by mucosal T_{RM} cells and may contribute to tissue retention by interaction with E-cadherin on the surrounding epithelial cells. In liver sinusoids, local T_{RM} cells upregulate LFA-1, which supports their tissue residence by binding to ICAM-1 on liver sinusoidal endothelial cells. In addition to these adhesion molecules, T_{RM} cells in many tissues are characterized by elevated transcript levels encoding for pro-inflammatory cytokines, e.g., IFN-γ and TNF-α, and protein expression of the cytotoxic serine protease granzyme B. Abbreviations: Runx3, Runt-related transcription factor 3; Blimp-1, B lymphocyte-induced maturation protein-1; Hobit, homolog of Blimp-1 in T cells; RBPJ, recombining binding protein suppressor of hairless; LFA-1, lymphocyte function-associated antigen-1; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α.

liver express lymphocyte function-associated antigen-1 (LFA-1), which is essential for these cells to mediate interactions with intercellular adhesion molecules on liver sinusoidal endothelial cells (18) (**Figure 1**). Many T_{RM} cells throughout tissues also express high levels of CD49a, which, in complex with β1 integrin, binds collagen within the extracellular matrix to establish tissue residency (35) (**Figure 1**). Therefore, elevated expression of adhesion molecules, such as CD103, LFA-1, and CD49a characterizes populations of T_{RM} cells and distinguishes them from circulating memory CD8 T cells.

The identification of human T_{RM} cells largely relies on phenotypic markers, due to difficulties in experimentally addressing the migratory behavior of human memory T cells *in vivo*. Considerable numbers of T_{RM}-type memory CD8 T cells co-expressing CD69 and CD103 have been found within human tissues, including skin, lung, liver, and intestines (33, 36–38), suggesting that humans also contain a resident compartment of memory CD8 T cells. These human T_{RM} cells share characteristics with their murine counterparts (33, 39, 40), as determined by transcriptional and phenotypic profiling. Similar to the transcriptional profile of murine T_{RM} cells, the core signature of human T_{RM} cells includes upregulated genes associated with the establishment of tissue residency such as CD49a and downregulated genes associated with tissue egress, e.g., S1PR1 and CCR7 (40).

Tissue-resident memory CD8 T cells are essential and sufficient to establish immediate protection against reinfection with pathogens (20, 41, 42). The remarkable effectiveness of T_{RM} cells to achieve clearance of infection and their potential protective

capacities in anti-tumor responses have spurred investigation into the regulatory mechanisms underlying the differentiation, maintenance, and effector functions of these memory CD8 T cells. Transcription factors play important roles in the regulation of memory T cells through their ability to modulate gene expression. Recently, we have identified homolog of Blimp-1 in T cells (Hobit) as a T_{RM}-specific transcription factor that together with related Blimp-1 essentially contributes to the differentiation and/or maintenance of T_{RM} cells (43). Besides Hobit and Blimp-1, other factors, including Runx3, Notch, aryl hydrocarbon receptor (Ahr), and NR4A1 are involved in the regulation of T_{RM} cells (**Figure 1**), suggesting that these cells are under the control of a network of transcription factors (37, 44–46). In this review, we will focus on the role of transcription factors during the different stages of T_{RM} differentiation and during the reactivation of T_{RM} cells upon pathogen re-challenge.

FROM NAÏVE TO MEMORY CELL—DIFFERENTIATION OF T_{RM} CELLS

The development of naïve CD8 T cells into effector T cells and subsequently into T_{RM} cells involves priming in the LN, migration from the LN to the peripheral tissues and the acquisition of a T_{RM} phenotype to establish local retention. Here, we will discuss the cell intrinsic signals and tissue-derived cues that instruct the generation and maintenance of T_{RM} cells.

Heterogeneity in Effector CD8 T Cells—T_{RM} Precursors

The "one cell, multiple fates" hypothesis describes the potential of a single naive CD8 T cell to generate diverse subsets of effector and memory CD8 T cells (47, 48). Studies using genetic barcoding and adoptive transfers of single naïve T cells have demonstrated that T_{CM} and T_{EM} cells can differentiate from the same naïve CD8 T cell. However, it was not addressed whether T_{RM} cells originate from the same naïve T cells as T_{CM} and T_{EM} cells. More recent studies using deep sequencing of the T cell receptor (TCR) β repertoire have revealed substantial overlap in TCR usage between T_{CM} and T_{RM} populations in a skin immunization model (49), suggesting that T_{CM} and T_{RM} cells may develop from a common progenitor. However, given that the naive CD8 T cell population may contain multiple clones bearing identical TCRs, the development of T_{CM} and T_{RM} cells from different precursors cannot be completely excluded.

After recognition of cognate antigen, naïve CD8 T cells first differentiate into effector CD8 T cells. Effector cells diversify into different subsets that include terminal effector cells (TECs) and memory precursor effector cells (MPECs). TECs are characterized by surface expression of killer cell lectin-like receptor G1 (KLRG1) (50). In contrast, memory precursors express very low amounts of KLRG1, but maintain expression of IL-7Rα (CD127) (51). The IL-7Rα^{hi} MPECs differentiate into long-lived memory CD8 T cell populations, whereas the majority of TECs undergoes apoptosis after clearance of the infection. While these studies showed that circulating memory cells develop from MPECs, it was not addressed whether this is the case for T_{RM} cells. Similar to the spleen, peripheral organs such as the skin and small intestine contain KLRG1$^+$ and KLRG1$^-$ fractions within the virus-specific effector CD8 T cell population after infection (25, 29). The cells that remain within the skin and small intestine at the memory stage lack expression of KLRG1, suggesting that tissue-residing T_{RM} cells develop from MPECs. Indeed, adoptive transfer of the KLRG1$^+$ and KLRG1$^-$ fractions confirmed that T_{RM} cells preferentially arise from KLRG1$^-$ MPECs (25). A regulatory role has been reported for transforming growth factor (TGF) β in controlling TEC cell numbers under acute inflammatory conditions (52). Therefore, local TGF-β signaling may drive the preferential development of MPECs in the small intestine, by selectively inducing apoptosis of the TEC fraction during clonal expansion. Recently, *Klrg1* lineage reporter mice have been developed to track the memory offspring of KLRG1$^+$ cells after *Listeria* infection. Fate mapping using the KLRG1 reporter mice showed that approximately half of the T_{RM} cells in the liver and small intestine originate from KLRG1$^+$ precursors (53). These findings suggest that the T_{RM} precursor population may contain MPECs that transiently expressed KLRG1 besides MPECs that never expressed KLRG1.

