RESIDENT MEMORY T CELLS: GUARDIANS OF THE BALANCE OF LOCAL IMMUNITY AND PATHOLOGY

EDITED BY: Nick P. Goplen, Toshinori Nakayama, Jie Sun and Shiki Takamura
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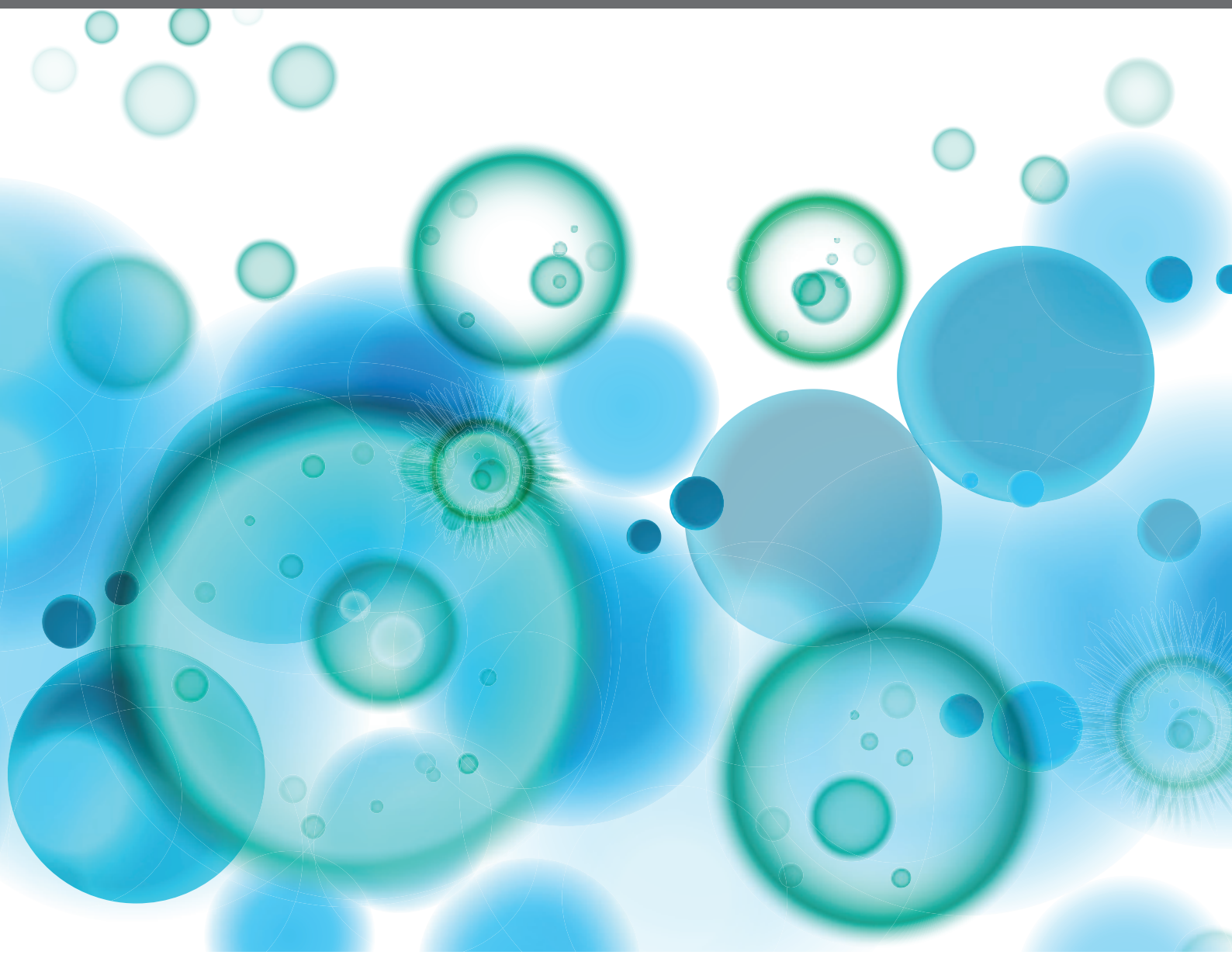

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RESIDENT MEMORY T CELLS: GUARDIANS OF THE BALANCE OF LOCAL IMMUNITY AND PATHOLOGY

Topic Editors:
Nick P. Goplen, Mayo Clinic, United States
Toshinori Nakayama, Chiba University, Japan
Jie Sun, Mayo Clinic, United States
Shiki Takamura, Kindai University, Japan

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Editorial: Resident Memory T Cells – Guardians of the Balance Between Local Immunity and Pathology – The Minority Report

Nick P. Goplen[1,2,3]*, *Shiki Takamura*[4], *Toshinori Nakayama*[5,6] *and Jie Sun*[1,2,3]

[1] Division of Pulmonary and Critical Medicine, Department of Medicine, Thoracic Disease Research Unit, Mayo Clinic, Rochester, MN, United States, [2] The Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, United States, [3] Department of Immunology, Mayo Clinic, Rochester, MN, United States, [4] Department of Immunology, Faculty of Medicine, Kindai University, Osaka, Japan, [5] Department of Immunology, Chiba University, Chiba, Japan, [6] Advanced Research and Development Programs for Medical Innovation-CREST (AMED-CREST), Japan Agency for Medical Research and Development, Chiba, Japan

Keywords: resident memory T (Trm), differentiation, maintenance, immune protection, pathology

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Edited and reviewed by:
Scott N. Mueller,
The University of Melbourne, Australia

****Correspondence:***
Nick P. Goplen
goplen.nicholas@mayo.edu

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Editorial on the Research Topic

Resident Memory T Cells – Guardians of the Balance Between Local Immunity and Pathology – The Minority Report

INTRODUCTION

Once T cell responses peak in response to early antigenic and pro-inflammatory programming, ~95% of the accumulated die, contracting clonally expanded pools. Survivors become long-lived memory T cells, the flavour of which is largely defined by migration patterns and epigenetic capacities for self-renewal and effector function. From mice to apes and humans, Tissue resident memory T cells (T_{RM}) that reside in non-lymphoid tissue constitute a previously unappreciated slice of the memory T cell pie. T_{RM} are intimately involved in dynamic secondary responses and lend considerably to their swiftness. This Research Topic reviews vastly different T_{RM} phenotypes and modes of retention, with conserved protective functions across tissues, models, and species while also exploring instances in which T_{RM} dysfunction may turn pathogenic and harm vital host organs or compromise barrier tissues. Predicting innocence or guilt in a heterogeneous amorphous pool of resident lymphocytes, is proving a formidable puzzle that may retard manipulation of T_{RM} for immunotherapies. Yet, with the recent surge in reports of pathogenic T cells in human disease and animal models, we are encroaching on foresight levels seen in the movie, Minority Report, a trajectory that may someday offer selective targeting of the trouble-makers before their crimes are committed.

For either T_{RM} function or dysfunction, reaching critical mass seems … critical. Whether the therapeutic goal is increasing or decreasing T_{RM} density, modes of differentiation and maintenance

in various tissues require further understanding. Mora-Buch et al. break down $CD8^+$ T_{RM} differentiation into stages by location, location, and location, starting with commitment issues in draining lymph nodes as early as stage zero. They also highlight work by Beura et al. demonstrating these commitment issues, in those that survive the initial trials, give way to increased fluidity in secondary T_{RM} responses. In a complementary review, Pritzl et al. give novel insight as to how the response to antigen, PAMPs/DAMPs, and tissue inherent signals might integrate to tune heterogeneous $CD8^+$ T_{RM} differentiation, maintenance, and function. They also make an irresistible rational argument to explore the involvement of the NF-κB-Eomes circuit in T_{RM} differentiation during clonal contraction. Importantly, they address how, for better or worse, timing of therapeutics could disrupt the status quo programming of T_{RM} differentiation.

Netherby-Winslow et al. extend these views on crucial signal integration and multidimensional $CD8^+$ T_{RM} differentiation to the central nervous system highlighting their hypothesis that TCR and inhibitory signals may be key to preventing brain pathologies under steady-state conditions. Indeed, collectively these reviews suggests inhibitory receptors may be a rheostat that modulate/appropriates response to antigen concentration, minimizing bystander damage, as has been postulated for T_{RM} in the brain (Netherby-Winslow et al.), lung (Qian et al.; Goplen et al.), and skin (Tokura et al.).

Du et al. submit a protocol of isolation from human skin biopsies that preserve *in situ* phenotypes, optimized for T_{RM} viability, functionality, and longevity *ex vivo* that may capture the usual, and potentially unusual, suspects. Attractively, the tissue digestion process also captures a wide array of local antigen presenting cells including Langerhans, potentially allowing for comparison of functional assays *in situ* versus *ex vivo*.

Despite their penchant for lodging in tissues, T_{RM} have been shown to be surprisingly motile in many environments and exhibit smooth sailing while performing their protective sentinel duties. How then do redundant layers of T_{RM} retention allow for ambulation within barrier and non-barrier tissues? Stein et al. explore recent data in their wheelhouse suggesting the tissue topography (degree of epithelialization) in combination with the array of integrins T_{RM} express, may govern these seemingly contradicting T_{RM} features of anchoring in place, but allowing for local T_{RM} drift in 2D and 3D space.

Perhaps in contrast to, or possibly in conjunction with their protective function and dynamic motility, pathologies involving T_{RM} dysregulation have been observed in a growing number of contexts from inflammatory bowel disease to rejection of transplants, psoriasis, asthma, and respiratory viral infections. Paap et al. tackle the complex role of T_{RM} in homeostatic control of the gastral intestinal tract. They highlight recent advances in chronic inflammatory bowel diseases (IBD), which provide an antigen-rich environment where lack of tolerance is one cell layer away from catastrophe. They give unique insight as to how current IBD therapies may fortuitously, but not purposefully, target intestinal T_{RM}. Hirahara et al. contrast anti-microbial protections in mucosal tracts afforded by CD4+ T_{RM} with the pathogenic potential of sub-populations in allergy models. From fibrosis inducing amphiregulin-positive and eosinophil sustaining IL-5-producing Th2 T_{RM} maintained in iBALT to their regulatory counterparts generated in the same models, they highlight a need to understand the heterogeneity and plasticity within the resident $CD4^+$ T cell compartment to combat mucosal diseases and enhance protection.

Continuing on the diversity and inclusion theme, Goplen et al. explore influenza infection from a polyclonal T_{RM} viewpoint and expose questions regarding heterogeneity that transgenic TCR models have not beckoned. For instance, $CD8^+$ T_{RM} within the same organ against the same pathogen, but with different antigen specificities, possess disparate: transcriptional signatures, phenotypes that may dictate sub-compartmental localization, maintenance requirements, and to some degree, functionality. Regardless the reasons for the inequalities (e.g. TCR signaling, location, etc.), recent work indicates this full spectrum of T_{RM} differentiation should be considered when formulating T_{RM} dependent pulmonary immunotherapies, particularly in those of advanced age, where lung $CD8^+$ T_{RM} may lose their protective function and adopt a pathogenic role sustaining chronic inflammation.

If such findings in aged mice were to have implications for COVID-19 in the elderly, they may play a role in uncovering treatments for "long COVID-19"; such possibilities are being explored. Both Goplen et al. and Qian et al. draw parallels from mouse influenza models to findings in human SARS-CoV2 specific T_{RM} reviewing their expected and tested protective capacity. Additional but congruent phenotypes in various Caronavirus family (SARS & MERS) studies, particularly, long-lasting fibrotic sequelae seen on CT scans up to 6 months post-infection are discussed. In influenza models, such long-term lesions are dependent on age-associated parenchymal CD8 T cells, suggesting they are responsible for some of the long-term physiologic impairment of the lung following severe viral pneumonia. Given the crux of vaccinating the elderly to relieve stresses of the current pandemic, it may therefore be fortuitous that intramuscular jabs are not expected to induce local T cell immunity to respiratory viruses, but further investigations are clearly warranted.

This topic collection of ten articles was undertaken to drill-down and refine tissue-specific nuances regarding resident memory CD4 & CD8 T cell differentiation, maintenance, function, and regulation, particularly as it relates to protecting the host from both antigen re-encounter and untoward immune responses. Many studies now agree, regimens that tune T_{RM} density in various tissues will usher in next-gen vaccines and immunotherapies with previously unrealized potential. Yet, as this Research Topic highlights, learning how to predict and target the criminals before the crime is committed while preserving protective capacity, may be a potential bottleneck in this endeavor. Experiments on the horizon will reveal the heterogeneic and plastic nature of T_{RM} differentiation and function that allow us to push past these boundaries and expose more nontrivial nuances to be surmounted. These reviews begin to contextualize the conditions, phenotypes, and

functions for which T_{RM} are guardians of their local environment or whether they wreak havoc in them.

We thank all the authors, reviewers, and the shoulders on which they stood, and hope you find this Research Topic a useful contribution to your field.

AUTHOR CONTRIBUTIONS

NG and JS conceived the Research Topic. NG organized the solicitation of submissions. All authors refereed the peer-review process for various items in the collection as noted. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Total Recall: Intestinal T_{RM} Cells in Health and Disease

*Eva-Maria Paap, Tanja M. Müller, Katrin Sommer, Markus F. Neurath and Sebastian Zundler**

Department of Medicine 1 and Deutsches Zentrum Immuntherapie, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Tissue-resident memory T cells (T_{RM} cells) have crucial functions in host defense in mucosal tissues. They provide local adaptive immune surveillance and allow the fast initiation of targeted adaptive immune responses in case of antigen re-exposure. Recently, an aberrant activation in the case of immunologically mediated diseases has been increasingly acknowledged. As the organ with the largest interface to the environment, the gastrointestinal tract faces billions of antigens every day. Tightly balanced processes are necessary to ensure tolerance towards non-hazardous antigens, but to set up a powerful immune response against potentially dangerous ones. In this complex nexus of immune cells and their mediators, T_{RM} cells play a central role and have been shown to promote both physiological and pathological events. In this review, we will summarize the current knowledge on the homeostatic functions of T_{RM} cells and delineate their implication in infection control in the gut. Moreover, we will outline their commitment in immune dysregulation in gastrointestinal chronic inflammatory conditions and shed light on T_{RM} cells as current and potential future therapeutic targets.

Keywords: tissue-resident memory T cells, intestine, inflammatory bowel diseases, infection control, therapeutic targets

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Edited by:
Jie Sun,
Mayo Clinic, United States

Reviewed by:
Nu Zhang,
The University of Texas Health Science Center at San Antonio, United States
Xenia Maria Ficht,
San Raffaele Hospital (IRCCS), Italy

***Correspondence:**
Sebastian Zundler
sebastian.zundler@uk-erlangen.de

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INTRODUCTION

Coordinated processes of the immune system require a tightly regulated interplay of various immune cell types and mediators. A particular feature of the adaptive immune system is the generation of immunological memory following antigen exposure leading to preparedness for the initiation of targeted immune responses in case of re-exposure. To this end, memory T cells are generated during a primary confrontation with an antigen. After its clearing, they survive as long-lived patrolling guards in particular compartments of the body.

Memory T cells are grouped into three main populations: central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and tissue-resident memory T cells (T_{RM}) (1–4). T_{RM} cells persist at epithelial surfaces including the gastrointestinal tract (GIT), skin, and lung as well as in non-barrier tissues such as the brain and the joints (3, 5–9). They are transcriptionally, phenotypically, and functionally distinct from recirculating central and effector memory T cells (10). Due to their localization at the interface between the host and the environment, they provide local adaptive immune surveillance for intruding cognate antigens, positioning them in the driver's seat for the re-initiation of immune responses to known antigens in mucosal tissues (11). The GIT disposes over

the largest surface of the body exposed to the external environment. This environment has a challenging composition including commensal, pathobiontic and sometimes pathogenic bacteria, viruses and, parasites as well as nutritional and potentially toxic antigens. Therefore, a closely regulated local immune system balancing tolerance and protection is essential and, as the first line of adaptive defence, T_{RM} cells play a key role in this context. This said, it is obvious that in addition to crucial functions in infection control, dysregulation of T_{RM} networks may also contribute to the development of diseases such as chronic inflammatory bowel diseases (IBD).

However, the role of T_{RM} cells in the intestine is not completely understood. In the following paragraphs, we will review the current knowledge on their implication in intestinal immune processes and also outline the putative contribution to pathological conditions as well as translational approaches to target T_{RM} cells.

PHENOTYPE OF INTESTINAL T_{RM} CELLS

T_{RM} cells have first been described in 2009 (4) and, early on, a specific profile of molecules associated with a T_{RM} phenotype was evident. More recently, Kumar and colleagues described a transcriptional and phenotypic signature that defines both $CD8^+$ and $CD4^+$ T_{RM} cells in humans and that is conserved across individuals and in mucosal and lymphoid tissues (12).

In general, the membrane protein CD69 is used to define both $CD8^+$ and $CD4^+$ T_{RM} cells. CD69 is a type II C-lectin receptor, which regulates, on the one hand, the differentiation of regulatory T cells and the secretion of cytokines like IL-17, IL-22, and interferon-γ (IFN-γ) and suppresses, on the other hand, the sphingosine-1-phosphate receptor 1 (S1PR1) [(13, 14), reviewed in (15)]. Mechanistically, CD69 interferes with the cell surface expression and function of S1PR1, which is essential for T and B cell egress from peripheral tissues, secondary lymphoid organs and thymus *via* chemotaxis towards S1P, which is present in high concentrations in the bloodstream (13, 16, 17). Moreover, a decreased expression of the transcription factor KLF2 in T_{RM} cells leads to the downregulation of S1PR1 (18). Together, the upregulation of CD69 and the downregulation of KLF2 and S1PR1 promote tissue retention of T_{RM} cells.

However, there is also evidence that CD69 is not expressed on all T_{RM} cells and—depending on the tissue—is not necessary for their generation. According to these studies, CD69 plays no discernible role for T_{RM} cell formation in the small intestine, while it is essential for T_{RM} cell development in the kidney in mice (19, 20).

Another important marker of T_{RM} cells is CD103, also called αE integrin. CD103 pairs with the β7 integrin chain and the heterodimer binds to E-cadherin, which is expressed on epithelial cells (21). Thus, this interaction constitutes an independent mechanism promoting mucosal retention. It was already shown in humans and in mice that the expression of CD103 is more predominant in $CD8^+$ T_{RM} cells than in $CD4^+$ T_{RM} cells (22–24). Moreover, in the human intestine, CD103 is not necessary for the persistence of $CD4^+$ and $CD8^+$ T_{RM} cells (6, 7, 22). Bergsbaken and colleagues even identified a preferential development of $CD103^-$ T_{RM} cells in inflammatory microenvironments within the mouse *lamina propria* upon infection with *Yersinia pseudotuberculosis* (Yptb) (22).

Further core phenotypic markers for human $CD8^+$ T_{RM} cells in multiple mucosal and lymphoid tissues include CD49a, CD101, and PD-1 (12), whereas CD161, a C-type lectin-like receptor seems to be specific for $CD8^+$ T_{RM} cells in the human gut (25, 26). Furthermore, the T_{RM}-specific gene signature includes the downregulation of lymph node homing molecules such as CD62L and CCR7, the upregulation of specific adhesion molecules like CRTAM, as well as the modulation of specific chemokine receptors including an increased CXCR6 and decreased CX3CR1 expression (12).

Several transcription factors have been implicated in the transcriptional control of T_{RM} cells leading to the expression of the above-mentioned molecules. In particular, Hobit together with Blimp-1 (PRDM1), Runx3, and Notch regulate the differentiation and maintenance of T_{RM} cells. Importantly, Hobit and Blimp-1 are known to synergistically control the expression of T_{RM} cell-regulated genes like CD69, KLF2, and S1PR1 (27–29). In this context, it is important to mention that Hobit expression is restricted to tissue-resident T cells [including T_{RM} cells, NKT cells, and some MAIT cells] in mice (27, 30), but not in humans. There, Hobit expression is also found in other T cell subsets with cytotoxic phenotype (31, 32).

Importantly, several cytokines like IL-15, IL-33, transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) were identified to play a role in the maintenance of T_{RM} cells (18, 33).

T_{RM} CELLS IN INTESTINAL INFECTION CONTROL

Especially in the GIT, T_{RM} cells are important in mediating fast and effective immune responses, when necessary. Thus, they crucially contribute to the maintenance of the local tissue homeostasis.