While T_{CM}, T_{EM}, and T_{RM} cells all appear to develop from MPECs, the timing of branching into the different memory subsets remains unclear. Single cell sequencing data of effector CD8 T cells after the first cell division have revealed only two separate populations that correspond to TECs and MPECs (54), suggesting that at this early stage MPECs form a uniform population. It is conceivable that heterogeneity within MPECs arises at later stages. Adoptive transfer experiments have shown that as early as 7 days after viral infection, effector cells within the spleen have lost the potential to contribute to T_{RM} formation in the intestinal epithelium, while these cells retain the potential to form circulating memory cells (14). These experiments suggest separation between the T_{CM}, T_{EM}, and T_{RM} lineages at the peak of the effector response. Consistent with this time frame of T_{RM} commitment, kinetic analysis of the upregulation of T_{RM}-associated molecules, e.g., CD69 and CD103, during CD8 T cell responses demonstrated that pathogen-specific CD8 T cells within the small intestine and skin acquire a T_{RM} phenotype between 1 and 2 weeks after infection (25, 29, 44, 55). In fact, transcriptional profiling of effector CD8 T cells in the small intestine after lymphocytic choriomeningitis virus (LCMV) infection has shown that the T_{RM}-associated program is largely established within 1 week (44).

Signals Driving T_{RM} Differentiation

Sensing of inflammation and tissue damage during priming of T cells provide important cofactors for the generation of T_{RM} cells. Activated CD8 T cells home to inflamed tissues and can subsequently form T_{RM} cells at these locations, even when antigen is not present locally (41). *In vitro* experiments suggest that inflammatory stimuli may also induce T_{RM} differentiation in the peripheral tissues. Inflammatory cytokines, including type I IFN, IL-33, and tumor necrosis factor-α (TNF-α), downregulate expression of the transcription factor Krüppel-like factor 2 (KLF2) and the tissue exit receptor S1PR1 and upregulate expression of CD69 on CD8 T cells (26, 56).

In vivo evidence supports such a role for pro-inflammatory cytokines including type I IFN and IL-12 in T_{RM} differentiation (57). Local inflammatory cues might contribute differently to the generation and persistence of mucosal and non-mucosal T_{RM} cells. Inflammatory cytokines such as IFN-β and IL-12 counter-regulate the induction of CD103 by TGF-β during CD8 T cell priming and support the formation and persistence of $CD103^{-}$ $CD69^{+}$ T_{RM} cells in the small intestine (58). Binding of pSTAT4, which can be induced by IL-12 or type I IFN, to the CD103 encoding gene suggests that sensing of inflammation might directly affect CD103 expression (58).

These inflammatory signals might guide T_{RM} generation at different stages of CD8 T cell differentiation, with initial cues for commitment to the T_{RM} lineage already being provided in the lymph node. A specialized population of lymph node residing and crosspresenting $CD8\alpha^{+}$ DCs can provide signals, including IL-12, IL-15, and co-stimulation *via* CD24, which contribute to optimal generation of T_{RM} cells (59). Circulating memory CD8 T cells do not share this requirement for $CD8\alpha^{+}$ DCs in the early stages, suggesting that these DCs specifically drive the formation of T_{RM} cells. Following these early events during priming, effector T cells are recruited to the infected tissue. The inflammatory chemokine receptors CXCR3 and CCR5 have been shown to contribute to the recruitment of T_{RM} precursors. CXCR3 enables T_{RM} precursor cells to respond to the IFN-γ inducible chemokines CXCL9 and CXCL10, which is critical for differentiation of T_{RM} cells in the skin (25). CCR5 ligands provided by pro-inflammatory macrophages are important to instruct recruitment of T_{RM} precursors into the vaginal mucosa (60). These pro-inflammatory signals can be provided by a local network of macrophages (57, 60, 61). Thus, it appears that inflammatory stimuli within the LN and from the local environment contribute to T_{RM} differentiation.

The presence of local antigen is not required to attract activated CD8 T cells into the inflamed tissue (41, 62). In the skin, these activated CD8 T cells can subsequently develop into T_{RM} cells in the absence of local antigen (41). However, T_{RM} cell formation after local skin infection is greatly enhanced in the presence of cognate antigen in the tissue microenvironment (63–65). In other tissues, such as the lung and central nervous system, establishment of T_{RM} cells requires cognate antigen recognition in the tissue (28, 62). In the salivary glands, T_{RM} cell formation depends on antigen in the CD4 T cell compartment, but not in the CD8 T cell compartment (66). The presence of local antigen may, therefore, not impact the size of the effector response in the tissue, but rather promote local retention and the formation of T_{RM} cells. The role of antigen after establishment of T_{RM} cells is less clear, but the long-term maintenance of the T_{RM} cell pool in the lung and small intestine appears to be independent of local antigen (56, 67). Next to antigen, costimulatory signals might contribute to the differentiation of T_{RM} cells. Recent work has demonstrated the requirement of intrinsic signals *via* the tumor necrosis factor (TNF) receptor family member 4-1BB for the generation of influenza-specific CD8 T cells in the lung, in contrast to secondary lymphoid tissues (68).

Next to inflammation and local antigen, the accompanying tissue damage might also contribute to T_{RM} generation. Immunization *via* skin scarification generates highly protective T_{RM} cells, compared to subcutaneous or intradermal injection (69) and lung-resident T cells localize at spots that show signs of recovery from previous tissue damage (70). The factors contributing to these effects are still unknown. Inflammation accompanying tissue damage could be partly responsible for the accumulation of T_{RM} cells at sites of tissue damage. Additionally, competition for survival factors during the reorganization of the tissue after injury might influence T_{RM} persistence (71). Data on the local composition of skin-resident T cells support this view. Pre-existing tissue-resident dendritic epidermal γδ T cells are depleted at sites of infection and are replaced by virus-specific CD8αβ T cells (72). To cope with the inflection-related changes in their microenvironment, T_{RM} cells might have developed tissue-specific adaptations. For example, lung T_{RM} cells constitutively express the interferon-induced transmembrane protein 3 (IFITM3), which facilitates their survival during secondary challenges with influenza (73).

Maintenance of T_{RM} Cells

Tissue-resident memory CD8 T cells can persist in tissues for long periods of time (13, 20, 57). Their location at distinct sites throughout the body suggests different requirements for their maintenance and specific adaptions to the local environments. The local presence of antigen, cytokines, chemokines, and tissue-specific metabolites are factors that contribute to T_{RM} maintenance.

Similar to recently and chronically activated T cells, T_{RM} cells demonstrate increased expression of activation-associated molecules, such as PD-1 and importantly CD69 (40, 43). However, persistent stimulation by antigen is not required for T_{RM} maintenance. In fact, the development of T_{RM} cells in the intestine is compromised after chronic viral infection compared to acute viral infection (56). In addition, T_{RM} cells can be formed and maintained by recruiting activated T cells into tissues *via* sterile inflammation (41), suggesting that T_{RM} cell persistence does not require local antigen in the peripheral tissues after infection.

Similar to circulating memory cells, T_{RM} cells upregulate receptors for IL-7 and IL-15 (39, 74), suggesting that these homeostatic cytokines contribute to antigen-independent maintenance of T_{RM} cells. Indeed, IL-7 and IL-15 produced within hair follicles maintain T_{RM} cells near these structures within the skin (75). IL-15 already plays a role during lodgment of T_{RM} cells, but the continued presence of IL-15 is essential for long-term T_{RM} maintenance within the skin (74). IL-15 may not be crucial for T_{RM} cells at other sites, as virus-specific T_{RM} cells within the intestines, pancreas, and female reproductive tract (FRT) are maintained independently of IL-15, in contrast to those in the salivary glands and kidneys (76). The involvement of other homeostatic cytokines in the maintenance of these IL-15-independent T_{RM} populations is currently unclear. T_{RM} cells require TGF-β for maintenance in the mucosa (25, 56, 77). TGF-β instructs the upregulation of CD103 that allows retention of T_{RM} cells in the epithelium, potentially through interactions with E-cadherin on epithelial cells (25, 56, 77). TGF-β is produced as part of an inactive complex together with latency associated protein

(LAP). Integrins such as $\alpha_V\beta6$ and $\alpha_V\beta8$, which are expressed on keratinocytes, are required to release LAP and activate TGF-β in the epithelium (78). These integrins may restrict the action radius of TGF-β close to the epithelial layer. T_{RM} populations underneath the epithelium such as those within the lamina propria of the intestine are independent of TGF-β and largely do not express CD103 (57). T_{RM} populations within internal organs such as the liver and the kidney also largely lack CD103 expression (17, 43), suggesting TGF-β-independent maintenance. Thus, with notable exceptions, T_{RM} populations are maintained on homeostatic cytokines similar to other memory cells and epithelial T_{RM} cells uniquely require TGF-β.

After development, T_{RM} cells form stable populations in many tissues, including skin, liver, and the small intestine, and provide long-term protection against reinfection (13, 17, 20, 41, 57). Maintenance of T_{RM} cells in these tissues appears to be independent of the recruitment of circulating cells, as adoptive transfer experiments have shown that circulating memory CD8 T cells do not convert into T_{RM} cells under steady state conditions (14). In contrast, influenza-specific T_{RM} cells in the murine lungs fail to survive long-term (67, 79). These T_{RM} cells appear to be continuously replenished *via* recruitment from the circulating memory CD8 T cell pool (67).

Tissue-resident memory CD8 T cells are present throughout the body at distinct sites in highly diverse environments that differ in oxygen and nutrient levels, exposure to microbiota, and the regenerative ability of the tissue. Given that T_{RM} cells are permanently residing within the peripheral tissues, they are strictly dependent on the resources within the local environment in contrast to circulating memory cells. Therefore, T_{RM} cells may require tissue-specific adaptations to cope with different conditions posed by the local microenvironment. Transcriptional profiling has revealed a T_{RM}-specific core signature shared between T_{RM} cells at different locations, including the lungs, liver, intestine, and skin (25, 43). In addition to this core signature, T_{RM} cells at different sites are characterized by tissue-specific gene expression profiles (25, 43). The distinct gene programs of T_{RM} cells include chemokine receptors and adhesion molecules that are required to address T_{RM} cells to different tissues. The chemokine receptors CCR8 and CCR10 and the adhesion molecule cutaneous lymphocyte antigen (CLA) are specifically upregulated on skin T_{RM}. CCR10 and CLA have also been functionally implicated in the localization of T_{RM} in the skin (25, 80). In contrast, CCR9 is specifically expressed on intestine-derived T_{RM} cells and may, together with the α4β7 integrin, drive localization of T_{RM} cells in the small intestine (14). Skin-resident T_{RM} cells have been described to rely on the uptake of exogenous fatty acids *via* the fatty acid binding protein (FABP) 4 and FABP5 in contrast to circulating memory CD8 T cells (81). The metabolic requirements of T_{RM} cells at other locations are not yet clear. Members of the FABP family are expressed in a tissue-specific manner (82), suggesting that populations within brain, liver, and intestine may take advantage of local opportunities to meet metabolic demands. Thus, the heterogeneity within T_{RM} populations at different locations may reflect strategies to optimally adapt to the local circumstances.

EFFECTOR RESPONSES OF T_{RM} CELLS UPON REACTIVATION

Numerous studies have highlighted the essential role of T_{RM} cells in providing efficient protection against local reinfections at barrier sites (20, 41, 42). Being situated at the front lines of the immune defense, T_{RM} cells are poised for early detection of recurring pathogens. Here, we will discuss the mechanisms by which T_{RM} cells protect against local infections and the fate of T_{RM} cells after antigen re-encounter.

Border Patrol

Despite their inability to recirculate throughout the body, T_{RM} cells retain the ability to migrate within their local environment. This has been most extensively studied for T_{RM} cells in the skin. These T_{RM} cells localize to the basal layer of the epidermis, where they migrate in the two-dimensional plane of the tissue. Skin T_{RM} cells display a dynamic morphology and continually project dendritic extensions in multiple directions (72, 83, 84) (**Figure 2**). In contrast, T cells in the underlying dermis exhibit an amoeboid shape, which resembles that of migrating lymphocytes in the secondary lymphoid organs. The migration of T_{RM} cells within the epidermis appears to be constrained by the local environment upon resolution of inflammation (72). These constraints only permit relatively slow migration of skin T_{RM} cells, thus promoting their long-term persistence at sites of prior infection (72), and enhancing their ability to scan the local environment for recurring pathogens. This local border patrol requires a density of T_{RM} cells of approximately 100 or more cells per mm^2 for complete coverage of the local area and to ensure early detection of cognate antigens (84).

Patrol of the local tissue environment by T_{RM} cells has also been demonstrated in other organs, e.g., in the FRT and in the liver (17, 18, 85). T_{RM} cells in these tissues show a higher motility compared to the epidermis, which may be related to the more relaxed constraints posed by the tissue architecture. In fact, the speed of T_{RM} cell migration in the FRT is dependent on the local collagen density (85). Local encounter of T_{RM} cells with their cognate antigen in the skin and FRT results in motility arrest and loss of their dendritic morphology (85, 86) (**Figure 2**). The immobilization is transient and T_{RM} cells resume their migratory behavior within 48 h after antigen re-encounter. Motility arrest upon antigen encounter is important for T cell activation. The transient stop allows for the formation of an immunological synapse between T cells and antigen-presenting cells, and enables T cells to acquire of signals for activation (87). Given that most non-lymphoid tissues are primarily surveyed by T_{RM} cells (21), border patrol by these memory cells likely plays an essential role for the local protection throughout the body. This property as motile sentinels places T_{RM} cells in the front lines of defense, enabling rapid responses to reinfection.

Early Effector Response of T_{RM} Cells Upon Reactivation

Tissue-resident memory CD8 T cells are among the first immune cells to act in response to pathogens that have been