During primary infection, whether viral, bacterial or parasitic, some memory T cells acquire a T_{RM} phenotype including differential protein expression as described above and are retained in the tissue, where they are able to survive long-term (4, 34, 35). There seems to be considerable heterogeneity in intestinal T_{RM} populations as recently suggested by two studies building on single-cell transcriptomics in mice (36, 37). After re-infection with a previously encountered pathogen, the presence of T_{RM} cells provides a short-cut with regard to the time-consuming processes involved in *de-novo* adaptive immune responses, i.e. antigen processing by antigen-presenting cells (APCs), APC migration to secondary lymphoid tissues, T cell recognition, co-stimulation with subsequent activation, and proliferation as well as recirculation and migration of effector T cells to the infected tissue [reviewed in (38–41)]. Instead, upon

antigen binding, T_{RM} cells are directly able to proliferate, to secrete pro-inflammatory cytokines such as IFN-γ or TNF-α and chemokines and to mediate cytotoxicity by secreting granzyme B and perforin to directly eliminate infected cells (**Figure 1**) [(5–7, 42), reviewed in (43)].

Interestingly, T_{RM} cells are not only generated at the site of primary infection but also seed distant locations. However, as shown by Sheridan and colleagues in mice, intestinal $CD8^+$ T_{RM} cells developing upon oral infection with *Listeria monocytogenes* are more robust and have another phenotype than intestinal T_{RM} cells developing upon intranasal or intravenous infection (44).

Due to the increased abundance of $CD8^+$ T_{RM} cells compared with $CD4^+$ T_{RM} cells, the former have been examined in much more detail in the context of intestinal infections. Yet, $CD4^+$ and $CD8^+$ T_{RM} cells share several similarities and $CD4^+$ T_{RM} cells crucially contribute to recall immunity by chemokine secretion and immune cell activation (45).

In summary, these observations suggest that T_{RM} cells might be important effectors of vaccination strategies in the gut. Consistently, a recent study showed that an oral typhoid vaccine was able to induce antigen-specific $CD4^+$ T_{RM} cells in the human small intestine (46). Additionally, transient microbiota depletion-boosted immunization in mice has been proposed as a strategy to optimize T_{RM} cell generation upon exposure with vaccine antigens (47).

Studies by Bartolomé-Casado et al. revealed that both $CD4^+$ and $CD8^+$ T_{RM} cells persist for years in the human small intestine. Both undergo tissue-specific changes, which make them polyfunctional T_H1 and T_C1 cells (6, 7). How this longevity of T_{RM} cells is ensured is not completely elucidated so far and the question arises whether the size of the T_{RM} population in a homeostatic state is regulated by a continuous supply of recirculating memory T cells or whether a well-balanced T_{RM} cell proliferation is sufficient for the maintenance of the T_{RM} cell population [reviewed in (43)]. However, low-level homeostatic cell proliferation has been described for T_{RM} cells, e.g. in the skin and female reproductive tract, but not for the GIT so far (5, 48).

In contrast to the view that T_{RM} cells are confined within "their" tissue, Fonseca and colleagues showed that there is also evidence for fully differentiated T_{RM} cells in mice, which re-differentiate and recirculate into lymphoid tissues (49).

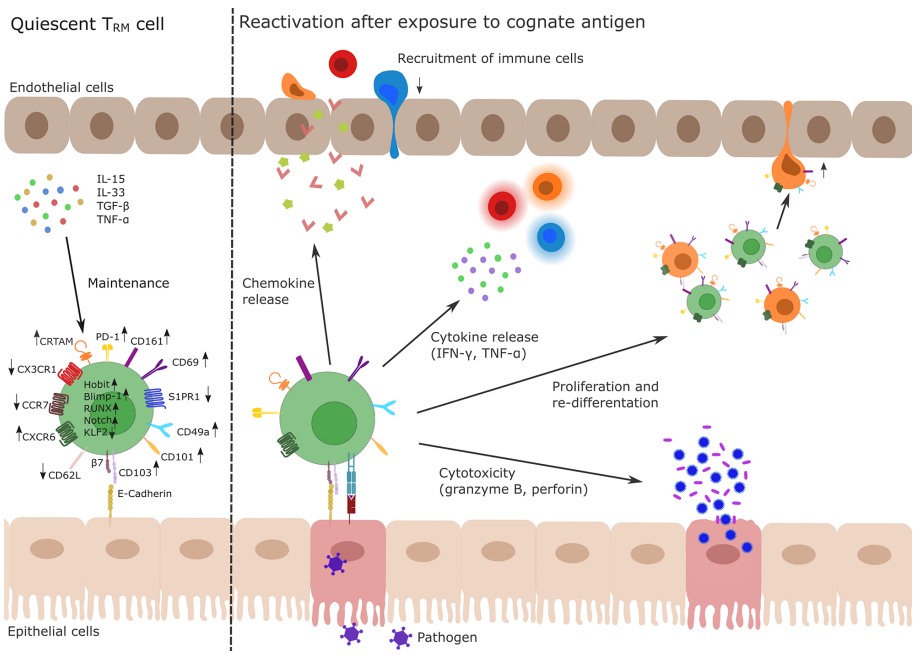

FIGURE 1 | Profile and function of T_{RM} cells. Left side: T_{RM} cells develop during primary infection. The differentiation and maintenance of T_{RM} cells is controlled by tissue-derived signals, e.g., TNF-α, TGF-β or IL-15 and IL-33 resulting in the up- and down-regulation of different genes *via* activity of the transcription factors Hobit, Blimp-1, Runx3, and Notch and the silencing of Klf2. In particular, upregulation of CD69 and CD103 and simultaneous downregulation of S1PR1 are key drivers of T_{RM} cell tissue retention. Other membrane molecules highly expressed in T_{RM} cells are CD49a, CD101, PD-1, CRTAM, and CXCR6 while CD62L, CCR7, and CX3CR1 show a decreased expression pattern in T_{RM} cells. Right side: After re-exposure to a cognate antigen (e.g., from a pathogen, shown in purple), T_{RM} cells are able to initiate a fast immune response. This includes chemokine release to recruit lymphocytes (indicated as red, orange, and blue immune cells) to the site of infection, release of pro-inflammatory cytokines (IFN-γ, TNF-α) to activate other cells as well as the production of the cytotoxic effectors perforin or granzyme B. There is also evidence for the ability of T_{RM} cells to proliferate or to re-differentiate (indicated as green and orange cells) and to leave the tissue (orange ex-T_{RM} cells; for details cf. main text). T_{RM}, tissue-resident memory T cell; TNF, tumor necrosis factor; TGF, transforming growth factor; IL, Interleukin; KLF, Krüppel-like factor; CD, cluster of differentiation; S1PR1, sphingosine-1-phosphate receptor 1; PD-1, programmed cell death protein 1; CRTAM, cytotoxic and regulatory T-cell molecule; CXCR, CXC-motif chemokine receptor; CCR, Chemokine receptor.

Moreover, it was shown that $CD4^+$ T_{RM} cells in the skin may have the ability to downregulate CD69 and subsequently exit the tissue (50). Very recently, this has been demonstrated for intestinal $CD8^+$ T_{RM} cells following oral *Listeria monocytogenes* re-infection. Using a Hobit reporter mouse strain, Behr and co-workers could elegantly show that ex-T_{RM} cells appeared in the circulation and were able to mount systemic and local immune responses (51).

Taken together, these data show that T_{RM} cells represent an important switch point in recall immunity. However, the presence of this cell type, which is able to mediate powerful immune responses also entails the risk that dysregulation and imbalance can lead to immune dysfunctions like allergic disorders or chronic inflammation.

T_{RM} CELLS IN INFLAMMATORY BOWEL DISEASES

In recent years, the implication of T_{RM} cells in pathological conditions has been increasingly acknowledged. In particular, they seem to play an important role in various cancer entities and several immune-mediated inflammatory disorders like psoriasis, vitiligo, psoriatic arthritis, and IBD (52–58). Whereas T_{RM} cells as tumor-infiltrating lymphocytes (TIL) are associated with a better prognosis in most cancer types (e.g. ovarian cancer, breast cancer, and gastric adenocarcinoma), $CD103^+$ TIL in colorectal cancer are associated with poor prognosis (56–59), suggesting that their impact is tissue-specific.

In the context of IBDs, an important role of T_{RM} cells has only recently emerged. Several studies indicate that the presence and generation of T_{RM} cells are involved in the pathogenesis of IBDs (**Table 1**). We were able to show that $CD69^+CD103^+$ cells with a T_{RM} phenotype are increased in the lamina propria of patients with ulcerative colitis (UC) and Crohn's disease (CD) and that high levels of $CD4^+$ T_{RM} cells in IBD patients are associated with early relapse. In mice, we observed that the key T_{RM} transcription factors Hobit and Blimp-1 are essential for experimental colitis since their absence protected from T cell transfer colitis, dextran sodium sulphate-induced colitis and trinitrobenzene sulfonic acid-induced colitis. Mechanistically, we could attribute this to an adaptive-innate crosstalk mechanism including chemokine release by T_{RM} cells and subsequent recruitment and differentiation of pro-inflammatory immune cells (55). Consistent with these results Bishu and colleagues reported, that $CD4^+$ T_{RM} cells are increased in CD compared with control patients and identified these $CD4^+$ T_{RM} cells as the major T cell source of TNF-α in the mucosa of CD patients. Furthermore, these cells produced more IL-17A and TNF-α in inflamed compared to healthy tissue (60). Bottois and colleagues profiled two distinct $CD8^+$ T_{RM} cell subsets in CD, defined by KLRG1 and CD103, which are both receptors of E-Cadherin. $CD103^+CD8^+$ T_{RM} cells in CD patients expressed T_H17-related genes such as CCL20, IL-22 and, IL-26 suggesting that they may trigger innate immune responses as well as the recruitment of effector cells. $KLRG1^+CD8^+$ T_{RM} cells were specifically elevated under inflammatory conditions and showed increased proliferative and cytotoxic potential (61). Furthermore, a recent study employing single-cell RNA-sequencing identified changes in the transcriptional profile of $CD8^+$ T_{RM} cell subsets in UC including a pro-inflammatory phenotype and increased expression of Eomesodermin (62). Similarly, Corridoni and colleagues reported that $CD8^+$ T_{RM} cells in UC express more GZMK and IL26, suggesting that altered $CD8^+$ T_{RM} cells are implicated in UC pathogenesis (63).

Yet, observations made by other groups support the notion that the picture is more complex. E.g., Noble et al. described reduced numbers of $CD103^+Runx3^+$ T_{RM} cells in CD and UC.

TABLE 1 | Overview of studies on the role of T_{RM} cells in IBD.

Organsim	Key conclusions on T_{RM} cells	Ref.
Human and Mouse	Human: → $CD69^+CD103^+$ cells with a T_{RM} phenotype are increased in the lamina propria of patients with ulcerative colitis (UC) and Crohn's disease (CD) → High levels of $CD4^+$ T_{RM} cells in IBD patients are associated with early relapse. Mouse: → T_{RM} cells expressing Hobit and Blimp-1 are key drivers of experimental colitis due to an adaptive-innate crosstalk mechanism	(55)
Human	→ Increased $CD4^+$ T_{RM} cell population in CD compared with control patients → Increased production of IL-17A and TNF-α by T_{RM} cells in inflamed compared to healthy tissue → Major T cell source of TNF-α in the mucosa of CD patients.	(60)
Human	→ Two distinct $CD8^+$ T_{RM} cell subsets in CD, defined by KLRG1 and CD103 → $CD103^+CD8^+$ T_{RM} cells: express T_H17-related genes such as CCL20, IL-22, and IL-26 → $KLRG1^+CD8^+$ T_{RM} cells: specifically elevated under inflammatory conditions, show increased proliferative and cytotoxic potential	(61)
Human	→ Changes in the transcriptional profile of $CD8^+$ T_{RM} cell subsets in UC: pro-inflammatory phenotype and increased expression of Eomesodermin	(62)
Human	→ $CD8^+$ T_{RM} cells in UC express more GZMK and IL26 → Altered $CD8^+$ T_{RM} cells may be implicated in UC pathogenesis	(63)
Human	→ Reduced numbers of $CD103^+Runx3^+$ T_{RM} cells with a probably regulatory phenotype in CD and UC: expression of CD39 and CD73, release of IL-10	(64)
Human	→ Decreased numbers of $CD103^+CD4^+$ and $CD103^+CD8^+$ T cells in active IBD → Rise of the numbers of these cells in the remission phase up to levels comparable with healthy controls.	(65)

They observed the expression of CD39 and CD73 on these cells as well as the release of IL-10 suggesting that these cells have a regulatory phenotype. They hypothesized that T_{RM} cells probably serve as gatekeepers by controlling the access of mucosal antigens to germinal centers in lymphoid tissue (64). Roosenboom and colleagues reported decreased numbers of $CD103^+CD4^+$ and $CD103^+CD8^+$ T cells in active IBD and found a rise of these numbers in the remission phase up to levels comparable with healthy controls. In addition, they observed a lower number of $CD103^-$ T cells in healthy controls and IBD patients in remission in comparison with active CD and UC patients (65). Importantly, this study was not specifically designed to assess T_{RM} cells. Thus, it seems possible that these data are actually indicative of a change in T_{RM} cell phenotype similar to some of the studies mentioned above.

Taken together, T_{RM} cells are undoubtedly involved in the pathogenesis of IBDs. However, different observations have been made with regard to their function and mechanisms. While these seem to be conflicting on first view, it is likely that they rather derive from different approaches to a complex issue. For example, considering that T_{RM} cell generation may occur following any recognition of a cognate antigen by a naïve T cell, it is also clear that —depending on co-stimulatory signals and the nature of the surrounding environment—different forms of T cell memory may be imprinted. Thus, it is not surprising that regulatory as well as pro-inflammatory T_{RM} phenotypes have been described depending on the markers chosen to identify the cells. In consequence, the reduction of regulatory-type T_{RM} cells is actually not at all contradicting other observations, such as perturbed T_{RM} cell phenotypes in IBD or increased pro-inflammatory T_{RM} cell populations. Yet, further investigations are necessary to answer the remaining open questions.

T_{RM} CELLS AS POTENTIAL THERAPEUTIC TARGETS IN INFLAMMATORY BOWEL DISEASES

Based on the above-mentioned reports T_{RM} cells seem to be a promising therapeutic target to treat UC and CD.

Specific approaches in that regard are still lacking and would require the identification of unique targets on or in T_{RM} cells as well as the selection of appropriate targeting strategies. However, the mechanism of the monoclonal anti-β7 integrin antibody etrolizumab, which blocks the αEβ7 and α4β7 integrin heterodimers might in part be explained by effects on T_{RM} cells. For example, this antibody has been shown to block the retention of $CD8^+$ T cells from patients with UC in a humanized *in vivo* cell trafficking model suggesting that it might also reduce the retention of T_{RM} cells in the gut (66). Moreover, *post-hoc* analyses of the successful phase II trial in UC showed that patients with high expression of CD103 were more likely to respond to etrolizumab therapy (67, 68). Etrolizumab recently completed an ambitious phase III trial program in UC, in which only two out of three induction trials and no maintenance trial reached the primary endpoint. However, the drug was efficient in several important secondary endpoints and was similarly effective as infliximab and adalimumab, underscoring its biological activity and warranting further research (69–72). Phase III trials in CD are still ongoing with promising results in an exploratory cohort (73, 74).

As mentioned above, the downregulation of S1PR1 is a hallmark of T_{RM} cells. In this context, it is tempting to speculate, which effect the class of S1PR modulators including ozanimod, etrasimod, and amiselimod, which are currently also investigated for application in IBDs might have on intestinal T cells (75, 76). While it is evident that they lead to sequestration of naïve T cells and T_{CM} cells in secondary lymphoid organs (77), one could also assume that they reduce recirculation of T cells from the tissue driving the retention of local non-T_{RM} T cells.

Some of the drugs already in use in IBD might also partly affect T_{RM} cells in the gut. For instance, the anti-α4β7 integrin antibody vedolizumab that blocks T cell homing to the gut *via* MAdCAM-1 might reduce the recruitment of pre-T_{RM} cells and, thus, prevent the seeding of new T_{RM} cells [reviewed in (78)]. The anti-IL-12/23 antibody ustekinumab is thought to block the generation and differentiation of T_H1 and T_H17 cells [reviewed in (79)]. This will certainly also affect T_{RM} cells with a T_H1 or T_H17 phenotype, e.g. the *de-novo* generation of such cells might be reduced or established T_{RM} cells might be subjected to plasticity due to an altered cytokine balance (80, 81). Another drug routinely used in UC is tofacitinib, which inhibits the Janus kinase (JAK) pathway (mainly JAK1 and JAK3) and, thus, abrogates signaling of numerous cytokines (82, 83). This also affects IL-15, which is known to participate in the maintenance of T_{RM} cells (18, 33, 84). In the skin, it has already been shown that targeting CD122, a subunit of the IL-15 receptor, is a potential treatment strategy for tissue-specific autoimmune diseases involving T_{RM} cell such as vitiligo (85).

Collectively, research on T_{RM} cells as a therapeutic target is still in its infancy. However, several currently used and developed drugs, particularly etrolizumab and S1PR1 modulators, might interfere with T_{RM} cells and it is likely that the coming years will reveal further details on their suitability for treating IBD.

CONCLUDING REMARKS

Over the last decade, T_{RM} cells have emerged as an important cell population in mucosal tissues controlling the initiation of secondary immune responses. Multiple efforts have led to a precise characterization of their phenotype and implication in infection control. Moreover, they have been increasingly associated with pathological conditions, in the case of the GIT, particularly with IBD. Although not all questions are already resolved, T_{RM} cells seem to control important steps in the pathogenesis of chronic intestinal inflammation and, thus, represent a potential target for future IBD therapy. Further research is necessary to better define their pathogenetic contributions and to develop targeted therapeutic approaches.

AUTHOR CONTRIBUTIONS

E-MP, TM, KS, MN, and SZ wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Balancing Inflammation and Central Nervous System Homeostasis: T Cell Receptor Signaling in Antiviral Brain T_{RM} Formation and Function

*Colleen S. Netherby-Winslow, Katelyn N. Ayers and Aron E. Lukacher**

Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, United States

Tissue-resident memory (T_{RM}) CD8 T cells provide early frontline defense against regional pathogen reencounter. CD8 T_{RM} are predominantly parked in nonlymphoid tissues and do not circulate. In addition to this anatomic difference, T_{RM} are transcriptionally and phenotypically distinct from central-memory T cells (T_{CM}) and effector-memory T cells (T_{EM}). Moreover, T_{RM} differ phenotypically, functionally, and transcriptionally across barrier tissues (e.g., gastrointestinal tract, respiratory tract, urogenital tract, and skin) and in non-barrier organs (e.g., brain, liver, kidney). In the brain, T_{RM} are governed by a contextual milieu that balances T_{RM} activation and preservation of essential post-mitotic neurons. Factors contributing to the development and maintenance of brain T_{RM}, of which T cell receptor (TCR) signal strength and duration is a central determinant, vary depending on the infectious agent and modulation of TCR signaling by inhibitory markers that quell potentially pathogenic inflammation. This review will explore our current understanding of the context-dependent factors that drive the acquisition of brain (b)T_{RM} phenotype and function, and discuss the contribution of T_{RM} to promoting protective immune responses *in situ* while maintaining tissue homeostasis.

Keywords: T cell receptor, PD-1, brain-resident memory CD8 T cells, virus, neuroinflammation

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Jie Sun,
Mayo Clinic, United States

Reviewed by:
Aaron Jon Johnson,
Mayo Clinic, United States
Lalit K. Beura,
Brown University, United States

***Correspondence:**
Aron E. Lukacher
alukacher@pennstatehealth.psu.edu

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INTRODUCTION

Development of long-lived T cell memory is vital to protection against microbial pathogens and cancer, and a goal of vaccination efforts. Initial work identified T_{CM} which, like naive T cells, survey secondary lymphoid organs, and T_{EM}, which circulate in the blood and non-lymphoid tissues. Because of their increased numbers over naïve T cell precursors to a particular antigen, and their lower threshold for

Abbreviations: APC, antigen-presenting cell; bTRM, brain tissue-resident memory CD8 T cell; CNS, central nervous system; ColIV, collagen IV; FAO, fatty acid oxidation; i.c., intracranial; ICOS, inducible T-cell costimulator; IL, interleukin; ITIM, immunoreceptor tyrosine-based inhibition motif; ITSM, immunoreceptor tyrosine-based switch motif; Klf2, Kruppel-like factor 2; LCMV, lymphocytic choriomeningitis virus; MCMV, mouse cytomegalovirus; MHC, major histocompatibility complex; MPEC, memory precursor effector cell; MuPyV, mouse polyomavirus; PD-1, programmed cell death protein-1; PD-L1, programmed death-ligand 1; pMHC, peptide:MHC complex; PML, progressive multifocal leukoencephalopathy; S1P, sphingosine-1-phosphate; S1P1, sphingosine-1-phosphate receptor 1; SHP2, Src homology 2 domain-containing phosphatase 2; SLEC, short lived effector cell; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; T_{EX}, exhausted T cell; T_{RM}, tissue-resident memory T cell; TCR, T cell receptor; VSV, vesicular stomatitis virus; WT, wild type.

activation and reduced dependence on costimulation, T_{CM} and T_{EM} respond rapidly to pathogen reencounter (1, 2). Nearly 20 years ago, evidence emerged supporting the idea that a population of memory T cells poised with an effector arsenal resided in non-lymphoid tissues (3). More recent evidence suggests that T_{RM}, like T_{CM}, are derived from a common naive T cell precursor after local antigen exposure (4). While sharing many effector capabilities with T_{EM}, T_{RM} differed from T_{EM} in expression of trafficking molecules and having a distinct gene expression signature (5). The classification of T_{RM} as a separate subset of CD8 T cell memory prompted new investigations to define the factors that contribute to T_{RM} development and maintenance, how T_{RM}-mediated immunity contributes to the dynamic immune response to microbial pathogens, and if T_{RM} function can be harnessed for a multimodal therapeutic approach to treat or prevent infection and cancer.

An additional layer of complexity is that T_{RM} are not a homogeneous subset, because tissue environments themselves impose tissue-specific heterogeneity to T_{RM}. Most T_{RM} characterization has been done in barrier tissues; far less is understood how T_{RM} establish themselves in non-barrier sites. In particular, the brain and spinal cord are especially sensitive to tissue injury and loss from pro-inflammatory mediators. Mouse models of CNS infection, including by vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), *Toxoplasma gondii*, murine cytomegalovirus (MCMV), and mouse polyomavirus (MuPyV), have identified T_{RM} in the brain that confer antigen-specific protection against reinfection (5–9). It is likely that brain-specific factors contribute to formation of T_{RM} and their functional attributes due to the exquisite need to balance immune activation and tissue preservation in the CNS.

The trajectory of T cell differentiation is initiated by TCR engagement, then modified by costimulation and inflammation (10). The integration and duration of these signals directs a naïve T cell toward effector or memory fates, with peptide:MHC (pMHC) ligand-TCR interaction being the critical first step that guides the memory response. The strength of signal transduction events orchestrated after TCR binding with its cognate pMHC regulates induction of transcription factors, tissue-trafficking adhesion molecules, and cytokine receptors required for T_{RM} generation. Thus, TCR signal strength per se dictates the quality and abundance of the resulting T_{RM} population (11, 12). Additionally, regulating TCR signaling *via* inhibitory receptors, such as programmed cell death protein-1 [PD-1(CD279)], may be essential for T_{RM} maintenance in particular tissues by operating as a rheostat to fine tune T cell activation and effector function. This review will focus on how TCR signaling shapes the T_{RM} pool and how inhibitory receptor signaling drives the balance between effector function and long-term maintenance in tissues, an issue of especial importance in the CNS.

T_{RM} IDENTIFICATION IN BARRIER VS. BRAIN TISSUE

T_{RM} are distinguished from circulating memory T cells by the expression of the integrins CD103 (αE subunit of the αEβ7 heterodimer) and CD49a (alpha subunit of the CD49a/CD29 heterodimer), as well as the C-type lectin CD69; these molecules act to direct and retain T cells in tissues (**Figure 1**). Additionally, T_{RM} are phenotyped by the absence of cell surface sphingosine-1-phosphate receptor 1 (S1P1), the CCR7 chemokine receptor, and CD62L (L-selectin); these molecules contribute to T cell homing to (CCR7, CD62L) and egress from (S1P1) lymph nodes (13). The activating transcription factor Kruppel-like factor 2 (Klf2) targets the S1P1 gene and Klf2 downregulation is also used to define T_{RM} (14). CD103 is a common marker for T_{RM} due to its association with epithelial localization and tissue retention (15), but the requirements for CD103 expression for T_{RM} development or maintenance is a topic of some debate (16).

A role for CD103 integrins in T_{RM} retention in epithelial sites, like skin, lungs, salivary glands, and intestinal and female reproductive tract mucosa makes intuitive sense, due to its binding to the epithelial junction protein, E-cadherin. CD103 expressing T cells, however, can also be found in locations distant from epithelium, such as the brain and other non-barrier tissues; the function of CD103 expressed by T_{RM} in these locations is unclear. Using peripheral infection and dendritic cell-mediated immunization, Urban et al. recently demonstrated that non-CNS infections generated CD8 bT_{RM}. Notably, few of these CD8 bT_{RM} expressed CD103 and donor $CD103^{-/-}$ CD8 T cells yielded CD8 bT_{RM} at the same levels as donor WT cells (17). These data indicate that CD103 is dispensable for generating CD8 bT_{RM}, which contrasts with the apparent requirement for CD103 for establishment of intestinal CD8 T_{RM} (18).

To this point, $CD103^-$ T_{RM} in the brain retain T_{RM} migratory and phenotypic properties (e.g., being tissue-sessile, $CD69^+$, and $CD49a^+$) as well as T_{RM} gene expression signatures (19). During persistent infection with MuPyV, a natural mouse pathogen, $CD103^+$ bT_{RM} are more efficient effectors (7), which is consistent with evidence of signaling from CD103:E-cadherin interactions enhancing CD8 T_{RM} function, cytoskeleton reorganization, migration, cytokine release, and cytotoxicity (20–22). Although members of the cadherin family have been implicated in regulating neuron synaptic plasticity and flow cytometric analysis has shown E-cadherin expression on certain immune cells like dendritic cells and even some T_{RM} (23–27), E-cadherin is predominantly expressed in epithelial tissues. With regard to $CD103^+$ CD8 bT_{RM}, however, there is little published data on E-cadherin expression in the brain, but it has been proposed that perhaps $CD103^+$ brain CD8 T cells are interacting with E-cadherin-expressing immune cells rather than epithelial cells (16, 28). Aberrant expression of E-cadherin has also been associated with a more aggressive tumor subtype (28), but whether chronic inflammation or cancer alters E-cadherin expression in neural tissue is an open question. Alternatively, another ligand in the CNS may bind CD103 integrins expressed by CD8 bT_{RM}. TGFβ is a well-documented inducer of CD103 on T_{RM} (18). TGFβ receptor signaling acting concomitantly with TCR stimulation may modulate CD103 expression levels. This possibility raises the broader issue of whether TGFβ and pMHC availability act together or independently to affect T_{RM} development, location, and function. Although CD103 expression seems to be specific to T_{RM}, it is variably expressed

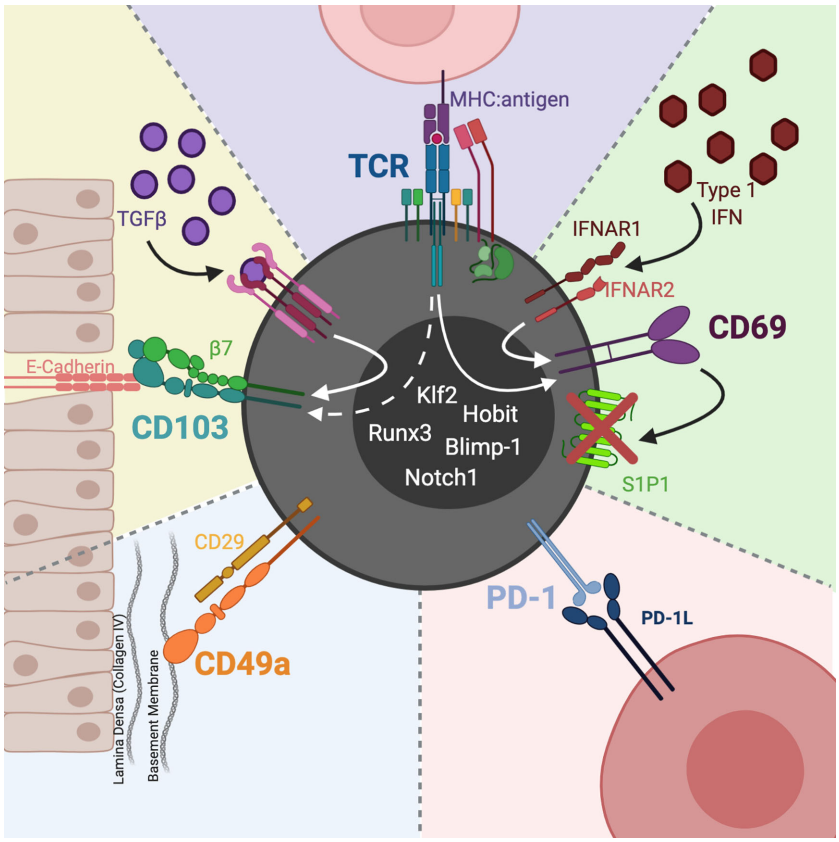

FIGURE 1 | CD8 T_{RM} phenotype and heterogeneity. CD103 is the receptor for the epithelial junction protein, E-cadherin. The CD103:E-cadherin interaction moors the T cell to the epithelial mucosa. TGFβ induces expression of CD103, whose levels may also be affected by TCR activation. CD49a partners with CD29 (integrin β1) to constitute the heterodimer VLA-1. VLA-1 binds collagen, with a predilection for Col IV in epithelial basement membranes. CD69 is a C-type lectin upregulated by type 1 IFNs as well as TCR activation. Once expressed, CD69 hinders T_{RM} egress by complexing with S1P1, leading to S1P1s internalization and degradation. In particular sites, such as the CNS, T_{RM} express PD-1 which acts to maintain functional T_{RM} and preserve tissue homeostasis. Downregulation of Klf2 and upregulation of Blimp-1, Runx3, Notch1, and Hobit transcription factors have also been used to define T_{RM}. Image created with BioRender.com.

by T_{RM} in different tissues and is arguably dispensable for T_{RM} functions. For example, CD103 blocking antibody does not negate the ability of lung CD8 T_{RM} to protect mice from lethal influenza infection (29). Thus, the requirements for CD103 for CD8 bT_{RM} maintenance, and the precise role TCR signaling plays in regulating CD103 expression warrants investigation. CD49a's role in T_{RM} development is less well defined than for CD103. CD49a does not directly attach to epithelia like CD103, but collagen IV (ColIV), its primary ligand, is positioned in the lamina densa layers of epithelial basement membranes (16, 30). The CD49a:ColIV interaction could then result in T_{RM} localization to the epithelium and subsequent tethering to CD103:E-cadherin. Furthermore, in influenza infection CD49a protects lung CD8 T_{RM} from apoptosis in part *via* interactions with collagen IV (31). A recent study shows that CD49a is required for T_{RM}-mediated protection from lethal influenza pulmonary infection (29). In the skin, however, CD49a seems to influence the effector function of T_{RM}, with $CD49a^+$ CD8 T_{RM} producing IFN-λ and $CD8^+$ $CD49a^-$ T_{RM} producing interleukin (IL)-17 (32). Although CD69 is often used as a marker of recent T cell activation, it is expressed by T_{RM} in most tissues including those of the CNS (33). CD69 is also upregulated by type I interferons independent of TCR engagement (34). CD69 binds to and induces degradation of S1P1, which enables T cells to migrate along sphingosine-1-phosphate (S1P) gradients (SIP is higher in lymphatics than tissues). The expression profile for CD69, CD103, and CD49a, however, is not exclusive to nor is it uniform across T_{RM}; disappointingly, there is no cleanly defined T_{RM} phenotype (15).

Identifying T_{RM} is made more challenging by evidence that T_{RM} can be phenotypically heterogeneous even in the same organ (15). In mice intracranially (i.c.) inoculation with an attenuated LCMV variant, only ~50% of the bT_{RM} are $CD103^+$ (9). During persistent infection with MuPyV, the vast majority of virus-specific CD8 T cells in the brain are $CD69^+$, but only ~40% expressed CD103 (19). In addition, the fraction of $CD103^-$ cells co-expressing $CXCR5^{hi}$ and TCF-1^{hi} cells was higher than the $CD103^+$ subpopulation. Elevation of both the transcription factor TCF-1 and the chemokine receptor CXCR5 on memory CD8 T cells has been linked to increased functional capability

during chronic infection (35). This is noteworthy since in chronic viral infections TCF-1 and CXCR5 aid in establishing a population of proliferation-competent memory CD8 T cell precursors to maintain a pipeline leading to end-stage exhausted T cells (T_{EX}) (36). The $CD103^+$ and $CD103^-$ subsets, interestingly, expressed similar levels of Ki67 expression and antigen-stimulation IFN-γ production, indicating comparable proliferative and functional capabilities, respectively; however, the $CD103^+$ subpopulation displayed higher effector activity (7, 19). A strategy to help reconcile these apparent discrepancies is to further stratify T_{RM} by overlaying expression of additional transcriptome molecules and cytokine receptors linked to T_{RM} differentiation, including Runx3, Notch, Hobit, and Blimp-1, as well as the receptors for IL-15, Type I IFN, TGF-β, and IL-12 (13, 37). Due to the phenotypic heterogeneity across T_{RM} populations and shared markers with other CD8 T cell subsets, more in-depth "clustering" of these molecules may help not only to ensure that a T cell is a bona fide T_{RM} but also to uncover additional breadth of T_{RM} diversity between and within tissues.

An under-appreciated feature of T_{RM} cells is the upregulation and maintenance of immune checkpoint molecules, particularly PD-1, in certain tissues and with particular viral infections (19, 38). T_{RM} generated in the skin after HSV-1 infection or the brain following MuPyV infection have increased surface expression of multiple inhibitory receptors in addition to PD-1, but retain at least partial functionality (7, 39). PD-1 is transiently expressed by CD8 effector T cells after antigen receptor signaling, but even here PD-1 inhibits functionality (40). The appellation "persistent infection" as a catchall belies the complexity of lifecycles by viruses that co-reside long-term with their hosts, such as latency-reactivation by herpesviruses vs. smoldering infections by papillomavirus and polyomaviruses. Whether bona fide memory T cells develop in the setting of persistent infection is often debated. Often overlooked, however, is the nature of the persistent infection, which depending on level, location, and timing of epitope availability may allow co-habitation by both memory and effector T cells. Compounding this complexity is that some viruses previously thought to be completely cleared after acute infection (e.g., influenza, VSV) leave residual T cell epitope-bearing antigen-presenting cells (APCs) for several weeks (41–43). Unremitting strong TCR stimulation in neoplasia and chronic viremia arguably should be considered separately from transient/low-level persistent viral infections, as the former typically render CD8 T cells profoundly dysfunctional and direct them toward an adaptive state of differentiation termed T_{EX} (44). Yet, even under these circumstances T_{EX} exert antiviral activity as evidenced by the outgrowth of CD8 T cell epitope escape variants in HIV infection (45, 46). Although PD-1, as well as CTLA-4 and TIM-3, are upregulated and sustained on the surface of CD8 T cells infiltrating tumors and in chronically infected tissues, these T cells can express molecules and gene signatures shared with T_{RM} (47, 48). Similar to its role in checking T cell-mediated autoimmunity, checkpoint inhibitors mitigate T cell-mediated immunopathology (19, 38, 49, 50). PD-1 expression as well as its role in the cell's functional adaptivity may distinguish T_{RM} from other memory CD8 T cell subsets that infiltrate the CNS (19, 38, 48).

TCR SIGNAL STRENGTH AS A DRIVER OF T_{RM} FATE AND FUNCTION

TCR signaling has been implicated in the formation of a diverse memory pool. From its initial description in the early 1980s (51), extensive research has been conducted on how signals induced when the TCR engages the pMHC complex directs effector memory differentiation and function. The relative "strength" of the TCR signal is the composite of affinity of the pMHC ligand for its cognate TCR, the amount of antigen presented on the surface of the APC (i.e., pMHC epitope density), the number of cell surface TCRs, and the duration of the TCR:pMHC interactions (52–54). The prevailing model holds that activation through the TCR orchestrates an instructional program that directs CD8 T cell expansion, effector differentiation, contraction and memory formation (55). In addition, co-stimulation through CD28, CD27, CD40, 4-1BB, and/or ICOS during priming of naïve T cells further tailors T cell fate (56–60). Cytokine input complements TCR activation to select differentiation programs and T cell longevity. For example, IL-12 promotes effector function and survival (61, 62), and IL-15 supports homeostatic maintenance of memory T cells (63–65). Kaech and colleagues have shown that a critical determinant whether a naive T cell becomes a short-lived effector cell (SLEC) or a memory precursor effector cell (MPEC) is the amount of IL-12 present during naïve T cell priming (66). IL-12 was found to regulate the level of expression of the T-box transcription factor T-bet (Tbx21) in a dose-dependent manner; high levels of T-bet instructed cells to become SLECs, and low T-bet expression favored MPEC development. Together with strength of TCR signaling, a complex tapestry of inflammatory signals and co-stimulation coalesce to influence the size and durability of a T cell memory response.

TCR signal strength also quantitatively and qualitatively shapes memory T cell differentiation. Disruption in TCR proximal signaling *in vivo* by mutating SLP-76 caused impaired Ca^{2+} influx and dampened T cell activation, without disrupting the expansion of CD8 T cells in response to acute LCMV infection (67). Weaker TCR stimulation in SLP-76 mutant mice biased CD8 T cells toward memory differentiation, with weak TCR stimulation favoring the production of cells with a $CD62L^{hi}$ T_{CM} phenotype. Our group found that CD8 bT_{RM} generated during persistent MuPyV infection possess high-affinity TCRs compared to counterparts in the spleen and kidney. Because virus-specific CD8 T_{EFF} also express high-affinity TCRs, we suggested that these cells were the progeny of high-affinity effectors recruited to the brain during the acute stage of infection (68). Indeed, we observed that there is a window of opportunity for immune cells, and possibly virus, to breach a blood-brain barrier rendered permeable during acute MuPyV encephalitis (69). A plausible possibility is that high-affinity TCRs enable CD8 bT_{RM} to detect low levels of viral antigen during persistent infection (68).

During MuPyV infection, our group reported that weaker TCR stimulation favored expansion of CD8 bT_{RM} having superior ability to respond to homologous MuPyV i.c. re-infection (11). Using site-directed mutagenesis to alter a subdominant epitope in a nonstructural viral protein of MuPyV, Maru et al. generated a panel of viruses with non-synonymous mutations in a CD8

T cell epitope to assess *in vivo* the impact of TCR stimulation strength per se on bT_{RM} differentiation. By using adoptively transferred CD8 T cells from a TCR transgenic mouse recognizing a subdominant epitope, these authors controlled the size, recruitment, and clonality of the naïve T cell response, and circumvented the confounding problems of changes in virus levels and inflammation over the course of infection. Although CD8 bT_{RM} generated in a setting of suboptimal TCR stimulation enjoyed a more robust ability to expand upon pathogen reencounter, no impact on effector function was observed. Similarly, Langlois and colleagues reported an advantage in forming influenza-specific lung CD8 T_{RM} after stimulation with low-affinity epitopes (12). Here, TCR transgenic OT-I CD8 T cells (specific for the H-$2K^b$-restricted SIINFEKL peptide from chicken ovalbumin residues 257–264) were adoptively transferred to mice infected with a recombinant influenza virus encoding native and altered OT-I epitopes. Although high- and low-affinity stimulated OT-I T_{RM} had similar phenotype and function, transcriptional profiling revealed that T_{RM} generated by low-affinity stimulation expressed increased pro-survival factors, which would favor long-term maintenance in tissues. CD8 bT_{RM} having high-affinity TCRs would likely be selected by suboptimal TCR stimulation allowing them to engage low-density epitopes or epitopes modified to limit binding to TCRs (70). The level and duration of TCR stimulation, in concert with tissue-specific cytokines, may result in upregulation of inhibitory receptors on CD8 T_{RM} to modulate their TCR signal strength, and thereby control their effector capabilities and survival (7, 71).

THE NEED TO REGULATE TCR SIGNAL STRENGTH IN bT_{RM}

Unchecked T cell activation can cause autoimmunity and immunopathology. To prevent this, inhibitory receptors constrain T cell effector functions and proliferation following TCR engagement and are upregulated in chronic infection and cancer, with the level of expression and number of inhibitory receptors dictated by the density and duration of cognate epitope (72). The importance of PD-1 and other inhibitory receptors in mitigating T cell function and prolonging longevity are well-established in animal models and humans, where blockade of PD-1 or PD-L1 reinvigorates T cell responses, reduces viral load, and/or boosts tumor control. PD-1 primarily regulates T cell activity by dampening intracellular stimulatory signals from the TCR/CD3 complex. When the PD-1 monomeric receptor engages its ligands PD-L1 (CD274)/PD-L2 (CD273), its cytoplasmic immunoreceptor tyrosine-based inhibition motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) domains are phosphorylated, resulting in binding by the Src homology 2 domain-containing phosphatase 2 (SHP2) (73). Subsequent SHP2 activation leads to tyrosine dephosphorylation of signaling molecules downstream of TCR and costimulatory receptors (74). PD-1 signaling can also result in metabolic reprograming; e.g., PD-1 signaling reduces Akt activity, suppressing mTOR (75). This effectively switches T cell metabolism from glycolysis to fatty acid oxidation (FAO). T_{RM} have a dynamic metabolic profile, but predominantly utilize oxidative phosphorylation (76). Skin CD8 T_{RM} make use of exogenous fatty acids for FAO (77). Whether CD8 bT_{RM} share this metabolic pathway remains to be determined.