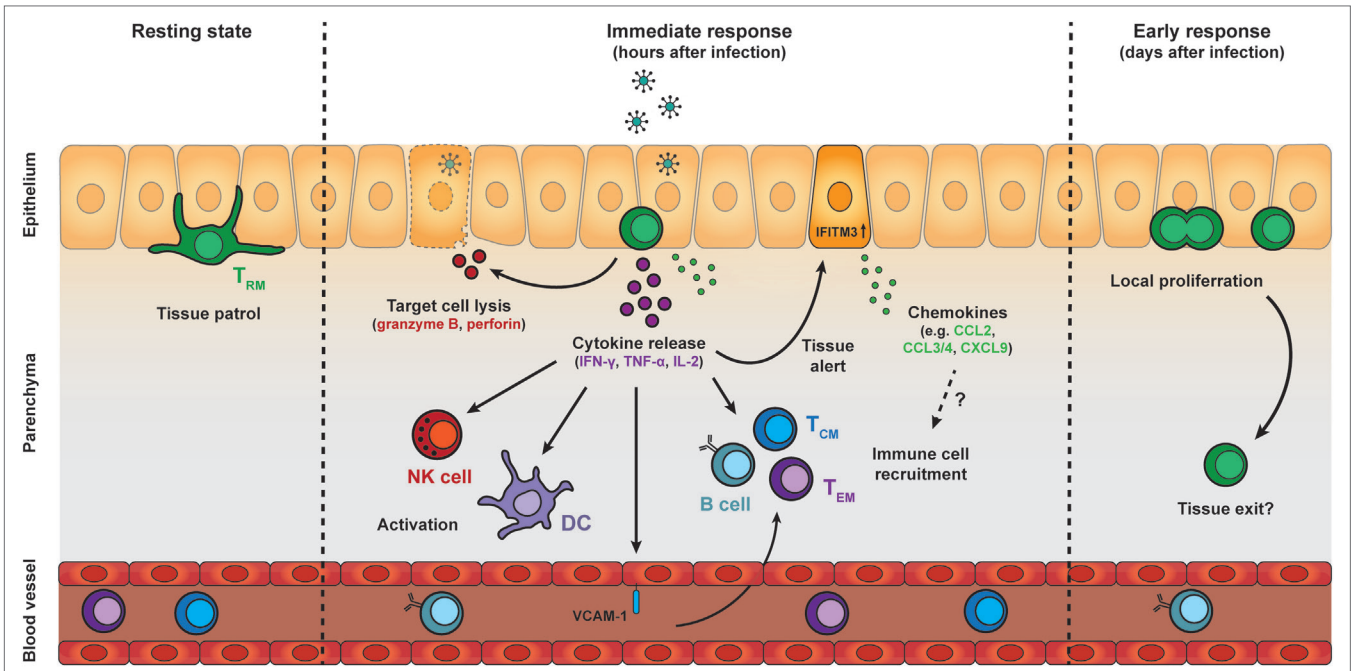

FIGURE 2 | Protective effector responses of epithelial T_{RM} cells upon secondary infection. T_{RM} cells in the epithelia continually patrol their local environment, projecting dendritic extensions in multiple directions. Upon pathogen challenge and antigen re-encounter, T_{RM} cells rapidly release pro-inflammatory cytokines, including IFN-γ, TNF-α, and IL-2, which induce several immune cell- and tissue-specific effects. Local cytokine release by T_{RM} cells results in recruitment and activation of natural killer (NK) cells and dendritic cells (DCs), as well as upregulation of VCAM-1 on endothelial cells in local blood vessels, which may enhance the recruitment of T_{CM}, T_{EM}, and B cells from the circulation. T_{RM} cell reactivation and cytokine release also induces a tissue-wide state of alert, resulting in upregulation of many innate immune response genes, including interferon-induced transmembrane protein 3 (IFITM3), and the increased local expression of inflammatory chemokines. The protective capacity of T_{RM} cells may also rely on perforin-mediated killing of target cells. One to two days after antigen re-encounter, T_{RM} cells undergo local proliferation. Further investigation is required to determine whether T_{RM} cells exit their local environment after reactivation. Abbreviations: IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; IL-2, interleukin 2; ICAM-1, vascular cell adhesion molecule 1; T_{CM} cell, central memory T cell; T_{EM} cell, effector memory T cell; CCL, C-C motif chemokine; CXCL9, C-X-C motif chemokine 9.

previously encountered in an antigen-specific manner. Upon activation, T_{RM} cells rapidly respond by the production of pro-inflammatory cytokines, including IFN-γ (**Figure 2**). In both mice and men, T_{RM} cells across different tissues express high transcript levels of these pro-inflammatory cytokines compared to their circulating counterparts (37, 40, 43, 88). These elevated transcript levels may endow T_{RM} cells with the potential to rapidly produce cytokines upon activation. In addition, post-transcriptional mechanisms have been shown to control cytokine production in CD8 T cells (89, 90), and may contribute to the fast responsiveness of T_{RM} cells. IFN-γ has direct antiviral properties, but is also important for the recruitment and activation of immune cells. The early release of IFN-γ by T_{RM} cells has been demonstrated to stimulate immune cells including DCs and NK cells (91). T_{RM}-derived IFN-γ also elevates expression of the homing molecule vascular cell adhesion molecule 1 on endothelial cells, and enhances the recruitment of circulating B cells and memory T cells from the bloodstream (60, 91, 92) (**Figure 2**). Furthermore, antigen recognition by T_{RM} cells potentiates the local expression of inflammatory chemokines in the tissue, including CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 (60, 91). High transcript levels of CCL3, CCL4, and XCL1 in quiescent T_{RM} cells suggest that T_{RM} cells participate themselves in the production of these chemokines (43, 88). T_{RM}-derived IFN-γ may also contribute to the release of IFN-γ-dependent chemokines, such as CXCL9 and CXCL10, from the surrounding tissue. These chemokines may trigger the attraction of innate myeloid cells, e.g., neutrophils and monocytes, to the site of infection, thereby further enhancing the immune response (93, 94). In addition, IFN-γ release by reactivated T_{RM} cells has been shown to induce a tissue-wide state of alert in the skin, resulting in elevated expression of many innate immune response genes, including IFITM3, in the tissue (95) (**Figure 2**). Under certain conditions, T_{RM} cells may even induce a body-wide state of alert to prevent viral spread (96). Interestingly, while the local activation of T_{RM} cells is pathogen-specific, the triggering of downstream immune responses can ultimately lead to near-sterile protection of the tissue against antigenically unrelated pathogens (92, 95). The importance of cytokine production by T_{RM} cells for tissue protection has also been demonstrated in the lung, where airway T_{RM} cells protect against respiratory influenza virus through production of IFN-γ (42). Similarly, IFN-γ production by brain T_{RM} cells is crucial for protection against intracerebral infections (97). Tissue-specific adaptations may exist in the secreted factors of T_{RM} cells at different locations (96). For example, lung-resident T_{RM} cells release IL-22 next to IFN-γ, while T_{RM} cells in the liver co-produce granulocyte-macrophage colony-stimulating factor and IFN-γ (96). These

differences in local cytokine repertoires may allow T_{RM} cells to tailor responses to their local microenvironment.

Protection against intracellular pathogens by effector CD8 T cells is partly mediated by the removal of infected cells through the targeted release of cytotoxic molecules, including perforin and granzyme B. After clearance of infection, the expression of cytotoxic molecules is strongly downregulated in circulating memory CD8 T cells. In contrast, T_{RM} cells in several tissues maintain high levels of granzyme B in the memory phase (17, 56, 97) (**Figure 1**). The constitutive expression of granzyme B suggests that T_{RM} cells can rapidly employ cytotoxic mechanisms to eliminate infected cells early after pathogen re-encounter. Indeed, T_{RM} cells in the brain can kill target cells and their protective capacity is dependent on perforin (28, 97). Granzyme B has furthermore been implicated in the remodeling of extracellular matrices (98, 99), suggesting that the serine protease may also contribute to the local migration of T_{RM} cells within tissues. Granzyme B-driven cytotoxicity may not be essential for T_{RM}-mediated protection at other sites, given that, for example, airway T_{RM} cells do not maintain expression of granzyme B and other cytotoxic mediators (42). The selective killing of infected cells by T_{RM} cells minimizes off-target immunopathology, but this protective mechanism may be overwhelmed by rapidly replicating pathogens. Under these conditions, the potential of T_{RM} cells to amplify immune responses through the release of pro-inflammatory cytokines and chemokines may be essential and offset the increased risk for collateral damage.