PD-1 expression by CD8 T_{RM} appears to be dependent on the tissue environment and the nature of the viral infection. What governs the stability of PD-1 expression and its role in T_{RM} function and maintenance is an area of active interest. In VSV infection, CD8 bT_{RM} express low levels of PD-1 transcripts but no detectable PD-1 protein, whereas bT_{RM} from mice infected with mouse cytomegalovirus (MCMV) or MuPyV are PD-1^{hi} (5, 6, 19, 78). Youngblood et al. established that the PD-1 promoter is dynamically epigenetically regulated, with the extent of demethylation of the PD-1 promoter correlating with the strength and duration of TCR stimulation. During acute LCMV infection, the PD-1 promoter is extensively demethylated and then remethylated upon viral clearance. During chronic LCMV infection, the PD-1 promoter remains demethylated in viral antigen-specific CD8 T cells (79). In MuPyV encephalitis, the PD-1 promoter is likewise heavily demethylated in $bT_{RM,}$ and undergoes only a partial remethylation in virus-specific T cells in the spleen (19). Interestingly, maintenance of PD-1 expression on MuPyV-specific CD8 bT_{RM} was found to be independent of cognate antigen or inflammation (19). In contrast, PD-1^{hi} CD8 T_{RM} in the lungs of influenza-infected mice are maintained by MHC class I signaling and CD80 and CD86 costimulation (80). PD-1 may serve to dampen the level of TCR signaling in CD8 bT_{RM}, allowing them to exert some antiviral activity and avoid apoptosis.

Because antigen is required for CD8 bT_{RM} formation but not PD-1 maintenance, it is possible that PD-1 is an important regulator of T_{RM} function specifically in the brain microenvironment. Memory CD8 T cells in the eye, an immune privileged organ, also express PD-1 (81). In a mouse model of coronavirus CNS infection, PD-1 expression on CD8 T cells limits immune pathology and axonal damage (82, 83). The concept that PD-1 expression plays an important regulatory role in the brain is strengthened by evidence that splenic CD8 T_{RM} lack PD-1 expression during persistent MuPyV infection and that PD-L1 blockade limits CD8 bT_{RM} effector function. bT_{RM} produce IFN-γ, which regulates microglial function (84). It is also possible that microglia in turn regulate T_{RM} homeostasis through PD-1:PD-L1 interaction. A complete understanding how PD-1 regulates deleterious CD8 bT_{RM} activation in the setting of persistent viral encephalitides or whether PD-1 may selectively inhibit neuropathological effector activities remains unclear.

PD-1: AN ARBITER OF NEUROPROTECTION

CD8 T cells expressing a T_{RM} phenotype (CD69, CD103) and PD-1 progressively accumulate in the brain parenchyma with aging. Cerebral ischemia promotes production of inflammatory

mediators by these CD8 bT_{RM} (85). Clonally expanded CD8 T cells with gene signatures for cytokine-producing effector memory cells expressing CD69 and VLA-1/-4 transcripts accumulate in the subventricular zone (SVZ) of aged brains, a neurogenic niche containing neural stem cells (NSC), neural progenitor cells (NPC) and microglia; notably, IFN-γ secreted by CD8 T cells inhibits proliferation of NSCs and NPCs (86). In MuPyV encephalitis, virus-specific CD8 T cells aggregate in the SVZ subjacent to infected ependyma and produce IFN-γ *in situ* (69, 87). It is tempting to speculate that SVZ-localized antiviral CD8 bT_{RM} produce IFN-γ, which is deleterious to neurogenic niches and contributes to cognitive decline in survivors of the life-threatening brain demyelinating disease progressive multifocal leukoencephalopathy (PML) caused by the JC polyomavirus (JCPyV). Following recovery from neuropathic flavivirus infection, IFN-γ from CD8 bT_{RM} has also been show to drive microglia to eliminate synapses in the hippocampus and cause spatial-learning defects (84). These findings raise the ominous spectre that activation of JCPyV-specific CD8 bT_{RM} after PD-1 blockade may compromise learning and memory in PML survivors.

Although PD-1 is highly expressed by CD8 bT_{RM} during encephalitis by MuPyV and MCMV (7, 19, 88, 89), these bT_{RM} do not display a clear exhaustion profile (19, 90, 91). Rather, PD-1 appears to operate in the brain primarily to balance bystander- and virus-induced inflammation and tissue damage against virus control by antiviral bT_{RM} cells (90, 91). In the pancreas, PD-1 ligand-expressing macrophages control the function of the PD-1^+ CD8 T_{RM} cells. PD-1 blockade of pancreatic CD8 T_{RM} cells significantly augmented their ability to produce IFN-α, TNF-α, and IL-2 upon TCR stimulation (90). In the lung, PD-L1 blockade promoted the expansion of T_{RM} and enhanced secondary protection to influenza infection, but also resulted in the development of inflammation-induced fibrotic injury (80). These results are mirrored in the brain. bT_{RM} in MuPyV-infected PD-$L1^{-/-}$ mice had a higher frequency of IFN-γ-producing cells than bT_{RM} from MuPyV-infected wild type (WT) mice (91). Furthermore, PD-1:PD-L1 interactions were found to quell inflammation in the pancreas and brain (90, 91). CD8 T_{RM} are detected in brains of patients dying of non-neurological causes. Interestingly, these T_{RM} are $CD103^+$ $CD69^+$ and highly express PD-1 and CTLA-4 (92). bT_{RM} in healthy human brains may be telltale signs of long-resolved infections. These bT_{RM} may also provide the "fertile field" for CNS autoimmune diseases, such as multiple sclerosis by secreting chemokines that attract circulating self-reactive T cells (93). Thus, expression of checkpoint inhibitory receptors, such as PD-1, may act to halt production of such chemokines and the potential for CNS autoimmune diseases. PD-L1 expression by MHC-I/II-expressing CNS-resident cells (e.g., microglia) may, in turn, be critical determinants of susceptibility to CNS autoimmunity. Collectively, these data support the likelihood that CD8 T_{RM} in the brain retain expression of checkpoint inhibitory molecules to limit tissue-injurious inflammation and preserve CNS integrity.

With the heightened effector functionality of T_{RM} consequent to interrupting PD-1 signaling, PD-1 or PD-L1 blockade could be anticipated to enhance T_{RM} response against persistently infecting viral pathogens. In a small randomized and placebo-controlled study, 3 out of 6 patients with hepatitis C virus given a new humanized ligand-blocking PD-1 antibody exhibited 4-log reductions in viral load, but this was associated with immunologic adverse events, including autoimmune thyroiditis (94). In a phase Ib study of patients with chronic hepatitis B virus (HBV) infection, nearly all of the patients given a single infusion of the PD-1 blocking antibody nivolumab experienced a decrease in HBV surface antigen (HBsAg) titers (95). Finally, in individuals with PML, a significant number of patients receiving anti-PD-1 had fewer cerebrospinal fluid JCPyV genome copies, elevated JCPyV-specific CD4 and CD8 T cell responses, and importantly, clinical improvement or disease stabilization (96, 97). A likely critical variable in the success of PD-1 blockade therapy is the severity of infection at the time of therapy initiation, with higher viral burden being associated with greater risk of immune-mediated complications. Although these studies do not directly assign effects of the PD-1:PD-1L blockade to $bT_{RM,}$ they demonstrate the importance of checkpoint inhibitor blockade as an anti-viral therapy in humans. Knowing that bT_{RM} have increased effectivity in mouse models lacking either PD-1 or PD-L1, a plausible hypothesis is that the antiviral effects of the PD-1:PD-1L blockade in humans could be due to resurrected effector activity by bT_{RM}.

Beyond affecting the functional capabilities of T_{RM} cells, recent reports suggest that PD-1 is involved in the development of T_{RM} in different tissues, including those in the CNS. During MCMV infection, $CD103^+$ $CD69^+$ bT_{RM} populations were sparse in PD-$L1^{-/-}$ and PD-$1^{-/-}$ mice compared to WT mice, implicating PD-1 signaling as a positive factor in development of bT_{RM} (89). PD-1 is involved in governing T cell activation, fate, function, and tolerance as well as immune homeostasis (98). Therefore, using a global PD-1 knock-out system could have altered the fate of all T cell subsets and not just that of the $bT_{RM.}$ Conversely, in response to MuPyV, a higher frequency of $CD103^+$ CD8 T cells populations were observed in brains of PD-$L1^{-/-}$ mice as well as in mice treated with PD-1 blocking antibodies compared to the WT mice (91). These conflicting findings raise the caveat that PD-1's role in the CNS can differ between viral infections and highlight the need for caution in extrapolating conclusions of immune responses across infection models. By extension, understanding how PD-1 controls T_{RM} development in different CNS viral infections should uncover novel insights in mechanisms of détente between viral control and collateral tissue injury by CD8 bT_{RM}.

CONCLUDING REMARKS

Accumulating evidence supports the concept that T_{RM} progenitors are generated early in the course of effector differentiation. An intriguing possibility is that factors such as TCR signal strength or differential expression of inhibitory receptors contributes to a nuanced differentiation spectrum that guides development of T_{RM}. Similar ideas hold true for T_{EX}. Recent work reveals that T_{EX} exist as a continuum from self-renewing "stem-like"

progenitors that progress to a nonproliferative terminal state which is vulnerable to death. T_{EX} at different stages vary in their ability to respond to immune checkpoint blockade therapy (36). MuPyV-specific CD8 bT_{RM} heterogeneously express many molecules associated with T_{EX} subsets (36, 87). Single-cell analysis of adaptive immune cells in ulcerative colitis patients suggests that transcriptional heterogeneity also exists in the T_{RM} compartment and its demarcation into distinct differentiation stages (99). Similarly, lung CD8 T_{RM} generated to influenza infection exhibit both exhausted and memory characteristics by phenotype, transcriptome, and function (80). The proportion of T_{RM} in each stage of differentiation, however, will certainly be altered by disease processes and possibly by immunomodulatory regimens as well. Recent work also demonstrates that the quality of functional CD8 T_{RM} responses in the influenza-infected lung is dependent on the type of cell presenting viral antigens (100). Furthermore, T_{RM} can also egress from tissues, convert into other memory subsets, and change their migratory behavior depending on the inflammatory context (101, 102). Together these findings contribute to an increasingly multidimensional view of the factors that drive T_{RM} formation, what constitutes tissue residence, and the role T_{RM} play in antiviral defense. Particularly important for persistent neurotropic viruses is to develop a comprehensive understanding how bT_{RM} balance virus control against neuropathology and to learn how this equilibrium is established for different viral infections.

AUTHOR CONTRIBUTIONS

CN-W wrote the original draft and revised the manuscript. KA wrote the original draft, revised the manuscript, and prepared the figure. AL revised this manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Legend of the Sentinels: Development of Lung Resident Memory T Cells and Their Roles in Diseases

Youkun Qian[†], *Yicheng Zhu*[†], *Yangyang Li*[*] *and Bin Li*[*]

Department of Immunology and Microbiology, Shanghai Institute of Immunology, Shanghai Jiao Tong University School of Medicine, Shanghai, China

SARS-CoV-2 is wreaking havoc around the world. To get the world back on track, hundreds of vaccines are under development. A deeper understanding of how the immune system responds to SARS-CoV-2 re-infection will certainly help. Studies have highlighted various aspects of T cell response in resolving acute infection and preventing re-infections. Lung resident memory T (T_{RM}) cells are sentinels in the secondary immune response. They are mostly differentiated from effector T cells, construct specific niches and stay permanently in lung tissues. If the infection recurs, locally activated lung T_{RM} cells can elicit rapid immune response against invading pathogens. In addition, they can significantly limit tumor growth or lead to pathologic immune responses. Vaccines targeting T_{RM} cells are under development, with the hope to induce stable and highly reactive lung T_{RM} cells through mucosal administration or "prime-and-pull" strategy. In this review, we will summarize recent advances in lung T_{RM} cell generation and maintenance, explore their roles in different diseases and discuss how these cells may guide the development of future vaccines targeting infectious disease, cancer, and pathologic immune response.

Keywords: tissue-resident memory T cells, lung, infection, asthma, cancer, vaccine

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***Correspondence:**
Bin Li
binli@shsmu.edu.cn
Yangyang Li
liyangyang@shsmu.edu.cn

[†]*These authors have contributed equally to this work*

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INTRODUCTION

The COVID-19 pandemic is ravaging the world. By the end of November 2020, there are over 60 million cumulative cases globally, and the number of deaths has exceeded one million (1). This disease is caused by SARS-CoV-2, which is mainly transmitted through air-borne droplets, leading to severe pulmonary diseases and systemic damage (2). Up to now, the treatment for COVID-19 is very limited, and no specific antiviral drug has been developed. Multiple candidate COVID-19 vaccines are undergoing clinical trials (3).

In general, most COVID-19 vaccines in clinical trials focus on humoral immunity, which exerts antibodies to prevent the virus from invading cells. However, antibodies alone may not be sufficient to prevent SARS-CoV-2 infection. One reason is that extracellular antibodies cannot completely clear the cells infected by virus (4). The final elimination of the virus depends on the supplement of cellular immunity, that is, the role of T cells, which help B cells produce neutralizing antibodies and can directly kill virus-infected cells. The second is that the memory B cell response tends to be short-

lived (5), whereas the T cell response can last for many years. Recent researches have demonstrated that patients who recovered from the severe acute respiratory syndrome (SARS) still had long-lasting memory T-cells but reduced antibody responses (6, 7). Therefore, vaccines against SARS-CoV-2 should focus on activating the adaptive branch of the immune system and explicitly focus on inducing long-term memory T cells. Given that many respiratory viruses are controlled by tissue immune cells that may not be present in the blood, the tissue-resident memory T (T_{RM}) cells infiltrated in the lungs that can recognize foreign antigens locally and provide a rapid immune response will be an area of concern.

Actually, CD8+T cells retained for a long time after influenza virus infection were observed in mouse lungs as early as 2001 (8). Extensive studies in mouse models have determined that the lungs are enriched in T_{RM} cells against a variety of viral and bacterial antigens brought by respiratory infections or vaccination. Specific T_{RM} cells were also detected in the respiratory tract of patients with influenza or tuberculosis (TB) (9). These pathogen-specific T_{RM} cells produced by prior exposure can control acute re-infections and achieve long-term immunity. In mouse model, an intranasal recombinant vaccinia virus boosting regimen has generated SARS-CoV-specific lung resident memory CD8+T cells. When re-stimulated, these T_{RM} cells can effectively release a variety of effector cytokines and cytotoxic molecules that prevent extensive virus replication and limit the alveolar damage (10). Another study suggested that the administration of SARS vaccine intranasally induced CD4+ T_{RM} cells in the respiratory tract of mice, which offered the protective immunity against death (11). Regarding SARS-CoV-2, recent published single-cell profiles have indicated that the CD8+ T cells in bronchoalveolar lavage fluids (BALFs) of patients with severe infection exhibited a less proportion of tissue-resident phenotypes than those in moderately infected patients (12). Hence a vaccine that induces the production of lung T_{RM} cells is an ideal candidate for generating a strong and rapid immune response against SARS-CoV-2.

There are other T_{RM} cells in the lungs with different roles, including T_{RM} cells that may cause pathological immune responses and tumor-infiltrating T_{RM} cells that can enhance anti-tumor immunity in the lungs (13). These T_{RM} cells under different immune microenvironment in the lungs act in various roles in immune defense, immune homeostasis, and immune surveillance. An in-depth understanding of the generation and maintenance of lung T_{RM} cells will provide new insights for the development of novel vaccine formation and delivery strategies and lung-specific immunoregulatory therapy.

This review will focus on the definition, generation, and different roles of lung T_{RM} cells in infection, pathological immune responses, and cancers, and discuss T_{RM} cell-related vaccination strategies combined with emerging cutting-edge discoveries.

HALLMARKS OF T_{RM} CELLS

T_{RM} cells, also known as non-circulating memory T cells, include both CD8+ and CD4+ subgroups. It refers to those memory T cells that occupy long-term residency in local tissues such as lung, intestine, and skin. Through cell labeling, parabiosis, tissue transplantation, and other methods, the circulation trajectory of cells can be observed to determine T_{RM} cells (14–16). However, it is still a challenge to clearly distinguish T_{RM} cells from other cells *in vitro* by surface markers.

In recent years, with the development of transcriptomics, T_{RM} cells have been found to have unique transcriptional profiles and functional characteristics. The main hallmarks of T_{RM} cells that distinguish it from other circulating memory T cells are the ability to adhere to peripheral tissues and the lack of homing signals. Based on the research on both mouse and humans, the most used phenotypic marker defining T_{RM} cell subsets is CD69. Due to the competitive protein-protein interaction between CD69 and sphingosine-1-P receptors (S1PR), it inhibits the expression of S1PR and prevents S1P-mediated egress (17, 18). These cells also lack CD62L and CC-chemokine receptor 7 (CCR7), both of which direct cells into lymphoid tissue (19). On the flip side, CD44 up-regulated by T_{RM} cells is the receptor for hyaluronic acid and other ligands expressed in peripheral tissues, which can induce the retention of memory T cells in peripheral tissues (20). As another key T_{RM} cell marker, the integrin αE:β7 (CD103) is mainly expressed on CD8+ T_{RM} cells and some on CD4+ T_{RM} cells, which binds E-cadherin and anchors cells around epithelial cells (21). It is worth noting that T_{RM} cells in lungs can be defined by several major surface markers, but this subset itself is still heterogeneous in some way. The transcriptome analysis reveals the inconsistent changes in gene expression among different cells (19, 22, 23). Further elucidation of detailed mechanism of T_{RM} cell formation and maintenance will add to understanding of the phenotype of lung T_{RM} cells under different pathophysiological conditions.

DEVELOPMENT OF LUNG T_{RM} CELLS

The development of lung T_{RM} cells can be divided into several steps: 1) activation in lymphoid tissues and migration into inflammatory lung tissue guided by local cytokines, 2) expression of homing molecules and specific transcription factors and differentiation into lung resident memory T cells, 3) local maintenance in specific niches and replenishment from T_{CM} cells (**Figure 1**). So far, the focus on specific transcription factors and cell surface receptors has gradually revealed details in the fate determination mechanism of lung T_{RM} cells.

Activation and Migration

The inability to recirculate between lung and lymph nodes or bloodstream is a key determinant of lung T_{RM} cells (24, 25). However, these cells did not start in the lung tissue but migrated into it later. Under normal conditions, naïve T cells consecutively circulate throughout the body. When infection occurs, dendritic cells (DCs) migrate from infected respiratory sites into mediastinal lymph nodes (MdLN) and activate naïve T cells. Among these migrant DCs there are two subsets, and only airway localized CD103+ DCs can fully induce the differentiation of

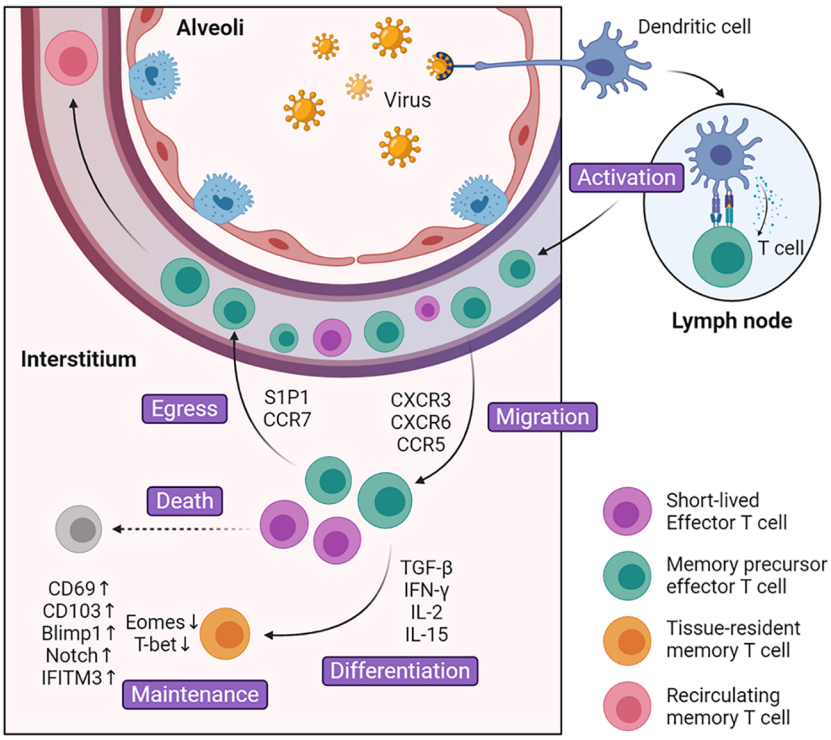

FIGURE 1 | Generation and maintenance of lung T_{RM} cells. During the activated phase of infection, dendritic cells present antigens to activate naïve T cells in the lymph nodes. These cells turn into effector T cells and up-regulate surface marker CXCR3, CXCR6, CCR5, which guide them into inflammatory tissues. After entering lung tissue, part of effector T cells is regulated by environmental signals including cytokines such as TGF-β and cognate antigens, and differentiate into lung T_{RM} cells. The rest of the effector T cells undergoes cell death or egress out of the lung. Compared with Teff cells, lung T_{RM} cells manipulate multiple surface markers and transcription factors that facilitate cell maintenance and survival.

naïve T cells into T_{eff} cells (26). Once activated, the T_{eff} cells up-regulate the expression of CXCR3, CCR5, and CCR4, which specifically guide T_{eff} cells into lung tissue and help control pathogen invasion (27–31). For example, after TB infection, chemokine ligand IP-10 in the lung increases significantly, which binds to CXCR3 and facilitates T cell migration (29). In addition, CD8+ and CD4+ lung T_{eff} cells are regulated differently and tend to localize in different regions. CD8+ T_{eff} cells are inclined to migrate to the collagen IV-rich region and CD4+ T_{eff} cells are more prone to be located in areas abundant in collagen I (32). Compared with CD8+ T cells, CD4+ T cells enter the lung tissues first and direct the localization of CD8+ T cells. CD4+ T cells fine-tune chemokine gradients in the microenvironment such as TGF-β, which promotes the production of CD103 and is crucial for CD8+ T_{RM} cell formation (33).

Differentiation

T_{eff} cells will not transform into lung T_{RM} cells immediately after entering the lung tissues. The tissue microenvironment has an important influence on the development of lung T_{RM} cells. In the early stage of infection, T_{eff} cells that migrate into the infection site will encounter redundant inflammatory signals, which guide T_{eff} cells towards terminal T_{eff} cells (34). They reduce local inflammation, help remold the microenvironment and make it more appropriate for the differentiation of lung T_{RM} cells. In the later stage, CD8+ T cells are recruited into tissue damage sites, which later developed into regenerative tissues termed as repair-associated memory depots (RAMDs). RAMDs provide environmental cues that help drive CD8+ T_{eff} cells into CD8+ T_{RM} cells and later become niches for CD8+ T_{RM} cells (35, 36). Predominant environmental cues include cytokines such as TGF-β, IL-33, TNF, IFN-γ, IL-15, and cognate antigens (18, 33, 37). TGF-β plays an important role in promoting the expression of T_{RM} cell marker CD103 and CD69. Together with IL-33 and TNF, TGF-β can provoke KLF2 down-regulation, which further down-regulates its target protein S1P1 and increases expression of CD69 (18). Furthermore, TGF-β down-regulate T-box transcriptional factor and promote the expression of CD103. T-box transcriptional factors are composed of eomesodermin (Eomes) and T-bet, and they vary in the degree of decline. While Eomes is effectively removed, T_{RM} cells maintain residual levels of T-bet which is important for T_{RM} cell survival (37). The decrease in production of T-box transcriptional factor is demonstrated in mature lung CD8+CD103+ T_{RM} cells (33, 37). Unlike CD8+ T_{RM} cells in other tissues like skin and vagina, where they can be generated with only local inflammatory signals (38), lung CD8+ T_{RM} cells must interact with cognate antigen before

differentiation. After the exposure to cognate antigen, CD8+ T_{eff} cells increase the expression of CD69, CD103, and collagen-binding integrin VLA-1 (39). T cell receptor (TCR) signaling can also induce Blimp-1 expression, which biased CD8+ T_{eff} cell differentiation towards T_{RM} cells rather than T_{CM} cells (40). It is surprising that pulmonary monocytes and type 1 regulatory T (T_{reg}) cells also contribute to the differentiation. Pulmonary monocytes are the major cells to present pathogen antigens, while type 1 T_{reg} cells promote the bioavailability of TGF-β (41, 42). As mentioned above, CD4+ T_{RM} cells have different development pathways compared with CD8+ T_{RM} cells. CD4+ T_{RM} cells express different cell markers and are affected by different cytokines (43). They have low expression of CD103, and their generation is not interfered by TGF-β, which has a great impact on the generation of CD8+ T_{RM} cell (44, 45). Beyond that, IL-2 and IL-15 were found to affect the differentiation of CD4+ T_{eff} cells in different subsets, respectively (44). Researches on differentiation of CD4+ T_{RM} cells are not as thorough as those on CD8+ T_{RM} cells, and there are still many points to be clarified.

Maintenance

While persisting in lung tissues, CD8+ and CD4+ T_{RM} cells will construct different structures that contribute to long-term survival. Most CD8+ T_{RM} cells reside in specific niches we refer to as RAMDs, which are constructed by tissue regeneration after tissue damage. These niches are significant for lung CD8+ T_{RM} cells. They may present cytokines that help lung CD8+ T_{RM} cell maintenance. Considering that the recovery of tissue damage takes a long time, the lung CD8+ T_{RM} cells may protect this vulnerable part from secondary infection (35, 36). Unlike CD8+ T_{RM} cells, lung CD4+ T_{RM} cells combine with B cells and other cells to form ectopic lymphoid tissue called inducible bronchus-associated lymphoid tissue (iBALT) that benefits cell survival. In iBALT, CD4+ T_{RM} cells surround B cell follicles, which facilitate rapid interaction with each other and provide a recall response toward potential infection (43, 46). Compared with circulating T_{EM} cells, lung T_{RM} cells displayed different patterns of genes and transcription factors that regulate the expression of cytokine receptors and adhesion molecules, most of which have been mentioned above. Single-cell sequencing found an important transcription factor Notch, which controls the expression of CD103 and the basic metabolic function of lung T_{RM} cells (47). The absence of Notch greatly reduces the population of lung T_{RM} cells. Another study indicated that lung T_{RM} cells were programmed to express IFITM3, which can protect them from secondary infection and improve survival (48). Except for cytokines and surface molecules, $M1^{hot}$ tumor-associated macrophages can also contribute to the maintenance of lung T_{RM} cells in tumor, possibly due to reduction in nutrition competition (49). In comparison with other tissue T_{RM} cells that may persist for a long time or even a lifetime, lung T_{RM} cells gradually disappear 4–5 months after infection. Lung T_{RM} cells that reside in the airway quickly decline due to the harsh environment, where amino acid starvation triggers the integrated stress response, leading to cell apoptosis (50). And those retained in the parenchyma decrease along with the shrink of RMADs. After full regeneration, most of the RAMDs will disappear, and only a minority of lung CD8+ T_{RM} cells may survive in iBALTs (35, 36). In order to compensate for the constant loss, airway T_{RM} cells are replaced primarily by recruitment from lung interstitium (51), and T_{RM} cells in interstitium receive continuous replenishment from circulating T_{EM} cells. T_{EM} cells are recruited and transformed into lung T_{RM} cells under the influence of TGF-β, IL-33, and TNF but antigen-independently. However, T_{EM} cells gradually lose their ability to migrate and convert into lung T_{RM} cells after infection (52). All in all, T_{RM} cells can only provide a short period of protection, which leaves the lung much more susceptible to further infection. However, this may be a designed mechanism for the prevention of pathological immune response.

LUNG T_{RM} CELLS AGAINST INFECTION

The lungs and respiratory tract, as part of direct access to the outside world, are easily exposed to various pathogens. Common pulmonary pathogens include influenza virus, respiratory syncytial virus (RSV), as well as Streptococcus pneumoniae, Klebsiella pneumoniae, Bordetella pertussis, and Mycobacterium tuberculosis. Under normal circumstances, the first infection caused by these pathogens will not only be cleared by the body's immune system but also induce memory T cells, some of which settle in the lungs as T_{RM} cells (**Figure 2**).

A large aggregation of studies has shown that the lung is rich in T_{RM} cells specific to a variety of pathogens such as viruses and bacteria. These T_{RM} cells have the potential to mediate immunity against different pathogens and protect the body from re-infection. It has been demonstrated that influenza-specific T_{RM} cells exhibited rapid and robust IFN-γ and TNF-α responses after restimulation *in vitro* (53, 54). In human RSV challenge model, cells with T_{RM} phenotype can be detected in BALFs, and the higher frequency of RSV-specific CD8+ T_{RM} is related to the decrease in the severity of disease and the viral load (55). CD4+ T_{RM} cells accumulate in the lungs after Bordetella pertussis infection. These cells are pathogen-specific and can secrete IL-17 and/or IFN-γ. A research observed that mice treated with the S1P antagonist Fingolimod (FTY720) to prevent lymphocyte migration into the lungs before initial infection with Bordetella pertussis were significantly more severely affected in the later stages of infection. However, in the case of re-infection, because the tissue-infiltrated T_{EM} cells have partially transformed into T_{RM} cells in the lung, they are not affected by Fingolimod treatment and can still quickly clear the bacillus. At the same time, the adoptive transfer of CD4+ T_{RM} cells from the lungs of mice in convalescence to uninfected mice can protect the latter from pathogens attack (56). All these evidences indicate that T_{RM} cells act as a pivotal role in the rapid response of secondary infection.

However, while T_{RM} cells eliminate invasive pathogens, the released proinflammatory factors such as IFN-γ or perforin and granzymes may damage normal cells, cause lung injury and lead

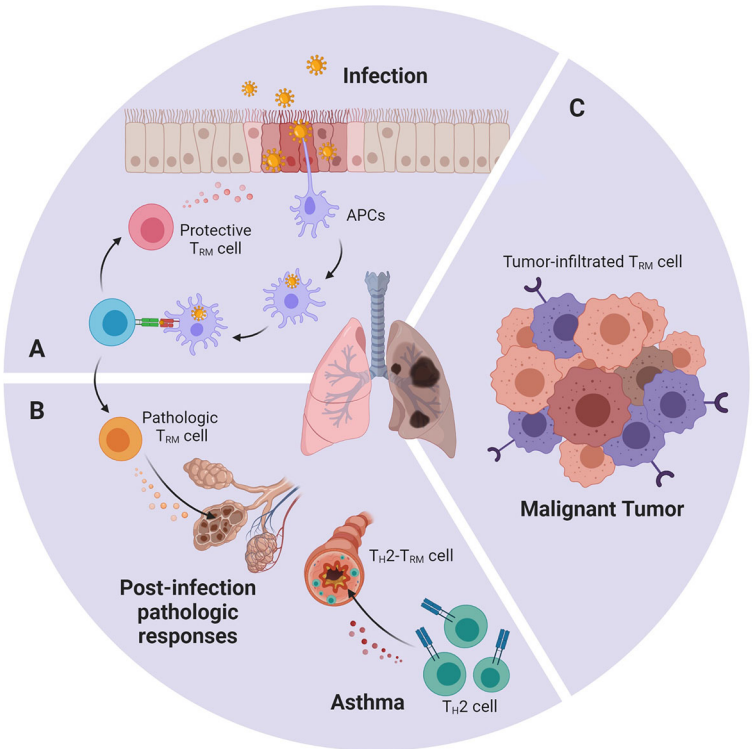

FIGURE 2 | An abstract figure of the role of T_{RM} cells in various lung diseases. Lung T_{RM} cells can: **(A)** rapidly respond towards invasive pathogens during re-infection, **(B)** cause pathologic immune response after overactivated by environmental stimuli or allergen **(C)** infiltrate in lung tumor and express cytotoxic molecules and effector cytokines.

to emphysema or fibrosis, even result in ARDS. Hence, an effective immune response to these infections requires precise immune regulation to eliminate pathogens while protecting the function of normal lung tissue. Many mechanisms exist in the lung to restrict the inflammatory response to acute infection, including inhibitory receptors, immunomodulatory molecules and cells like FOXP3+CD4+ T_{reg} cells (57). Under stable conditions, a large number of T_{reg} cells is reserved in the lung and IL-10 expression is significantly increased after influenza infection (58). In RSV-infected mice, the TCR of T_{reg} cells can specifically recognize the viral epitope-MHC II complex. Immunization of mice with this epitope can reduce clinical manifestations and immunopathology without virus clearance defects (59). In addition, PD-L1 and PD-L2 are expressed in alveolar epithelial cells and are significantly up-regulated to control inflammation in RSV infection (60). However, some studies held that this may limit the formation and development of T_{RM} cells and cause negative effects (61). The detailed mechanisms of lung T_{RM} cell function and immune homeostasis are not yet fully understood, and future improvement in the number and stability of T_{RM} cell population must be carried out on the premise that prevents re-infection of the virus and does not impair the respiratory health of the host.

LUNG T_{RM} CELLS IN PATHOLOGIC IMMUNE RESPONSE

As mentioned above, sometimes T_{RM} cells may cease to be the protector and become part of the destructor, and thus attack normal tissue and induce chronic inflammatory diseases (13) (**Figure 2**). After acute influenza infection, antigen deposits in the lung for 2–3 months. In young mice, the persistent presentation of the antigens may induce part of the T_{RM} cells to exhibit exhausted-like phenotype. This phenotype is thought to help maintain lung's immune balance and prevent damage. If PD-L1 antibody is used to blockade PD-L1 and PD-1 interaction, exhausted-like T_{RM} cells would rejuvenate, express more cytokines, and enhance their heterogeneous protection against infection. But they would also cause pulmonary pathological change and fibrosis (62). In elderly mice, increased expression of TGF-β in the environment led to accumulation of T_{RM} cells in the lungs. However, these T_{RM} cells have low effector activity due to intrinsic defects and fail to enhance the protective function, but can instead lead to chronic inflammation and fibrotic sequela (63). Also, it has been discovered that T_H2-T_{RM} cells are closely related to asthma (64). They release specific cytokines that recruit eosinophils and maintain mast cells in the airway, which result in

the inflammatory response. Using a mouse model exposed to house dust mite (HDM), T_H2-T_{RM} cells that specifically respond to HDM are identified. These T_H2-T_{RM} cells are developed from HDM-specific CD4+ T_{eff} cells and are mediated by IL-2 signaling. IL-2 up-regulates chemokine receptors such as CCR4 and CXCR3 that improve migration into the lung, as well as programs related to tissue intention (64). A recently published paper further reports that these T_H2-T_{RM} cells highly express CD44 and ST2, and can reside in lung tissue and maintain their memory towards allergen for the whole life of a mouse (65). Once re-exposed to allergen, T_H2-T_{RM} cells robustly proliferate near airways, produce type 2 cytokines, enhance eosinophil activation, and promote peribronchial inflammation. They together with circulating memory T_H2 cells perform nonredundant function in the induction of asthma (66, 67).

LUNG T_{RM} CELLS IN ANTI-TUMOR IMMUNITY

Accumulating evidence suggests that T_{RM} cells are important in anti-tumor immunity (**Figure 2**). It is suggested that a part of the tumor-infiltrating lymphocytes (TILs) isolated from several cancers displays a similar transcriptomic and phenotypic feature with T_{RM} cells. Some refer to it as T_{RM}-like TILs (9), but here we still call it "lung tumor T_{RM} cells", as the consensus in most articles. These lung tumor T_{RM} cells predict a better survival outcome in early-stage non-small-cell lung carcinoma (NSCLC) patients, as well as increased intraepithelial lymphocyte infiltration (68). Single-cell and bulk transcriptomic analysis reveals that lung tumor T_{RM} cells have slightly different transcriptomes compared with other lung T_{RM} cells. They express similar surface marker CD103, CD69, CD49a, and they also up-regulate Notch and Runx3. But lung tumor T_{RM} cells express more cell cycle-related genes, such as CD39, CXCL13, CCL3, and TNFSF4, indicating that they belong to a new subset (22). Comparing samples from different lung cancer patients, the T_{RM} cells of advanced lung cancer are mostly exhausted, while the function of early-stage lung tumor T_{RM} cells is relatively heterogeneous (69). Among them, CD103+CD8+ T_{RM} cells are found to release more cytokines, proliferate faster, and exhibit better anti-tumor performance (70). It is described that CD103 can connect with E-cadherin on tumor cells, which induces cytotoxic granule polarization at the immune synapses (71, 72). CD103 also facilitates T_{RM} cells to reside near tumor tissues (73). In contrast with previous studies, lung tumor T_{RM} cells show the diffuse expression of inhibitory receptors, but do not exhibit the exhausted phenotype. And instead, transcription factor Eomes is found to negatively correlate with T_{RM} cell function (69, 74). Single-cell analysis even discovered a PD-1+TIM-3+IL-7R- T_{RM} cell subset expresses high levels of inhibitory receptors, but remains the ability to proliferate rapidly *in situ* and displays enhanced capacity to express key cytotoxic molecules and effector cytokines (22). Since TIM-3+IL-7R- T_{RM} cells are the major cells expressing PD-1, and CD103+CD8+ T_{RM} cells show positive responses towards anti-PD-1 and anti-PD-L1 monoclonal antibodies, the researchers believe that these cells may be the major subset that reacts in anti-PD-1 therapy (22, 68, 70). In combination with the performance of T_{RM} cells in different stages of lung cancer, it has been speculated that T_{eff} cells were influenced by tumor antigens and cytokines such as TGF-β, up-regulate CD39 and CD103, and converted into CD103+ T_{RM} cells. They exercise their anti-tumor function diligently. If, for one reason or another, the tumor is not eliminated, the local microenvironment as well as the repetitive TCR stimulation may trigger their exhaustion program and they finally become hypofunctional T_{RM} cells (69, 75).

VACCINATION STRATEGIES INDUCING LUNG T_{RM} CELLS

The growing literature that considers T_{RM} cells are indispensable in eliminating infectious pathogens and controlling tumor progression has led to increasing interest in the induction of T_{RM} cells by vaccination for disease treatment and prevention. Compared with circulating T cells or B cells, activated T_{RM} cells are more focused in killing virus-infected cells in target tissues, which help complement neutralizing antibodies and reduce antibodies titer threshold needed to control virus (4, 76, 77).

There are two main strategies to establish T_{RM} cell pool within lung tissues. The first approach applies a one-step method to directly induce antigen-specific lung T_{RM} cells by vaccine vectors (78, 79). For this approach, the route of immunization is very important. Direct intranasal or intrapulmonary route provides better protection compared with commonly used intraperitoneal, intramuscular, or subcutaneous administration route (80, 81). Intranasal administration but not injection of live-attenuated influenza virus has shown the capacity to generate long-term CD4+ and CD8+ T_{RM} cells and provide heterosubtypic protection to nonvaccine influenza strains in mice (82). Intratracheal and intranasal rather than subcutaneous inoculation of Bacille Calmette-Guérin (BCG) also results in generation of T_{EM} and T_{RM} cells in the lung, which remedy the low efficacy of parenteral BCG vaccination to prevent pulmonary TB (83). In a preclinical head and neck cancer model, local T_{RM} cells can be induced and tumor growth can be controlled in mice immunized with the cancer vaccine (STxB-E7) by intranasal route (84). Another approach is a two-step method that combines conventional elicitation of systemic T cell response with the recruitment of these cells into target tissues, which are referred to as "prime and pull" (85). Actually, in a very early stage, scientists have discovered that mucosal boosting with the same vaccine after systemic priming can elicit more CD4+ and CD8+ lung T_{RM} cells compared with only mucosal or systemic vaccination (80). There is also evidence indicates that compared with the original "prime and pull" strategy used in genital tract, the pull step applied in lung disease should use pathogen antigens instead of proinflammatory chemokines. This is because only pathogen antigens can maintain the recruited T cells in airway lumen and persevere immune protection over time (86). Intranasal administration of a novel recombinant anti-TB vaccine (SeV85AB) after subcutaneous immunization with BCG uses this way to provide larger immune protection for lungs than either

SeV85AB or BCG alone (87). As opposed to vaccines that directly provide the pathogen antigens like SeV85AB, recent research developed an "antibody-targeted vaccination (ATV)" for the pull step. It connects antigen with antibody that targets lung DC cells, give raise to local antigen presentation, and improve activation of lung T_{RM} cells (88). Pulmonary surfactant-biomimetic liposomes containing stimulator of interferon genes that target alveolar epithelial cells give a new way to recruit CD8+ T_{RM} cells and provide long term wide-spectrum protection (89). These methods may also be used in inducing tumor antigen presentation and lung tumor T_{RM} cell function.

In summary, multiple studies have proved that T_{RM} cells can be induced by vaccination to make a difference in preventing pathogens or controlling tumor growth. However, many problems remained to be solved, for example, how to attract T_{eff} cells into target areas not close to mucosal, and how to maintain long-term lung T_{RM} cells (79). Systemic approaches should also be developed to evaluate the safety and efficiency of these vaccines and prevent overactivation of T_{RM} cells resulting in pathologic immune responses (90).