Proliferation and Maintenance of the Local T_{RM} Repertoire

The protective capacity of memory CD8 T cells depends on their robust proliferation upon recall to establish an army of secondary effector cells. The large number of effector cells can be crucial to counter rapidly replicating and spreading pathogens. In particular, T_{CM} cells have a robust proliferative capacity (100–102). These memory cells patrol secondary lymphoid organs and are, therefore, ideally positioned at these distal sites to the infection to mount secondary responses. T_{EM} cells, which survey peripheral tissues and have limited access to the LN, undergo less pronounced proliferation upon re-challenge (100–102). Using intravital imaging, it has been demonstrated that T_{RM} cells in the skin and FRT undergo local proliferation *in situ* within the first days after antigen re-encounter (85, 86). Potential changes in phenotypic markers on reactivated T_{RM} cells and timespan limitations for intravital imaging pose challenges for long-term follow-up of secondary T_{RM} responses. Despite these technical difficulties, it appears that pre-existing T_{RM} cells within peripheral tissues are the main origin of local proliferative recall responses (**Figure 2**). In line with this, the secondary T_{RM} population arising after pathogen clearance primarily develops from pre-existing T_{RM} cells (85, 86). Recruited circulating memory CD8 T cells also contribute to secondary effector responses (68) and the formation of secondary T_{RM} cells, albeit to a lesser extent (85, 86). However, these memory cells appear to have a limited potential to form T_{RM} cells, at least compared to naïve CD8 T cells (103). The importance of the recruitment of circulating memory cells into the secondary T_{RM} pool may reside in the introduction of new specificities to the local repertoire. Despite local proliferation, reinfection does not numerically increase the pool of local T_{RM} cells (86), suggesting that limits exist in the number of T_{RM} cells that can populate the peripheral tissues. If that is indeed the case, then secondary T_{RM} cells may compete for available niches, which may re-shape the local repertoire after reinfection (71). Previously, it has been demonstrated that circulating memory T cells undergo qualitative changes after successive infections (104, 105). In this context, it will be interesting to investigate the quality, function, and longevity of these secondary T_{RM} cells compared to primary T_{RM} cells.

Tissue Exit and Contribution to Systemic Responses

While local reinfection results in the recruitment of circulating memory T cells to the tissue, locally proliferating T_{RM} cells may in turn downregulate their tissue residency program and egress from the peripheral tissues. Secondary lymphoid organs, including lymph nodes (LN) that drain tissues, are mainly populated by circulating naïve and memory T cells, but also harbor T_{RM} cells (34). Recent work has shown that the T_{RM} cell population in the draining LN increases after a secondary challenge in the skin or the FRT and that these secondary T_{RM} cells are derived from reactivated T_{RM} cells in the non-lymphoid tissue (22). This demonstrates that, upon antigen exposure, T_{RM} cells possess the ability to leave their local environment and enter other tissues, where they can form secondary T_{RM} cells. It remains to be determined whether T_{RM} cells can also disseminate beyond the local draining LN and form secondary memory cells in anatomically distinct tissues (**Figure 2**). Consistent with a contribution of T_{RM} cells to systemic secondary responses, adoptively transferred intestinal T_{RM} cells can acquire properties of circulating memory CD8 T cells upon re-stimulation (55). Further work is required to address whether *in situ* reactivated T_{RM} cells also differentiate into circulating effector and memory cells during secondary responses. After tissue exit, reactivated T_{RM} cells may return to their tissue of origin. Previous work has demonstrated that re-stimulated memory CD8 T cells have a homing bias to their tissue of origin (27, 106), suggesting that reactivated T_{RM} cells may retain an imprint that permits re-entry into their former tissue of residence.

TRANSCRIPTIONAL CONTROL OF T_{RM} DIFFERENTIATION AND FUNCTION

The transition of naïve CD8 T cells into effector and memory cells is a tightly coordinated differentiation process under the control of transcription factors. Upon activation, naïve CD8 T cells upregulate a transcriptional program that drives their differentiation into effector CD8 T cells, thus enabling the establishment of immune responses against pathogens. After clearance of infection, T_{CM} and T_{EM} cells downregulate the effector program and partially re-acquire transcriptional regulators of naïve CD8 T cells to assist in the long-term maintenance of these memory

CD8 T cells. In contrast to circulating memory T cells, T_{RM} cells retain immediate potential to exert effector functions and do not re-establish body-wide immune-surveillance. Therefore, growing evidence suggests that T_{RM} cells require a specific program of transcriptional regulation. Here, we summarize data on the role of T_{RM} cell-specific transcription factors as well as on how transcription factors with a crucial role for effector CD8 T cell differentiation regulate T_{RM} cell generation and maintenance. Finally, we will discuss the transcriptional regulation of T_{RM} effector function and T_{RM} differentiation upon activation in secondary responses.

Transcription Factors Regulating Tissue Residency

Gene expression analysis of circulating memory CD8 T cells and T_{RM} cells has revealed transcription factors with T_{RM}-restricted expression profiles (**Figure 3**). One of these T_{RM}-specific transcription factors is Hobit. Hobit is upregulated in murine T_{RM} cells within skin, lungs, liver, kidney, small intestine, and brain, suggesting that Hobit is widely expressed throughout T_{RM} populations (25, 43, 88). These Hobit$^+$ T_{RM} populations include CD103$^+$ T_{RM} cells within epithelial tissues and CD103$^-$ T_{RM} cells within internal organs, underlining that the transcription