CONCLUDING REMARKS

It is now obvious that lung T_{RM} cells are an important part of the adaptive immune response within lung tissues. Although we have a rudimentary understanding of lung T_{RM} cells, they remain shrouded in mystery, waiting to be discovered more. While mentioning the migration, activation, differentiation, and maintenance of lung T_{RM} cells, main steps are outlined but there are still huge empties in the details. Do lung T_{RM} cells undergo pre-differentiation in lymph nodes before infection (91)? Which cytokines, transcription factors, and surface molecules are more decisive in the migration, formation, and maintenance of lung CD4+ or CD8+ T_{RM} cells? Are there different subtypes of lung T_{RM} in different lung tissue structures (such as in interstitium and parenchyma)? To answer these questions, more advanced techniques such as single-cell RNA-sequencing that identifies cell-cell interaction and TCR lineage tracking may be used.

A better understanding of these issues will undoubtedly help better manipulate lung T_{RM} cells to prevent or treat disease. Therapy focusing on lung T_{RM} cells in tumor and pathologic immune response is still in a nascent state. Besides direct activation or transmission of tumor-specific T_{RM} cells, currently there are vaccines that activate antiviral lung T_{RM} cells near tumor tissue (92), which reverse the immunosuppressive microenvironment, and may pave the way for later cell therapy. Drugs that prevent lung T_{RM} cell formation or function may also be useful in suppressing the immune response to lung transplantations or preventing lung sequela after respiratory infection in the elderly (63). Of course, T_{RM} cells in the lungs are mostly deemed to fight off lung infections. During the COVID-19 pandemic, lung T_{RM} cells are particularly important in the first line of defense against re-infection of SARS-CoV-2. Actually, influenza viruses have never been conquered, not only because of its versatility, but also because the immune memory only lasts for a short time in lung. To fight them, one possible solution is to improve the "width and depth" of the function of vaccines that induce lung T_{RM} cells. The width refers to the prospect that the same vaccine can induce lung T_{RM} cells that resist a wide range of virus strains in response to virus variability (88). The depth hopes that the induced T_{RM} cells can remain in the lungs for nearly lifelong, enhancing the killing effect and duration of protection of the vaccine (79). More insight and precise manipulation of the fate of lung T_{RM} cells will help to better develop novel immunomodulators to treat lung diseases by T_{RM} cells, and thus to exert the rapid and powerful action in critical illnesses such as COVID-19 pandemic.

AUTHOR CONTRIBUTIONS

YQ and YZ contributed to the central idea and coordinated the writing of the manuscript. YQ, YZ, YL, and BL read, discussed, and revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Pathophysiology of Skin Resident Memory T Cells

Yoshiki Tokura[1,2*], *Pawit Phadungsaksawasdi*[1], *Kazuo Kurihara*[1], *Toshiharu Fujiyama*[1] *and Tetsuya Honda*[1]

[1] Department of Dermatology, Hamamatsu University School of Medicine, Hamamatsu, Japan, [2] Department of Cellular & Molecular Anatomy, Hamamatsu University School of Medicine, Hamamatsu, Japan

Tissue resident memory T (T_{RM}) cells reside in peripheral, non-lymphoid tissues such as the skin, where they act as alarm-sensor cells or cytotoxic cells. Physiologically, skin T_{RM} cells persist for a long term and can be reactivated upon reinfection with the same antigen, thus serving as peripheral sentinels in the immune surveillance network. $CD8^+CD69^+CD103^+$ T_{RM} cells are the well-characterized subtype that develops in the epidermis. The local mediators such as interleukin (IL)-15 and transforming growth factor (TGF)-β are required for the formation of long-lived T_{RM} cell population in skin. Skin T_{RM} cells engage virus-infected cells, proliferate *in situ* in response to local antigens and do not migrate out of the epidermis. Secondary T_{RM} cell populations are derived from pre-existing T_{RM} cells and newly recruited T_{RM} precursors from the circulation. In addition to microbial pathogens, topical application of chemical allergen to skin causes delayed-type hypersensitivity and amplifies the number of antigen-specific $CD8^+$ T_{RM} cells at challenged site. Skin T_{RM} cells are also involved in the pathological conditions, including vitiligo, psoriasis, fixed drug eruption and cutaneous T-cell lymphoma (CTCL). The functions of these T_{RM} cells seem to be different, depending on each pathology. Psoriasis plaques are seen in a recurrent manner especially at the originally affected sites. Upon stimulation of the skin of psoriasis patients, the $CD8^+CD103^+CD49a^-$ T_{RM} cells in the epidermis seem to be reactivated and initiate IL-17A production. Meanwhile, autoreactive $CD8^+CD103^+CD49a^+$ T_{RM} cells secreting interferon-γ are present in lesional vitiligo skin. Fixed drug eruption is another disease where skin T_{RM} cells evoke its characteristic clinical appearance upon administration of a causative drug. Intraepidermal $CD8^+$ T_{RM} cells with an effector-memory phenotype resident in the skin lesions of fixed drug eruption play a major contributing role in the development of localized tissue damage. CTCL develops primarily in the skin by a clonal expansion of a transformed T_{RM} cells. $CD8^+$ CTCL with the pagetoid epidermotropic histology is considered to originate from epidermal $CD8^+$ T_{RM} cells. This review will discuss the current understanding of skin T_{RM} biology and their contribution to skin homeostasis and diseases.

Keywords: skin, resident memory T cell, skin immunity, psoriasis, vitiligo, cutaneous T cell lymphoma, fixed drug eruption

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***Correspondence:**
Yoshiki Tokura
tokura@hama-med.ac.jp

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INTRODUCTION

The number of T cells infiltrating in the skin is nearly twice as many as that in the peripheral blood, and the majority of these cells are effector memory T cells (1). T cells in the skin include αβ T cells accounting for up to 99% and γδ T cells for around 1% (2). Thus, the skin is a homing organ for T cells in physiological and pathological conditions related to adaptive immune response. Before the discovery of resident memory T (T_{RM}) cells, it was supposed that T cells infiltrating in inflamed or infected tissue transiently reside and undergo apoptosis or exit the tissue after clearance of inflammation or infection. Skin T_{RM} cells are a memory T cell subset that provides local surveillance and do not migrate out of the skin. This memory subset has distinct behavior and transcriptional profile that distinguish T_{RM} cells from other memory T cell compartment.

Tissue T_{RM} cells reside in peripheral, non-lymphoid tissues such as the skin, where they act as alarm-sensor cells or cytotoxic cells (3, 4). Physiologically, skin T_{RM} cells persist for a long term and can be reactivated upon reinfection with the same antigen, thus serving as a part of an immune surveillance network. $CD8^+CD69^+CD103^+$ T_{RM} cells are the well-characterized subtype that develops in the epidermis, although $CD4^+$ T_{RM} cells are documented in certain conditions. Local signaling by IL-15 and TGF-β is required for the formation of these long-lived memory cells (5).

Skin T_{RM} cells play a critical defensive role against skin infections. In addition to this essential physiological role, they are also involved in the pathological conditions (6), as exemplified by psoriasis. The functions of these T_{RM} cells seem to be different, depending on each skin disease. The T_{RM} cell-inducing skin diseases have currently extended from fixed drug eruption to psoriasis and cutaneous T-cell lymphoma, and even to vitiligo. In this review, we will discuss recent insights into skin T_{RM} cells, with emphasis on their pathogenic roles in these heterogeneous skin disorders.

TISSUE T_{RM} CELLS

T_{RM} cells, which lack the ability of recirculation *via* the bloodstream and reside in the tissue, exist in various tissues in various organs. However, the phenotypes of T_{RM} cells in each tissue, such as surface markers, the longevity, and the signals for their survival are not uniform and highly heterogeneous. Insights into T_{RM} cells in various tissues have mostly been obtained from mouse studies, and the data of human T_{RM} cells are relatively scarce, because of the technical difficulties in obtaining samples and taking enough number of cells from small biopsy samples in human. It is considered that both $CD8^+$ T_{RM} and $CD4^+$ T_{RM} cells exist, but the property is best defined for $CD8^+$ T_{RM} cells. In this section, we will briefly introduce the characteristics of T_{RM} cells in various tissues, mainly focusing on $CD8^+$ T_{RM} cells in mice (**Table 1**).

The surface markers and longevity of $CD8^+$ T_{RM} cells are critical issues and have been studied in mouse tissues. One of the most important functions of T_{RM} cells is the defense against pathogens such as viruses, bacteria, fungi, and parasites, all of which commonly invade to our body through barrier tissues. Consistently, T_{RM} cells are observed in barrier tissues such as the skin, intestines, lung, and female reproductive tract (25, 26). T_{RM} cells are also detected in non-barrier tissues such as the central nervous system, liver, and salivary glands (25, 26). Furthermore, T_{RM} cells are present in lymphoid tissues, some of which are derived from non-lymphoid tissues (27). CD69 and CD103 are the key surface markers of T_{RM} cells in general, however, the expression patterns of these markers are various depending on the tissues, and even show heterogeneity in the same tissue. CD103 is expressed in T_{RM} cells in most tissues such as the skin and central nervous system, but T_{RM} cells lacking CD103 have been reported in some tissues including intestines (28) and liver (29). CD69, a C-type lectin, is expressed in most T_{RM} cells. CD69 is supposed to work as a stop signal that prevents tissue egress of T_{RM} cells by antagonizing sphingosine-1-phosphate receptor 1 (S1PR1). However, a substantial proportion of T_{RM} cells in the pancreas, salivary glands, and female reproductive tract was reported to be negative for both CD69 and CD103 (30).

TABLE 1 | Resident memory T cells in various tissues in mice and humans.

Tissue of residency	Type of T_{RM} reported in mice or human		Possible involvements in human diseases
	CD4 T_{RM}	CD8 T_{RM}	
Skin		✓	Fixed drug eruption (7)
		✓	Psoriasis (8)
		✓	Vitiligo (9)
		✓	Alopecia areata (10)
		✓	HSV infection (11)
	✓		Candida infection (12)
	✓		Leishmania infection (13)
	✓	✓	CTCL (14)
Gut	✓	✓	Inflammatory bowel disease (15, 16)
Lung	✓	✓	Influenza (17)
	✓	✓	RSV infection (18)
	✓		Allergic asthma (19)
Synovial bursa	✓	✓	Rheumatoid arthritis (20)
Central nervous system		✓	Multiple sclerosis (21)
		✓	Schizophrenia (22)
Kidney		✓	Lupus nephritis (23, 24)

Abbreviations: ATLL, Adult T-cell leukemia/lymphoma; CCL, Chemokine ligand; CLA, Cutaneous lymphocyte-associated antigen; CTCL, Cutaneous T-cell lymphoma; CTLs, cytotoxic lymphocyte; CXCL, CXC chemokine ligand; CXCR, CXC chemokine receptor; DCs, Dendritic cells; DETCs, Dendritic epidermal T cells; FABPs, Fatty acid binding proteins; FFA, Free fatty acid; HSV, Herpes simplex virus; IFN, Interferon; IL, Interleukin; iNOS, Inducible nitric oxide synthase; KLRG1, Killer cell lectin-like receptor subfamily G member 1; LN, Lymph node; MF, Mycosis fungoides; MPECs, Memory precursor effector cells; PD-1, Programmed cell death protein 1; PDE4, Phosphodiesterase 4; PD-L1, Programmed cell death ligand 1; S1PR1, Sphingosine 1-Phosphate Receptor 1; SLECs, Short-lived effector cells; SLOs, Secondary lymphoid organs; SS, Sézary syndrome; TCM, Central memory T cell; TEM, Effector memory T cell; TMM, Skin-tropic migratory memory T cell; TPM, Peripheral memory T cell; TRM, Resident memory T cell; Th, Helper T cell; Treg, Regulatory T cell; TCR, T-cell receptor; TILs, Tumor-infiltrating lymphocytes; TIP-DCs, TNF-α iNOS producing dendritic cells; TNF, Tumor necrosis factor; VLA, Very late antigen protein.

Longevity, which can be defined as the persistence of T_{RM} cells in the tissues, may be also quite different between tissues (4). It has been reported that T_{RM} cells in the lungs and liver persist for weeks to months (31, 32), while T_{RM} cells in the skin remain numerically stable for months to years (33–35), suggesting a tissue specificity of longevity. Longevity is the net effects of several factors such as recruitment, maintenance, division, death, egress, and competition. The extent of the effects of each factor is various depending on the tissues. For example, at the steady state, the ratio of T_{RM} cells that uptake BrdU over 7 days is 0%–5% in the lung (36) and skin (37), while $Ki67^+$ T_{RM} cells in the brain is reported around 9% (38), suggesting the various proliferation ability of T_{RM} cells depending on the tissues. As for the maintenance signals of T_{RM} cells, IL-15 is one of the most important one. Indeed, IL-15 is required for the maintenance of T_{RM} cells in the skin (39), liver (40), salivary glands and kidney (41). However, this is not the case for T_{RM} cells in the female reproductive tract, pancreas, small intestines, and secondary lymphoid organs (SLOs) (41). Expression of CD103 may also be important for the persistence of T_{RM} cells in several tissues such as the skin (39) and the gut (42). TGF-β is necessary for the development of T_{RM} cells in the skin (39), gut (43), and lung (44), while not required for the development of T_{RM} cells in lamina propria of intestine (28). Thus, T_{RM} cells in each tissue possess their own characteristics. Because the environment in each tissue such as available cytokines and nutrients are various, T_{RM} cells seem to adapt to unique local environment to survive.

In human, T cells showing surface markers similar to murine T_{RM} cells have been detected in various tissues, suggesting that T_{RM} cells also exist in human. It is considered that T_{RM} cells play crucial roles for the protection of the host against pathogens, as well as the development of inflammatory diseases. T_{RM} cells in the skin are probably the best studied population in human T_{RM} cells. In the genital skin after human simplex virus (HSV) infection, virus-specific $CD8^+$ T cells persist at the epidermal-dermal junction (11). Involvement of T_{RM} cells is suggested in the development of various inflammatory skin diseases, such as psoriasis, vitiligo, and drug eruption, which will be discussed later. T_{RM} cells are also detected in the gut, and are suspected to contribute to the development of Crohn's disease (15). In the lung, $CD69^+$ or $CD103^+$ $CD8^+$ T_{RM}-like cells are detected in patients with influenza or respiratory syncytial virus infection (17, 18). Other than these tissues, existence of T_{RM} cells has been reported in the female reproductive tract after the vaccination targeting human papilloma virus 16 (45) and liver in hepatitis C infection (46), suggesting the importance of T_{RM} cells in the protective immunity in human as well.

$CD4^+$ T_{RM} cells are usually found within the tissue parenchyma, such as the dermis in the skin. Compared with $CD8^+$ T_{RM} cells, little is known about the characteristics and functions of $CD4^+$ T_{RM} cells. However, this subset may also play important roles in the protective immunity against pathogens in several tissues (47). In mice, the protective roles of $CD4^+$ T_{RM} cells have been reported in *Leishmania major* infection in the skin (48), herpes simplex virus infection in the genital mucosa (34), *Chlamydia trachomatis* infection at the reproductive mucosa (49), and *Streptococcus pneumonia* infection in the lung (50). It remains to be clarified whether those $CD4^+$ T_{RM} cells are really resident in tissues or just a subset of memory $CD4^+$ T cells which spend an extended period time in the tissue before circulation.

IDENTIFICATION AND DEFINITION OF SKIN T_{RM} CELLS

As discussed above, the markers that identify tissue T_{RM} cells may differ among the tissues. The characteristic behavior and markers of skin T_{RM} were well studied in murine models. In human, it is technically difficult to address the migratory behavior of skin T_{RM} cells in an *in vivo* system. The resident memory properties of human skin T cells are largely described on $CD8^+$ T cells with surface markers similar to those of murine T_{RM} cells (23, 51). In this section, we review the current evidence of skin T_{RM} identification, which mostly came from the murine study, and their relevance in human (**Figure 1**).

Precursors of Skin T_{RM} Cells

Naïve $CD8^+$ T cells proliferate and differentiate into a pool of effector cells upon recognition of cognate antigen. During the effector phase, $CD8^+$ effector cells can be divided into short-lived effector cells (SLECs) and memory precursor effector cells (MPECs) (52). SLECs are characterized by $KLRG1^{hi}$ IL-$7R\alpha^{lo}$(CD127), while MPECs are $KLRG1^{lo}$ IL-$7R\alpha^{hi}$. The fate decision of SLECs/MPECs depends on a sum of inflammatory signals that create a T-bet gradient, in which a low-level magnitude promotes MPECs fate during T cell priming (52). Almost all SLECs undergo apoptosis, whereas MPECs turn into heterogenous populations of long-lived memory $CD8^+$ T cells after clearance of infection (52). In early skin infection of herpes simplex virus, skin-infiltrating T cells are mainly $KLRG1^+$ effector cells, while at the memory phase, the remaining memory T cells in the skin bear negative or low expression of KLRG1. Consistently, the adoptive transfer study of $KLRG1^-$ T cells confirmed that $KLRG1^-$ MPECs gave rise to T_{RM} cell populations in the skin (39). Memory T cells also express CD45RO but not CD45RA. Skin-infiltrating T cells isolated from normal human skin were almost all $CD45RO^+$ memory T cells (1). Collectively, skin T_{RM} cells possess the memory precursor phenotype, $KLRG1^-CD127^+CD45RO^+CD45RA^-$.

Skin-Homing Molecules on T_{RM} Cells

Skin-infiltrating memory T cells express a distinct homing receptor called cutaneous lymphocyte-associated antigen (CLA), which binds to E-selectin and P-selectin and allowing CLA^+ T cells to enter the skin (1). Nearly all CLA^+ effector memory T cells are resident in human skin during steady state (1). Chemokine receptor (CCR)10 is one of the essential chemokine receptors for skin homing of T cells (53), as CCR10-deficient mice showed a reduction of $CD8^+$ T cells in the skin (54). Similarly, $CD8^+$ T cells lacking CCR10 impaired

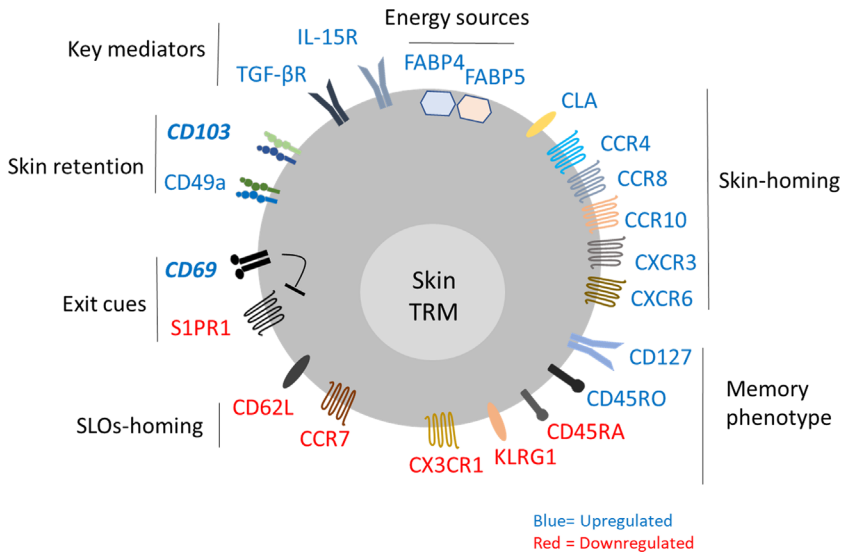

FIGURE 1 | Characterization of skin resident memory T cell.

their T_{RM} forming capacity (55). CXCR6 is expressed on skin T_{RM} cells in human (1) and mice (56), and CXC chemokine ligand (CXCL)16, a ligand for CXCR6, is expressed on epidermal keratinocytes and can be released as a chemoattractant (57). T cells lacking CXCR6 had low capacity to form T_{RM} cells in the skin, whereas $CXCR6^{-/-}$ and wild-type T cells were not different in number in the SLOs. Consistently, direct injection of $CXCR6^{-/-}$ $CD8^+$ T cells into the skin also decreased T_{RM} formation, suggesting that CXCR6 is important for retention rather than recruitment of $CD8^+$ T cells to the skin (55). CCR4 is an essential skin-homing molecule for the migration of T cells to the skin (58) and highly expressed on skin T_{RM} cells (1). Mogamulizumab, a humanized anti-CCR4 antibody, was approved for mycosis fungoides (MF) and Sézary syndrome (SS), which are a malignancy of skin-homing malignant T cells (59). However, the exact role of CCR4 on skin CD8 T_{RM} formation is not clear. Previous studies showed that CXCR3 expression is necessary for T_{RM} cell precursors to enter the epidermis, and $CD8^+$ T cells lacking CXCR3 resulted in less formation of $CD103^+$ T_{RM} cells in mice (39). Skin $CCR8^+$ T cells show phenotypic, functional, and transcriptomic profiles compatible with T_{RM} cells (60). CCR8 is expressed on half of cutaneous memory T cells, whereas very few CCR8 is expressed on circulating memory T cells (61). The ligand for CCR8, CCL1, is preferentially expressed in human skin, and keratinocyte-derived prostaglandin E_2 and vitamin D3 can induce CCR8 expression by $CD8^+$ T cells, suggesting that it may involve in T_{RM} localization in skin (62, 63). However, the role of CCR8 is currently unclear, since T cells lacking CCR8 can migrate and are maintained in the skin as usual in mouse epidermis following viral skin infection (55). Collectively, CCR10 (53, 64), CCR4 (58), CCR8 (60, 62), and CXCR3 (39) enable memory T cells to migrate to the skin, CLA allowing them to enter the skin (1), and CCR10 and CXCR6 (55) contribute to T_{RM} formation in the skin.