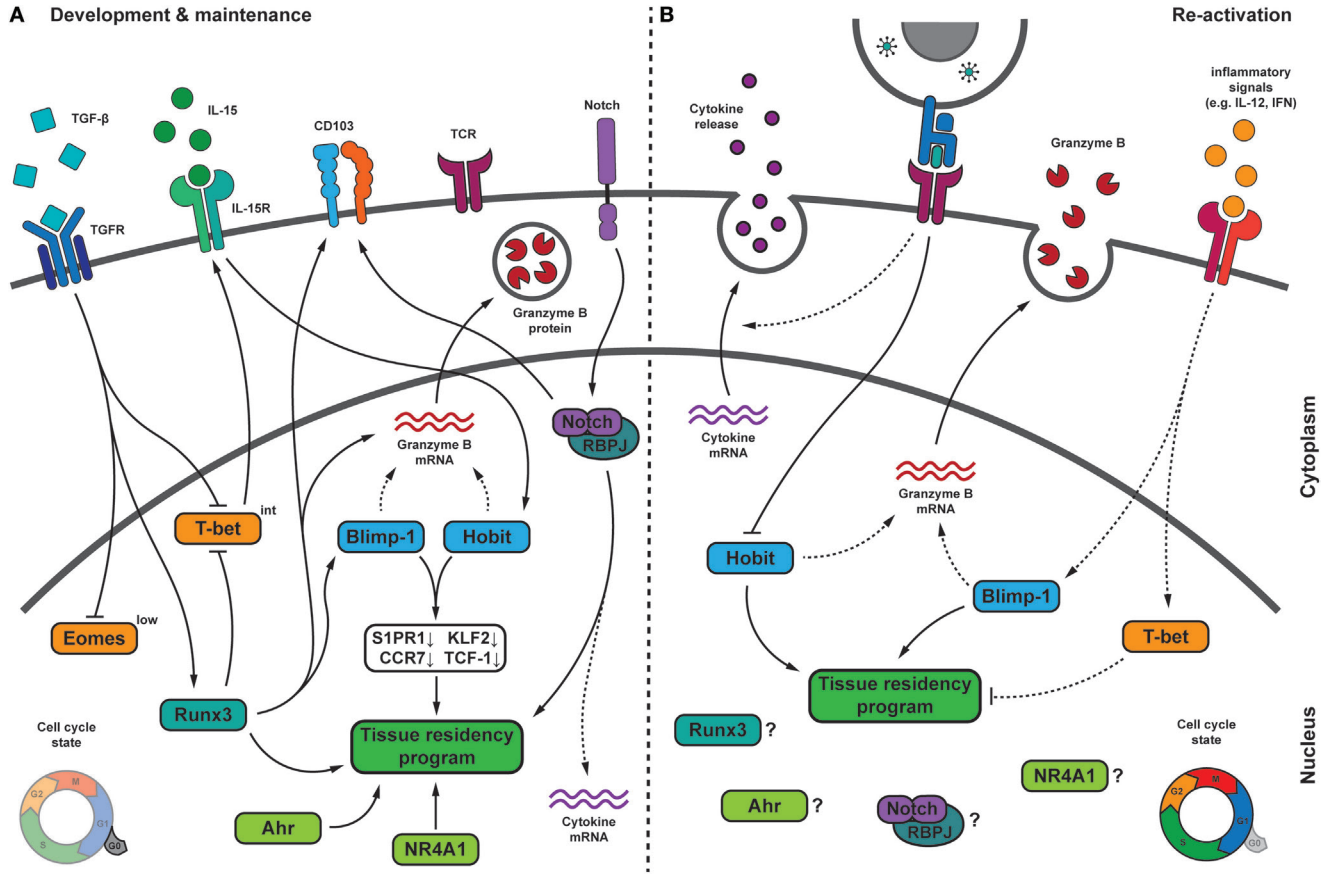

FIGURE 3 | Transcriptional regulation of T_{RM} cells during development, maintenance, and upon pathogen re-challenge. **(A)** During their formation, T_{RM} cells receive multiple signals from the tissue microenvironment that integrate into a transcriptional program, which drives T_{RM} differentiation and maintenance. In several tissues, transforming growth factor β (TGF-β) signaling drives the downregulation of the T-box transcription factors Eomesodermin (Eomes) and T-bet. Residual T-bet expression is required for IL-15Rβ expression. The transcription factor homolog of Blimp-1 in T cells (Hobit) can be induced in an IL-15-dependent manner and, together with its homolog Blimp-1, represses the expression of S1PR1, CCR7, Krüpple-like factor 2 (KLF2), and TCF-1, which is crucial for tissue residency. Blimp-1 and Hobit may also contribute to granzyme B maintenance in T_{RM} cells. The transcription factor Runx3, which can be induced by TGF-β signaling, is crucial for the establishment and maintenance of many aspects of T_{RM} cells, including granzyme B and CD103 expression. Runx3 has been shown to induce Blimp-1. Notch may regulate expression of the adhesion molecule CD103, is essential for maintenance of T_{RM} cells and might contribute to the elevated transcript levels encoding for pro-inflammatory cytokines in T_{RM} cells. Other factors regulating T_{RM} cells include the nuclear receptor NR4A1, and the aryl hydrocarbon receptor (AhR). During quiescence, T_{RM} cells show low proliferative activity. **(B)** Following pathogen re-encounter, T_{RM} cells are exposed to antigen-dependent T cell receptor (TCR) triggering and a variety of inflammatory signals. TCR triggering in T_{RM} cells may result in downregulation of Hobit, thereby weakening its contribution to maintenance of tissue residency. Inflammatory signals, such as IL-12 and type I interferons (IFN), can induce expression of Blimp-1 and T-bet. While increased Blimp-1 expression might fortify T_{RM} features, elevated levels of T-bet could interfere with tissue residency. T_{RM} cells rapidly release pro-inflammatory cytokines upon reactivation in an antigen-dependent manner. Upon re-infection, T_{RM} cells undergo local proliferation. Dashed lines indicate relations that require further investigation. Abbreviations: Blimp-1, homolog of B lymphocyte-induced maturation protein 1; IL, interleukin; S1PR1, sphingosine-1-phosphate receptor 1; CCR7, C-C chemokine receptor type 7; TCF-1, T cell factor 1; Runx3, Runt-related transcription factor 3; RBPJ, recombining binding protein suppressor of hairless; NR4A1, nuclear receptor subfamily 4 group A 1.

factor is ubiquitously expressed in murine T_{RM} subsets. In addition, other tissue-resident lymphocytes such as natural killer T (NKT) cells and innate lymphoid cells 1 express Hobit, suggesting that Hobit is a central regulator of the tissue-residency program of lymphocytes (43). Due to limitations in access to peripheral tissues, analyses of Hobit expression in human T_{RM} cells have not been as extensive as in mice. In line with findings in mice, a substantial proportion of $CD69^+$ CD8 T cells within the human liver expresses Hobit at the protein level (38, 107). Transcriptional profiling also revealed that $CD69^+$ CD8 T cells in human lungs express Hobit in contrast to their $CD69^-$ counterparts, although expression levels are low compared to murine T_{RM} cells (40). We have previously described that $CD45RA^+$ $CD27^-$ effector and $CD45RA^-$ $CD27^-$ effector memory CD8 T cells in human peripheral blood also express Hobit (108). Therefore, despite the presence of Hobit in subpopulations of human T_{RM} cells, no strict association of Hobit with tissue residency exists in human CD8 T cells.

In mice, Hobit specifically instructs the differentiation and/or maintenance of T_{RM} cells, but the transcription factor does not operate alone. Hobit is highly homologous to Blimp-1 and both factors co-operate in the transcriptional regulation of T_{RM} cells. Hobit and Blimp-1 both recognize a "GAAAG" containing binding motif and share the majority of their DNA-binding sites, suggesting that the related factors collaborate through competitive regulation at overlapping target genes. Hobit and Blimp-1 lock T_{RM} cells into the tissues, as these transcription factors instruct shutdown of exit pathways through CCR7 and S1PR1, thus preventing T_{RM} cells from re-entering the circulation (43). In circulating memory cells, the transcription factor KLF2 drives the expression of S1PR1 to provide access to the blood or lymph (109–111). Downregulation of S1PR1 and KLF2 is essential for T_{RM} differentiation, as evidenced by forced expression of S1PR1 that completely prevents the generation of T_{RM} cells (26). The Wnt signaling associated transcription factor TCF1 is involved in maintenance of the distinct phenotype of T_{CM} cells, including upregulation of CD62L and CCR7 (112). Hobit and Blimp-1 directly bind within the *Klf2* and the TCF1 encoding *Tcf7* locus and within the loci of the downstream targets S1PR1 and CCR7, suggesting that these transcription factors efficiently downregulate tissue exit pathways at multiple levels (26, 43) (**Figure 3**). The expression of Hobit in circulating human effector-type and effector memory-type CD8 T cells is enigmatic, given that Hobit in mice directly suppresses expression of tissue exit receptors. Although S1PR1 and CCR7 are nearly absent in quiescent human effector CD8 T cells (113), the putative repressive actions of Hobit on these pathways in long-lived human effector CD8 T cells appear insufficient to retain these cells within the peripheral tissues.

Other T_{RM}-specific transcription factors contribute to the regulation of T_{RM} cells. Expression of the Ahr has been identified in T_{RM} populations of the lungs, skin, and small intestine, but not in circulating memory CD8 T cells (25). In line with its expression pattern, Ahr specifically regulates the persistence of T_{RM} in the skin after HSV infection (72). Ahr is a ligand-operated transcription factor that responds to the presence of dietary components (45), but its ligands in virus-specific T_{RM} cells within the skin are unknown. The transcription factor NR4A1 is also expressed in T_{RM} cells in contrast to circulating memory CD8 T cells (46). NR4A1 is specifically involved in the development and/or maintenance of T_{RM} populations, in particular those in the epithelium and lamina propria of the small intestine (46). The downstream targets of Ahr and NR4A1 in T_{RM} cells have not been identified. Therefore, it remains unclear which aspects of T_{RM} differentiation are regulated by these transcription factors.

Regulation of T_{RM} Cells by Transcription Factors of Effector CD8 T Cells

Runx3, T-bet, Blimp-1, and Notch are each individually important in driving terminal differentiation of effector CD8 T cells and in the acquisition of important effector functions including the production of IFN-γ and/or cytotoxicity (50, 114–117). T_{RM} cells maintain direct effector function into the memory phase, suggesting a requirement for the persistent activity of these transcription factors. Indeed, Runx3, T-bet, Blimp-1, and Notch have also been implicated in the development and/or in the maintenance of T_{RM} cells (37, 43, 44, 74) (**Figure 3**).

Runx3 drives the generation of the CD8 T cell lineage in the thymus and is broadly expressed in peripheral naïve, effector, and memory CD8 T cells (118, 119). Runx3 pairs with the obligatory factor core binding factor of the Runx family that stabilizes binding of Runx proteins, including Runx3, to DNA (120). Functional profiling of CD8 T cell responses demonstrated that Runx3 expression is more relevant in T_{RM} cells than in circulating memory CD8 T cells (44). The transcriptional activity of Runx3 is already apparent at the effector stage in putative T_{RM} precursors, suggesting that Runx3 drives the formation of T_{RM} cells. Runx3 remains essential during the memory phase, implicating a continued role for Runx3 in the maintenance of T_{RM} cells (44). Virus-specific and tumor-specific T_{RM} cells in different tissues and settings require Runx3 for development, exemplifying Runx3 as an important transcriptional regulator of T_{RM} cells. Overexpression of Runx3 is sufficient to repress the expression of signature genes of circulating memory CD8 T cells and to promote the expression of residency signature genes including that of CD103 (44, 121). Collectively, these observations suggest that Runx3 has a primary role in the transcriptional regulation of T_{RM} differentiation. Runx3 may act upstream of Hobit and Blimp-1 in T_{RM} cells, given that the transcription factor induces expression of Blimp-1 and enhances accessibility to motifs shared by Hobit and Blimp-1 (122).

Notch is a surface receptor that interacts with the membrane-bound ligands Jagged and Delta-like on antigen-presenting cells (123). After ligand-induced activation, Notch is cleaved by γ-secretase, which enables its intracellular domain to translocate to the nucleus. Following translocation, Notch associates with the DNA-binding factor recombining binding protein suppressor of hairless (RBPJ) to form a transcriptional activator (124). Notch signaling orchestrates the maintenance of $CD103^+$ T_{RM} cells in the lungs after influenza infection (37). TGF-β-driven upregulation of Notch ligands within the epithelium may provide a mechanism to activate Notch specifically at these sites (125, 126). Notch appears to directly regulate expression of CD103 (37), thus

facilitating binding of T_{RM} cells within the epithelium. In addition, downstream targets of Notch include the glycerol transporter aquaporin-3, solute carriers for amino acids and other nutrients, suggesting that Notch contributes to the maintenance of T_{RM} cells through regulation of their metabolism (37).

T-bet is a T-box factor family member, which drives expression of the IL-15 receptor in circulating memory CD8 T cells (127) and appears to have a comparable role in T_{RM} cells (74). Similar to circulating memory CD8 T cells, T_{RM} populations in several, but not all tissues, require the homeostatic cytokine IL-15 for long-term maintenance (74, 76). Underlining its subordinate role in T_{RM} cells, it has been reported that T-bet acts at a lower level of expression in T_{RM} cells than in effector or circulating memory CD8 T cells. T_{RM} cells also completely lack the T-bet-related T-box factor Eomesodermin (Eomes) that, similarly to T-bet, can support IL15 receptor expression in circulating memory CD8 T cells (74). Overexpression of T-bet or Eomes abrogates differentiation of T_{RM} cells in skin and lungs, suggesting that high-level expression of these transcription factors is incompatible with long-term survival of T_{RM} cells (74, 128). The expression of T-bet is suppressed in T_{RM} cells in a TGF-β- and Runx3-dependent manner (44, 74). Downregulation of T-bet may dampen its suppressive impact on the CD103 encoding *Itgae* locus, where T-bet is able to bind at sites that overlap with the TGF-β-driven Smad proteins (128). Therefore, reduction of T-bet expression may limit interference with TGF-β-driven induction of CD103 expression in T_{RM} cells, while the residual T-bet expression may be sufficient to upregulate IL-15 receptor in T_{RM} cells and to receive IL-15-dependent survival signals for homeostasis (128).

Taken together, transcriptional regulation of T_{RM} cells includes the up-regulation of T_{RM}-specific transcription factors, suppression of transcription factors important for circulating memory T cells, and the maintenance of transcription factors involved in effector differentiation.

Transcriptional Regulation of Direct Effector Functions of T_{RM} Cells

Tissue-resident memory CD8 T cells rapidly exert effector functions upon activation, suggesting that transcription factors that regulate the expression of cytotoxic and pro-inflammatory molecules may also be active in T_{RM} cells. Interestingly, transcription factors that are important for T_{RM} development also play crucial roles in the regulation of effector functions.