Retention Mechanisms of Skin T_{RM} Cells

The retention properties of skin T_{RM} cells have been widely explored in a murine model. The most recognized markers of skin T_{RM} cells in both humans and mice are CD103 and CD69, which are responsible for T_{RM} retention (65). CD103 is an α-chain of the integrin αEβ7and binds to E-cadherin expressed by keratinocytes (**Figure 2**) and is the most common and widely accepted T_{RM} marker. CD103 expression on $CD8^+$ T_{RM} is dependent on the TGF-β (39, 66), which is activated by keratinocyte integrins αvβ6 or αvβ8 (67). In mice lacking this keratinocyte-integrin, T_{RM} cells are unable to express CD103 and cannot persist long term in epidermis (67). CD103 on CD8 T_{RM} cells mediate cell adhesion to the epidermis and thus promote local retention (55). Similarly, $CD103^{-/-}$ $CD8^+$ T cells can enter the epidermis but unable to persist long term in the skin as T_{RM} cells (39, 55). TGF-β induces CD103 expression on activated $CD8^+$ T cells, but not $CD4^+$ T cells, and leads to CD103-mediated adhesion of $CD8^+$ T cells, but not $CD4^+$ T cells, to monolayer human keratinocyte cultures (68). This may explain the reason why $CD4^+CD103^+$ T cells can exit in the skin, but $CD8^+CD103^+$ T_{RM} cells cannot. However, another study showed that TGF-β also induces CD103 expression on $CD4^+$ T cells and mediates cell adhesion to keratinocyte (14). This discrepancy is possibly due to different experimental setups and T cell stimulation methods, and further studies are needed to confirm the function of CD103 on $CD4^+$ T cells. Indeed, $CD4^+CD103^+$ cells can be found in human circulation but not $CD8^+CD103^+$ cells (69). Moreover, CD69 expression is very dynamic and can be easily induced *in vitro* upon stimulation (70). By using qPCR, the expression of TGF-β in psoriatic skin is

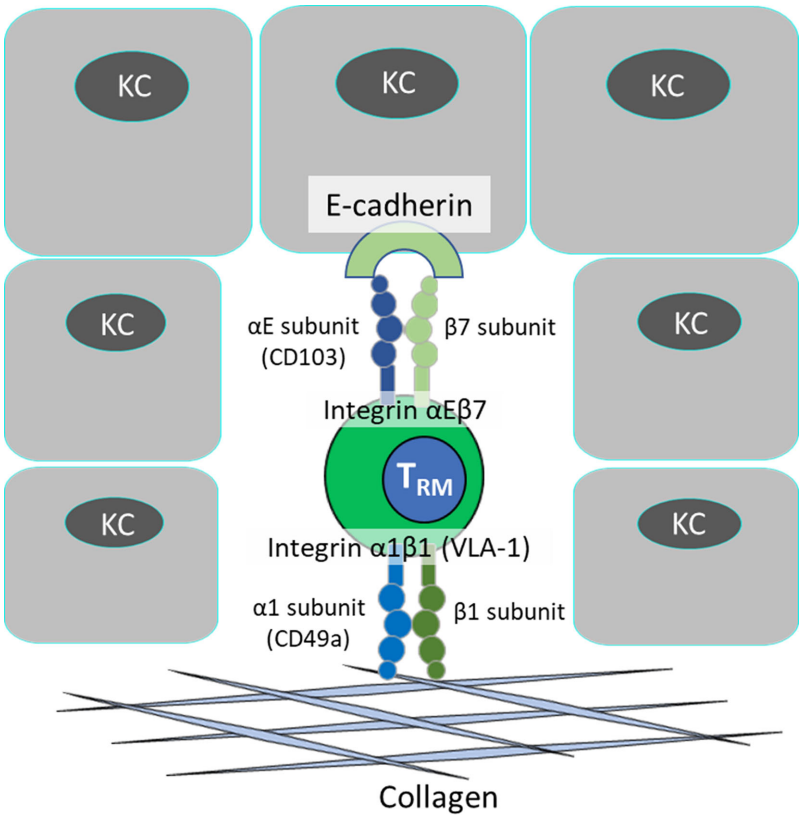

FIGURE 2 | Adhesion of T_{RM} cell in the skin.

comparable to normal skin, implying that increment of $CD103^+$ T cells in psoriasis does not stem from general upregulation of TGF-β expression (68). In tumor context, the interaction between αE(CD103)β7 on tissue-infiltrating lymphocytes and E-cadherin on tumor cells induces cytolytic granule polarization and subsequent exocytosis, leading to tumor cell lysis (71). This suggests that CD103 also exerts some biological activity in addition to the adhesion property.

CD69 is involved in the residency status of T_{RM} cells by downregulating sphingosine 1 phosphate receptor (S1PR1)-mediated tissue egress (72, 73). The vast majority of skin T_{RM} cells in both mice and humans express CD69 (14, 39, 74). The induction of CD69 expression is strongly influenced by antigen stimulation and exposure to pro-inflammatory mediators (72). CD69 is upregulated shortly after memory T cells reaching the skin and CD69 expression is critical for early T cell retention rather than recruitment of T cell into skin (39, 72). However, a recent parabiosis study demonstrated that CD69 expression is inadequate to define a stable residence (27).

α1(CD49a)β1 integrin is one of the T cell receptors for collagen IV, originally termed as Very Late Antigen (VLA)-1. CD49a is upregulated following T cell activation and can be found on circulating T cells before they enter into the skin (75). CD49a-expressing $CD8^+$ T cells are enriched in the epidermis of human and mouse skin (8, 37). In an HSV infection mouse model, CD49a increased T_{RM} effector function and promoted T_{RM} persistence in the skin, but not required for $CD8^+$ T cell to entry into the epidermis (75). In contrast, in the xenotransplantation model of psoriasis, blocking CD49a inhibits T cell migration into the epidermis, resulting in a decrease of T_{RM} cells and prevention of psoriasis development (76). IL-12 and TGF-β can upregulate CD49a expression on $CD8^+$ T cells (75). Not only $CD8^+$ T_{RM} cells but also $CD4^+$ memory T cells poised for Interferin (IFN)-γ production preferentially express CD49a in human (74, 77). Since IL-12 can induce IFN-γ production and CD49a expression, it is tempting to speculate that in the psoriasis context, IL-17A-producing T_{RM} cells, which preferentially express IL-23R (74), downregulate their CD49a due to a greater influence of IL-23 over IL-12.

Collectively, CD69 is critical for initial formation of T_{RM} cells shortly after T cells enter in the skin, while CD103 is required for T cell adhesion and long-term retention of T_{RM} cells. Ultimately, both CD69 and CD103 are required for T_{RM} formation in the skin. In addition, CD49a regulate the persistence, morphology and effector function of $CD8^+$ T_{RM} cells in the skin.

Characteristics of $CD4^+$ Skin T_{RM} Cells

Compared with $CD8^+$ skin T_{RM} cells, the characteristics and behavior of $CD4^+$ skin T_{RM} cells have been less understood,

and probably, they are quite different between mice and humans and remain controversial. In human skin, $CD4^+$ T cells can be found in both epidermal and dermal compartments (14), whereas $CD4^+$ T cells in murine skin are predominantly in the dermis. In fact, human skin has a thicker epithelial layer and lower density of hair follicles that are crucial for residency of $CD4^+$ T_{RM} in mouse skin (78, 79).

Earlier studies showed that the motility of skin-infiltrating $CD4^+$ T cells are higher than that of $CD8^+$ T cells, and they equilibrate with circulating T cell pool at steady state (78, 80). Skin $CD4^+$ memory T cells preferentially accumulate around the hair follicle isthmus and constantly move back and forth to the circulation (78). After cutaneous HSV infection, two distinct HSV-specific memory T cell subsets were found in the skin; the slow-moving $CD8^+$ T cell population resided in the epidermis, particularly at the site of infection, whereas dynamic $CD4^+$ T cell population rapidly trafficked through the dermis and showed recirculation pattern (80). Indeed, we have previously demonstrated a substantial recirculation of $CD4^+$ T cells in the skin to the draining lymph nodes, using a photo-convertible system of Kaede-transgenic mice (81).

A recent study using mice parabiosis experiment identified the $CD4^+$ T_{RM} population with prolonged residency in non-lymphoid tissue, which was separated from the circulation and shared transcriptional signatures with $CD8^+$ T_{RM} cells. However, this study showed only a limited period of 4 weeks of the extent of residency (82), because the prolonged parabiosis was associated with great equilibration for skin $CD4^+$ T cells (78). Another study using alemtuzumab, an antibody targeting CD52 and depleting circulating T cells, showed that $CD4^+CD69^+CD103^+$ and $CD4^+CD69^+CD103^-$ persist in the skin without replenishment of the circulating compartment, suggesting that they are T_{RM} populations. Similarly, in *in vivo* studies, $CD4^+CD69^+CD103^+$ T cells possibly represented a non-migrating resident $CD4^+$ T cell population in the dermis (12, 83). However, the dynamic observation of $CD4^+$ T_{RM} cells in the skin, particularly in human, is technically challenging, and their migratory behavior cannot be excluded. In contrast, the xenografting model with human skin showed that $CD4^+CLA^+CD103^+$ T_{RM} cells down-regulate CD69 expression, exit from the skin, and reach into the circulation (69). These cells in the blood and skin are clonally related and share their function and transcriptional profiles. $CD4^+$ T_{RM} cells were reported to play a role against skin infection with *L. major* (13) and *C. albicans* (12). Recently, resident memory Th2 cells in the lung exhibit a distinct CD4 population and play a critical role in an allergic asthma murine model (19). Furthermore, in experimental colitis, $CD4^+$ T_{RM} cells play a crucial role in the regulation of intestinal inflammation, and they were found in the colon of inflammatory bowel disease patients (16). These studies support the existence and critical role of CD4 T_{RM} cells in tissue-specific immune and inflammatory diseases.

Originally, T_{RM} cell was defined as a memory T cell population that persists long-term in peripheral tissue and do not migrate back to the circulation. According to this definition, not all skin-infiltrating T cells are resident memory T cells. There are only a fraction of these cells that represent the authentic T_{RM} population. A similar definition may be applied to $CD4^+$ T_{RM} cells. In fact, the residence is difficult to quantify, and there are no perfect markers to define a permanent resident T cell. CD103 and/or CD69 may not be sufficient for defining the residence status of skin infiltrating T cells, especially $CD4^+$ T cells (14, 84). Collectively, it is tempting to postulate that $CD4^+$ T_{RM} cells are generally more dynamic and have a distinct migratory behavior compared to $CD8^+$ T_{RM} cells in human skin. Meanwhile, in some inflammation or infection context, CD4+ T_{RM} cells play a crucial role and may persist in the skin for an extended period.

DEVELOPMENT OF SKIN T_{RM} CELLS

A different subset of memory $CD8^+$ T cells contribute to an immune memory response in different aspects and locations. Once naïve $CD8^+$ T cells are activated, they differentiate into pooled effector $CD8^+$ T cell populations, which are composed of SLECs and MPECs. MPECs are characterized by $CD127^{hi}KLRG1^{lo}$ populations, while SLECs are $KLRG1^{hi}$ populations. After clearance of inflammation or infection, the majority of SLECs undergo apoptosis, whereas MPECs turns into a heterogeneous subset of memory T cells (85). Historically, memory T cells were divided into central memory (T_{CM}) cells that express high lymphoid homing molecules and recirculate through SLOs, and effector memory T (T_{EM}) cells that lack lymphoid homing molecules (86). From the current literature, memory T cells can be broadly divided into four main populations in the murine model. (1) T_{CM}: expressing lymph node (LN) homing molecules ($CCR7^+CD62L^+CX3CR1^-$) and mainly surveying SLOs. (2) T_{EM}: expressing $CCR7^-CD62L^-CX3CR1^+$ and predominantly surveying the blood. (3) peripheral memory T cells (T_{PM}): expressing $CCR7^+CD62L^-CX3CR1^{int}$ and preferentially patrolling peripheral tissues and migrate to blood and LN. (4) T_{RM}: persisting for a long term in peripheral tissues.

By immunizing mice with a protein antigen, chemical hapten, or non-replicating virus, T_{RM} cells from the treated skin and distant skin as well as the draining and distant LNs contain identical TCR cells in both T_{RM} and T_{CM} compartment, suggesting that T_{RM} and T_{CM} cells may be derived from common naïve T cell precursors (87). However, equal contribution of individual naïve clones to formation of T_{RM} subsets has not been definite. Using a lineage-tracing technique to track individual naïve $CD8^+$ T cells responding to skin vaccination, it was shown that individual T cell clones contribute differentially to the formation of T_{RM}-poised effector T cell subset, which has a capacity to subsequently form T_{RM} population (88). The propensity to form T_{RM} populations is disparately distributed over T cell clones, implying that this fate must be committed before clonal expansion. The heterogeneity of circulating vaccine-specific effector T cell pool can be divided into four distinct populations based on the gene expression profiles, including effector cell, intermediate cell, circulating memory T cell-like

precursor, and T_{RM}-like precursor. This study revealed the existence of T_{RM} cell precursor in circulation and their commitment to T_{RM} cells before entering into the skin (88).

The existence of pre-commitment T_{RM} cells in circulation was further supported by an elegance study on the role of dendritic cell in T_{RM} cell formation (89) (**Figure 3**). This study revealed that the formation of skin T_{RM} cells requires interaction between naïve $CD8^+$ T cells and migratory dendritic cells (DCs) from the skin at a steady state. This process depended on the presence of TGF-β, which activates V-integrins on migratory DCs. In fact, lack of V-integrins on $CD11c^+$ DCs resulted in a substantial reduction in epidermal $CD8^+$ T cells, but did not affect dermal $CD8^+$ T cells or other skin immune populations. The expression of a V-integrins on DCs during immune homeostasis, but not in priming state, was required for pre-conditioning naïve $CD8^+$ T cells for effective T_{RM} cells formation (89). Therefore, T_{RM} fate decisions on T cells seem to happen earlier than expected, and this event appears to be controlled primarily by a cross-talk between local skin and draining LNs *via* DCs. Indeed, DCs are able to instruct T cells to migrate to a specific location. For example, DCs in skin-draining LNs and mesenteric LNs induce the expression of tissue homing molecule that elicits tropism for skin and gut, respectively (90, 91). Earlier studies showed that individual naïve T cells contribute differentially to short-term effector cells and long-term memory cells, and the fate of each naïve T cells is unpredictable (92). However, the subsequent study revealed the clonal bias of T_{RM} precursors within heterogenous memory populations (88).

Non-specific inflammation is sufficient to attract $CD8^+$ T cells into the inflamed tissue and adopt T_{RM} cells in the skin (93, 94), suggesting that T_{RM} cells in the skin do not require cognate antigen for their establishment. Basically, the skin immune cells respond to an invader such as hapten and secrete pro-inflammatory cytokines that induce dendritic cell migration and maturation (95). Endothelial cells increase the expression of adhesion molecules; CD54 (ICAM-1) and CD106 (VCAM-1), which guide T cell entry into the tissue. In addition, chemokines, Chemokine ligand (CCL)2 to 5, CXCL9, and CXCL10 are secreted from keratinocyte and innate immune cells, and this initial step is induced by a non-specific inflammation process and is a fundamental mechanism to recruit T cells into inflamed skin (96). However, the presence of cognate antigens enhances T_{RM} cell formation. Moreover, antigen challenges at the skin lead to generalized seeding of antigen-specific T_{RM} cells, which are found at the highest density at sensitizing area (39, 87).

MAINTENANCE OF SKIN T_{RM} CELLS

A whole-genome bisulfate sequencing study suggests that T_{RM} cells have a high plasticity and a development potential comparable to T_{CM} and T_{EM} cells, indicating that they are not terminally differentiated (97). In addition, T_{RM} cells can proliferate *in situ* in response to viral challenge, further supporting their as yet undifferentiated status (94). Different factors are required for maintenance of T_{RM} cells, depending on individual tissues (98). Skin $CD8^+$ T_{RM} cells can be maintained in the skin for a long period (65, 87). Several factors, including local antigens, cytokines, and metabolites, contribute to T_{RM} maintenance (**Figure 4**). A disparate level of skin residency may exist in skin T_{RM} cells. While certain subsets of skin T_{RM} cells have long-term residency, other subsets transiently reside in the skin and possibly migrate out to the circulation.

Effects of Cognate Antigens

Although local antigen is not required for skin recruitment of circulating $CD8^+$ T cells to obtain the T_{RM} phenotype, antigen exposure greatly amplifies the number of $CD8^+$ T_{RM} cells (99). Local antigenic challenge induces antigen-specific T_{RM} cell proliferation, and they are maintained as epidermal T_{RM} pool (94). Intriguingly, the subsequent pool of T_{RM} cells after antigen reencounter is generated mainly from the pre-existing T_{RM} cell

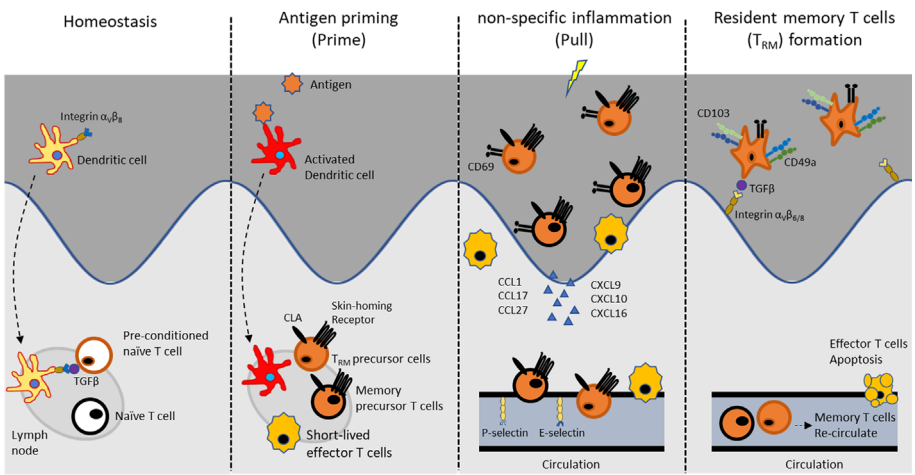

FIGURE 3 | Development of skin T_{RM} cell.

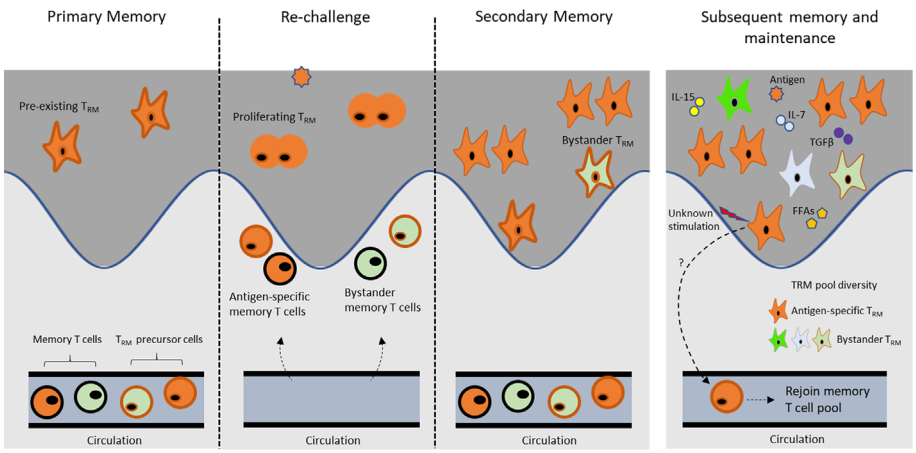

FIGURE 4 | Maintenance of skin T_{RM} cell.

population, rather than from circulating memory T cell compartment (94, 100). A self-sustained capacity of T_{RM} cells in the skin seems to be independent of $CD4^+$ helper T cells and $CD11c^+$ cells (100). The contribution of circulating memory T cells in the local immune response may depend on the density of the pre-existing T_{RM} population, suggesting the flexibility of circulating T_{CM} cells to support T_{RM} population. Moreover, even with the newly seeded, unrelated T_{RM} population in the skin, the number of pre-existing T_{RM} cells remain largely unchanged. Initial activation of skin T_{RM} cells requires antigen recognition, which represents T_{RM}-mediated skin protection and is ultimately changed to an antigen independent reaction (101). T_{RM} cells thus exert a protection capacity, depending on their local density in skin (94). A question arises as to how local antigen influences composition of skin T_{RM} cells from a pool of polyclonal skin-infiltrating memory precursors during active infection or inflammation. It has been revealed that local antigen-dependent cross-competition contributes to shaping the polyclonal T_{RM} cell repertoire in the skin, whereas this event is not observed in SLOs (102). Therefore, the local antigen-dependent self-amplification and cross-competition processes may serve as a mechanism to modulate local T_{RM} composition in response to a variety of invaders and responsible for maintenance of T_{RM} cell population in skin.