In contrast to circulating memory T cells, T_{RM} cells maintain expression of the cytotoxic mediator granzyme B at the protein level, which provides them with the potential to contain infection at early stages through the elimination of infected cells. Runx3 has been shown to induce expression of granzyme B in T_{RM} cells, directly implicating the transcription factor in the regulation of cytotoxicity in these memory T cells (44). A role for Runx3 in the instruction of lytic activity through the upregulation of granzyme B and perforin expression has been previously established in effector CD8 T cells (114, 129). Runx3 directly binds at the granzyme and perforin loci, but also recruits Eomes for synergistic activity at the perforin locus in effector CD8 T cells (114). Mucosal T_{RM} cells do not express Eomes (74), suggesting that in these cells the activity of Runx3 is Eomes-independent. The Runx3-driven program of cytotoxicity in effector CD8 T cells may also involve the upregulation of Blimp-1 expression (129). Blimp-1 and its homolog Hobit have been directly implicated in the regulation of cytotoxicity in effector CD8 T cells (115, 116) and in NKT cells (130), respectively. Blimp-1 drives the acquisition of granzyme B in effector CD8 T cells after acute infection with LCMV and influenza (115, 116). Hobit is required for NKT cells to upregulate granzyme B after stimulation with pro-inflammatory cytokines such as type I IFN and after infection with mCMV (130). The role of Hobit and Blimp-1 in the regulation of cytotoxicity in T_{RM} cells remains to be investigated. The transcriptional regulation of cytotoxicity in T_{RM} cells involves the long-term maintenance of cytotoxic molecules during steady state. Currently, it is not clear how the transcriptional network of T_{RM} cells achieves the retention of cytotoxic molecules into the memory phase. Constitutive expression of Runx3, Blimp-1, and Hobit in T_{RM} cells may be required for persistent expression of granzyme B and other cytotoxic molecules in these memory T cells (**Figure 3**).

Tissue-resident memory CD8 T cells are able to mount rapid cytokine responses upon reactivation, which at least in part resides in their superior capacity to retain mRNA molecules encoding pro-inflammatory cytokines, including IFN-γ (43, 88). The transcriptional network underlying the persistence of mRNA of pro-inflammatory cytokines has not yet been established. Important transcriptional regulators of IFN-γ include T-bet and Eomes (127, 131), but these T-box transcription factors are downregulated in T_{RM} cells in mice and humans (37, 74, 128), suggesting that they do not play a dominant role in T_{RM} cells. Runx3 has been described to regulate IFN-γ, TNF-α, and IL-2 in effector CD8 T cells (114), but is not essential for the regulation of cytokine production by T_{RM} cells (44). Although Notch ligands induce IFN-γ expression in human T_{RM} cells, Notch deficiency only marginally reduces the expression of IFN-γ in murine T_{RM} cells (37). It is possible that the absence of an essential role in the regulation of IFN-γ production for any of these transcription factors relates to redundancy between the IFN-γ-driving molecules.

Taken together, the overlap in the transcriptional programs of effector CD8 T cells and T_{RM} cells suggest a high degree of conservation in the regulation of their effector capacities. Understanding the interplay between the different transcriptional programs in the maintenance of the poised effector state of T_{RM} cells is crucial to further unravel the underlying transcriptional network.

Transcriptional Regulation of T_{RM} Cells Upon Re-Stimulation

While the transcriptional program of T_{RM} generation and maintenance is starting to become clear, it is currently not known how transcription factors regulate T_{RM} functions after reactivation during reinfection. Based on the available information in circulating CD8 T cells, we can speculate on how the signals received by T_{RM} cells during infection may influence their transcriptional program (**Figure 3**).

The transcription factor Hobit is specifically expressed by T_{RM} cells and other tissue-resident lymphocytes including NKT cells during quiescence. Antigen recognition by NKT cells leads to immediate downregulation of Hobit (130). Hobit expression might be similarly regulated in T_{RM} cells. Downregulation of Hobit after TCR activation might allow T_{RM} cells to release effector molecules and undergo proliferation. Additionally, the loss of the tissue-residence transcription factor Hobit might enable T_{RM} cells to leave the tissue, enter the circulation, and migrate to secondary lymphoid organs. In memory CD8 T cells, the sensing of inflammation alone without cognate antigen recognition is sufficient to induce upregulation of effector molecules such as granzyme B (132). IFN-α receptor 1 and signal transducer and activator of transcription 1 are critical in this bystander cytotoxicity of circulating memory CD8 T cells. In NKT cells, Hobit is crucial for the ability to respond to inflammatory cytokines and type I interferon-driven granzyme B upregulation (130). Similarly, Hobit expression may also drive the innate functions of T_{RM} cells after recognition of inflammation.

As pointed out above, many of the transcription factors, which are induced during priming of naïve CD8 T cells and upregulated in effector cells, are also critical for T_{RM} formation and maintenance. Blimp-1 and T-bet are highly expressed in effector T cells and maintained at a lower level in memory CD8 T cells (50, 115, 116). Upon reinfection, reactivated memory cells form secondary effector cells that phenotypically and transcriptionally resemble primary effector cells, e.g., high expression of T-bet. Recognition of IL-12 by memory CD8 T cells during recall responses is one of the main drivers of T-bet upregulation (133). Blimp-1 expression may be similarly regulated, as Blimp-1 is induced by pro-inflammatory cytokines including IL-12 *in vitro* (134). The data suggest that Blimp-1 and T-bet are upregulated in T_{RM} cells in response to inflammation and/or TCR triggering. Given its crucial role in T_{RM} differentiation, increased expression of Blimp-1 may manifest tissue-resident features upon reinfection. At the same time, concurrent inflammation-induced upregulation of T-bet may interfere with maintenance of tissue residency, as elevated levels of T-bet are incompatible with T_{RM} formation (74). The role of the transcription factors Ahr and NR4A1 during activation of memory CD8 T cells is less clear. The expression of Ahr is increased upon activation of memory T cells (135). Also NR4A1 expression is upregulated after TCR triggering (136), but appears to exert a regulatory role after activation, as the transcription factor can maintain T cells in a quiescent state *via* the suppression of IRF4 (137). These data suggest that changes in the transcriptional programming of T_{RM} cells likely occur upon reactivation. Further research is required to determine how the transcriptional network of T_{RM} cells controls their function and differentiation upon re-challenge with antigen and/or inflammation during infection.

CONCLUDING REMARKS

The unique properties of T_{RM} cells compared to circulating memory CD8 T cells have sparked interest in the development of therapeutic approaches that induce T_{RM} formation, especially in the context of future vaccination strategies (138, 139). Given their superior protective capacity at barrier sites, local establishment of T_{RM} cells constitutes an attractive approach to confer long-lasting tissue immunity. Recent work has demonstrated the potency of vaccine-induced T_{RM} cells in providing protection against heterotypic viral challenges (140) and local tumor development (141, 142). In line with this, the improved survival rates of patients with tumors containing large quantities of T_{RM}-type cells highlights T_{RM} cells as a potential target in the treatment of cancer (143–145). A better understanding of the transcriptional network underlying the differentiation and function of T_{RM} cells may assist in unlocking these potent memory cells for therapeutic purposes.

AUTHOR CONTRIBUTIONS

FB, AC, RS and KG drafted and edited the manuscript. FB drafted and edited the figures and figure legends. All authors approved the work for publication.

FUNDING

RS is supported by a Veni fellowship from The Netherlands Organisation for Health Research and Development (ZonMW). FB and KG are supported by a Vidi fellowship from ZonMW. KG is also supported by a fellowship from the Landsteiner Foundation for Blood Transfusion Research.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.