Fatty Acids for the Maintenance of Skin T_{RM} Cells

One of the basic needs for life is food. The skin has a unique microenvironment where lipids are rich even with shortage of nutrients. Skin T_{RM} cells reside in the epidermis, and thus, they are relatively independent from blood circulation. Although nutrients may diffuse from the dermis to the epidermis, the local energy source seems to be required for T_{RM} cells. Fatty acid binding proteins (FABPs) are a group of intracellular molecules that mediate lipid trafficking and metabolism (103). FABPs originally consist of adipose FABP (A-FABP) and epidermal FABP, which encoded by *Fabp5*. E-FABP is expressed on keratinocytes and immune cells, including T cells and macrophages (104). High-fat diet upregulated E-FABP expression and promote skin inflammation, suggesting the role of lipid metabolism in immune regulation (105). Recently, it was shown that $CD8^+$ T_{RM} cells utilize exogenous lipids in the skin as an energy source for their survival. T cells lacking Fabp4 and Fabp5 cannot uptake and utilize exogenous free fatty acid (FFA), which results in a reduction of long-term survival and impaired functional properties of $CD8^+$ T_{RM} cells *in vivo*. This deficiency has no effect on T_{CM} cell survival. Interestingly, the significance of lipid metabolism for T_{RM} survival is increased over time, suggesting metabolic adaptation to the skin environment. It is proposed that $CD8^+$ T_{RM} cells utilize local lipid as an energy source to maintain their functional competence and longevity in the skin. Similarly, $CD8^+$ T_{RM} cells in the skin also increase the expression of FABP4 and FABP5 (106). It seems that the impact of FABP deficiency is not only limited to $CD8^+$ T_{RM} cells but also affects $CD4^+$ T cells and DCs. Upregulation of FABPs on $CD4^+$ T cells promotes IL-17 expression, while the loss of FABPs is associated with enhanced expression of FoxP3 (104), suggesting the role of E-FABP and Th17/Treg balancing. In addition, FABP-deficient mice showed an altered antigen-presenting function of dendritic cells and macrophages (107). The limitation of energy resources in the epidermal niche possibly influences the T_{RM} cell density and survival. A recent study demonstrated that $CD8^+$ T_{RM} cells displace pre-existing dendritic epidermal T cells (DETCs) from the epidermis because they have a superior metabolic fitness (108).

Cytokines

Despite the likeness between IL-15 and IL-2, including shared receptor subunit, IL-15 has a perceptible difference in immunomodulatory properties (109). Basically, IL-15 promotes proliferation and survival of circulating memory $CD8^+$ T cells but did not affect regulatory T cell populations in human (110, 111). IL-15 deficient mice showed a reduction of $CD8^+$ T_{RM} cell number (39, 112) but slightly increased $CD4^+$ T_{RM} cells in the

skin, while the numbers of $CD8^+$ T cells and $CD4^+$ T cells in SLOs were not different between IL-15-deficient and WT mice (112). Keratinocytes at hair follicle has been shown as the main source of IL-15 for maintaining $CD8^+$ T_{RM} cells in the skin. In addition to IL-15, IL-7 from hair follicle also influence on both $CD8^+$ T_{RM} and $CD4^+$ T_{RM} cells persistent in the skin. However, the requirement of IL-15 for T_{RM} maintenance may vary depending on the tissue and context of inflammation (41). Apart from maintenance property, IL-15 strongly induces perforin and granzyme B expression in $CD8^+CD103^+CD49a^+$ T_{RM} cells but not in $CD8^+CD103^+CD49a^-$ T_{RM} cells isolated from normal human skin (74). TGF-β is a pleiotropic cytokine that is produced in an inactive form that requires specific integrins on keratinocyte to activate them (113). Activated-TGF-β induces $CD8^+$ T_{RM} cells to express CD103, which is mandatory for their retention and long-term persistence in the skin (39, 55). Collectively, keratinocytes play an important role in establishing long-term T_{RM} cell populations by providing local mediators like IL-15, IL-7, and activated TGF-β.

SKIN T_{RM} CELLS IN CUTANEOUS DEFENSE SYSTEM AGAINST PATHOGENS

Although the pathophysiological roles of skin T_{RM} cells encompass several aspects (65), they serve primarily as a critical component of cutaneous immune defense. T_{RM} cells act as peripheral sentinels providing rapid immune response against invading pathogens (114). Infection with pathogenic microorganisms leads to directed homing of T cells to the appropriate tissues, such as the skin. Subsequently, most antigen-specific memory T cells reside in the non-lymphoid organs, convey tissue-resident memory, and mount durable protective immunity in the skin.

Virus is a major pathogen to which skin T_{RM} cells respond, and a number of valuable findings have been obtained from studies on virus infection. T_{RM} cells can autonomously regulate the local T_{RM} composition to mediate immunosurveillance independently of circulating memory T cells (94, 100). Skin T_{RM} cells are activated and proliferate *in situ* upon encounter with virus-infected cells, and do not migrate out of the skin. As a consequence, secondary T_{RM} cell populations were mainly derived from pre-existing T_{RM} cell populations and the precursors recruited from the circulation. In subsequent infections, the pre-existing skin T_{RM} cell populations are not displaced by the newly generated T_{RM} cells, enabling multiple T_{RM} cell specificities to maintain a diverse immune response within the tissue (94). Consistently, mucosal T_{RM} cells are highly motile, but pause and undergo *in situ* division after local antigen challenge. T_{RM} cell reactivation triggers the recruitment of recirculating memory T cells that undergo antigen-independent T_{RM} cell differentiation *in situ*. The proliferation of pre-existing T_{RM} cells dominates the local mucosal recall response and contribute most substantially to the boosted secondary T_{RM} cell population (100).

$CD8^+$ T_{RM} cells seem to play a major role in cutaneous defense against virus. After resolution of skin vaccinia virus infection, antigen-specific circulating memory $CD8^+$ T cells migrate into the skin. Memory T cells that reside at these surfaces provide a first line of defense against subsequent infection (6, 115, 116).

The local cytokine environment within the skin determines the differentiation state and persistence of the central and peripheral memory-T-cell pool (67). $CD8^+CD103^+$ T_{RM} cells develop in the skin from epithelium-infiltrating precursor cells that lack expression of the effector-cell marker. Following the entry of the T cells into the epidermis, the local mediators such as IL-15 and transforming growth factor (TGF)-β are required for the formation of long-lived T_{RM} cell population in skin (39). The retention of tissue-resident memory T cells is mediated by TGF-β, which up-regulates CD103 expression and down-regulates CCR7 expression. Besides microbial pathogens, topical application of chemical allergen to skin causes delayed-type hypersensitivity and amplifies the number of antigen-specific $CD8^+$ T_{RM} cells at challenged site (117). Expanded T_{RM} $CD8^+$ T cells in the skin are derived from memory T cells recruited out of the circulation. Expanded T_{RM} $CD8^+$ T cells significantly increase anti-viral protection.

In addition to $CD8^+$ cells, $CD4^+$ T_{RM} cells are also involved in microbial defense. $CD4^+$ T_{RM} cells play a role in cutaneous fungal infection (12). *Candida albicans* (*C. albicans*) is a common dimorphic fungal pathogen to which human subjects are exposed early in life, and by adulthood. In a *C. albicans* infection mouse model, dermal γδ T cells producing IL-17 are the main effector cells in the initial infection, and then, αβTh17 effector T cells become predominant. By day 30 after infection, the $CD4^+$ T_{RM} cells become the main population of IL-17-producing T cells that react to *C. albicans*. Between 30 and 90 days after infection, these reactive $CD4^+$ T cells acquire expression of CD69 and CD103, the retention markers, and reside in the papillary dermis. These T_{RM} cells are more effective to eradicate *C. albicans* than recirculating T cells (12).

Recently, the preclinical studies on T_{RM}-targeted vaccination have shown a favorable outcome. Intranasal (118) and mucosal (119) administration of vaccine generated protective T_{RM} cells in the lung and airway of mice. Direct vaccination (118, 119) or delivery vaccine vectors to a specific tissue (120, 121), rather than parenteral route, generated antigen-specific T_{RM} cells, thereby mediating effective protection independent of circulating memory T cells. In addition, a "prime and pull" strategy (122), which combines vaccination with local application of chemokines, effectively generated T_{RM} cells. These studies suggest that protective T_{RM} cells can be generated through vaccination, especially tissue-targeted approaches that give a better protection than ordinary parenteral route. Since the skin is an accessible tissue for administration of vaccine, a question arises whether immunization through the skin can generate T_{RM} cells in other organs or barrier tissues. In fact, the smallpox vaccine, which is one of the most effective vaccine in history, was delivered by skin scarification (123). In a murine model, the

localized virus skin infection (35) or skin immunization (87) can generate antigen-reactive T_{CM} cells and skin T_{RM} cells that reside within the entire skin and possibly in the lung (124). Besides, the combination of "prime and pull" with a prime boost approach was reported to be very effective to produce protective T_{RM} cells (125). These suggest the possible role of the skin as a T_{RM}-targeted vaccination strategy. Further understanding of how skin dendritic cells shape the T_{RM} precursor pool (89), which have a potential to transform into tissue-specific T_{RM} cells, may provide a crucial information for the development of T_{RM}-targeted vaccination. Furthermore, skin resident memory T cells also play a protective role in skin infection, such as HSV (35), *C. albicans* (12), leishmania major (13), and in skin cancers, such as melanoma (126) and squamous cell carcinoma (127). They also play a pathogenic role in some autoinflammatory skin diseases; vitiligo (9, 128), psoriasis (8) and alopecia areata (10). Thus, the vaccination-induced T_{RM} cell strategy may also have a potential to become a novel therapeutic approach to protect the skin from infection, prevent tumor growth, or suppress autoreactive immune responses.

SKIN T_{RM} CELLS IN PSORIASIS

Psoriasis is a common chronic inflammatory skin disease, and the pathogenesis underlying psoriasis has been extensively studied (**Figure 5**). $CD4^+$ T cells producing interleukin (IL)-17, named Th17 cells, play an essential role in its pathogenesis (129). Th17-derived cytokines, IL-17A, IL-17F and IL-22, induce epidermal acanthosis, which represents an intriguing histological finding of psoriasis and results from the proliferation of epidermal keratinocytes. These mediators stimulate keratinocytes to produce TNF-α, IL-8, and vascular endothelial growth factor, thereby promoting inflammation, neutrophil recruitment, and angiogenesis (129). For maintenance of Th17 cells, IL-23 is required and secreted from inflammatory DCs or TNF-α and iNOS-producing DCs (TIP-DCs) Psoriasis and other Th17-mediated skin diseases (129). Epidermal Langerhans cells are another source of IL-23 in a certain condition (130). Keratinocytes are also activated by their own cytokines, such as IL-17C, IL-36, and TNF-α, in an autocrine manner (131, 132). In addition, antimicrobial peptides released from keratinocytes and (IFN)-α from plasmacytoid DCs has been considered to play initiative roles for the development of psoriatic lesions (133). Meanwhile, a self-regulatory autocrine mechanism is disturbed in epidermal keratinocytes of psoriasis patients (134).

The cytokine network in psoriasis has been proven by the therapeutic effectiveness of biologic antibodies that block individual cytokines, including TNF-α, IL-23/IL-12p40, anti-IL-23p19, IL-17A, and IL-17 receptor (135). Although biological drugs are effective, there are variations in the responsiveness between patients (136). Moreover, upon withdrawal of the biologics, the skin lesions often recur. Psoriasis plaques are seen in a recurrent manner especially at the originally affected sites (137). Thus, even after clearance of skin lesions, some immunocompetent cells possibly remain in the previously affected, currently normal-appearing skin. A number of studies have suggested the pathogenetic role of skin T_{RM} cells in psoriasis (8, 74), particularly as a strong candidate that evokes recurrence (2). Notably, T_{RM} cells in psoriatic skin can produce certain cytokines and decreased in number after improvement (74). $CD8^+$ T_{RM} cells reside even in disease-naïve, non-lesional sites of psoriasis patients possibly in correlation with disease duration (138).

The skin T_{RM} cells are positive for tissue-retention markers CD103 and CD69, but negative for lymphoid homing markers CD62L and CCR7 (139). Double immunofluorescent staining for CD3, CD4, or CD8 (red) along with CD103 (green) is shown, and the merged yellow color represents cells positive for both (**Figure 6**). $CD3^+$ T cells infiltrate into both epidermis and dermis, and majority of the T cells in the epidermis co-expressed CD103. $CD4^+$ cells mainly infiltrate in the dermis and scarcely express CD103. $CD8^+$ cells infiltrating in the epidermis are positive for CD103, while those in the dermis

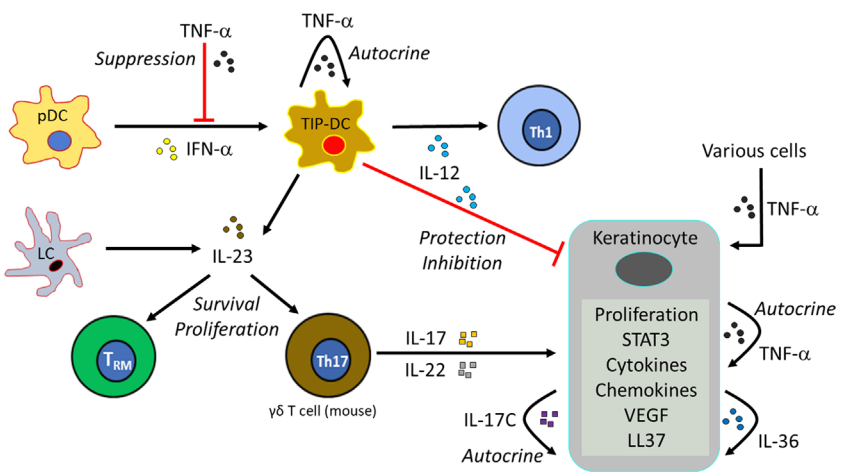

FIGURE 5 | Mechanism of psoriasis.

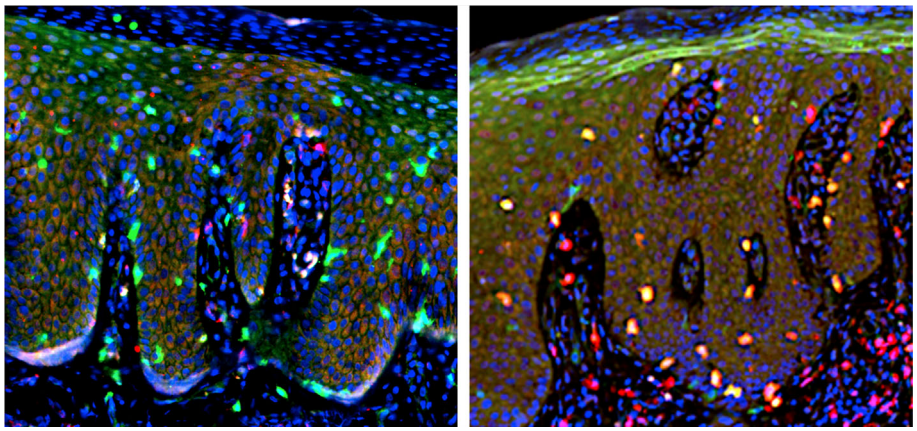

FIGURE 6 | Double immunofluorescent staining. Left: CD4 (red) and CD103 (green). Right: CD8 (red) and CD103 (green). Merged yellow color (right) indicate cells positive for both CD8 and CD103, representing T_{RM} cells.

were mostly $CD103^-$. Thus, the majority of epidermal T cells are $CD8^+CD103^+$ T_{RM} cells and a small number of $CD4^+CD103^+$ T_{RM} cells infiltrate in the dermis. A few $CD8^+CD103^+$ T_{RM} cells are present in the papillary and subpapillary layers. The number of $CD8^+CD103^+$ T_{RM} cells in the epidermis tends to correlate with the epidermal thickness (70), suggesting the role of T_{RM} cells in the formation of psoriatic lesions.

When $CD103^+$, $CD103^-$, $CD69^+$, and $CD69^-$ T cells were isolated and expanded *ex vivo* with anti-CD3/CD28 Ab and IL-2 (140–142), the positive and negative expression of CD103 was unchanged (70). However, CD69 expression can be changed bidirectionally by cultivation, suggesting the unsteady, fluctuated expression of CD69. By using skin-derived, *ex vivo* expanded T cells (140–142), we conducted to characterize the cytokine profile of $CD103^+$ skin T_{RM} cells, especially, epidermal $CD8^+CD103^+$ T_{RM} cells (39, 74). In T cell samples expanded from psoriasis lesional skin, a part of $CD8^+$ T cells co-expressed CD103, and this $CD8^+CD103^+$ T cells are considered to be epidermal T_{RM} cells. $CD4^+CD103^+$ cells are present at a much lower frequency. $CD103^+$ T cells were mostly $CD8^+CD45RO^+CD45RA^-CD69^+$ memory T cells with a skin-homing potential, i.e., partially $CCR6^+$ and mostly $CCR7^-CD62L^-$. They contained both $CXCR3^+CD49a^+$ and $CXCR3^-CD49a^-$ populations. These findings are in accordance with the importance of $CD8^+$ T cells in psoriasis pathogenesis (138, 143–145).

The cytokine production pattern of skin T_{RM} cells has been a crucial issue, because their function is generally determined by the released cytokines. Skin T_{RM} cells remain longer in the same position than effector memory T cells (51) and produce certain cytokines in relation to psoriatic etiology (39, 74, 146). $CD103^+$ T_{RM} cells produce IFN-γ, IL-17A, and IL-22 (39, 74, 147). In the *ex vivo* expanded T cells, certain populations of $CD8^+CD103^+$ T cells produce IFN- γ, IL-17A or IL-22, while $CD4^+CD103^+$ T cells scarcely elaborate these cytokines. In $CD8^+$ T cells, $CD103^+$ T_{RM} cells more frequently produce IL-17A than $CD103^-$ T cells. Thus, $CD8^+CD103^+$ T_{RM} cells efficiently produce IL-17A.

The sorted $CD103^+$ cells expressed CXCR3 or CD49a at a frequency of 28%, sharing the feature with Tc1 or reported IFN-γ-producing T cells (39, 74). The counterpart cells were CD49a negative or low, supposedly corresponding to IL-17A-producing T cells (39, 74). Taken together these observations, $CD8^+CD103^+$ T_{RM} cells can be divided into two types: $CD49a^-IL\text{-}17A^+$ and $CD49a^+IFN\text{-}\gamma^+$ types. It is assumed that the former type is closed associated with psoriasis, while the latter type play a role in vitiligo (74).

Skin T_{RM} cells are associated with not only the development of psoriasis (39, 138, 139), but also its clinical course. T_{RM} cells producing IL-17A in resolved psoriasis epidermis could be associated with early relapse (148), and $CD8^+$ T_{RM} cells with IL-17A-producing potential in disease-naïve, non-lesional sites possibly correlate with disease duration (138). Thus, IL-17A-producing $CD103^+$ T_{RM} cells may have an influence on the future clinical course of psoriasis. We surveyed the 10 patients as to whether oral cyclosporine, oral phosphodiesterase 4 (PDE4) inhibitor or systemic biologics was initiated within one year after the biopsy. The results showed that the patients having entered these advanced therapies possessed higher frequencies of $CD8^+CD103^+IL\text{-}17A^+$ T_{RM} cells (70). Among $CD103^+$ T cells, the frequencies of $CD8^+CD103^+IL\text{-}17A^+$ and $CD4^+CD103^+IL\text{-}17A^+$ cells tended to be higher in the advanced therapy group than in the non-advanced therapy group. The $CD8^+$ T_{RM} cells showed a high frequency compared with the $CD4^+$ T_{RM} cells. Thus, IL-17A-producing $CD8^+CD103^+$ T_{RM} cells may be associated with a progressive clinical course of psoriasis rather than the severity of skin lesions. One can speculate that upon provocation of the skin with stimulants causing Köbner phenomenon, reactivated $CD8^+CD103^+$ T_{RM} cells initiate the psoriatic condition with IL-17A.

SKIN T_{RM} CELLS IN VITILIGO

Vitiligo is an autoimmune skin pigmented disorder mediated by autoreactive IFN-γ- producing $CD8^+$ T cells that attack

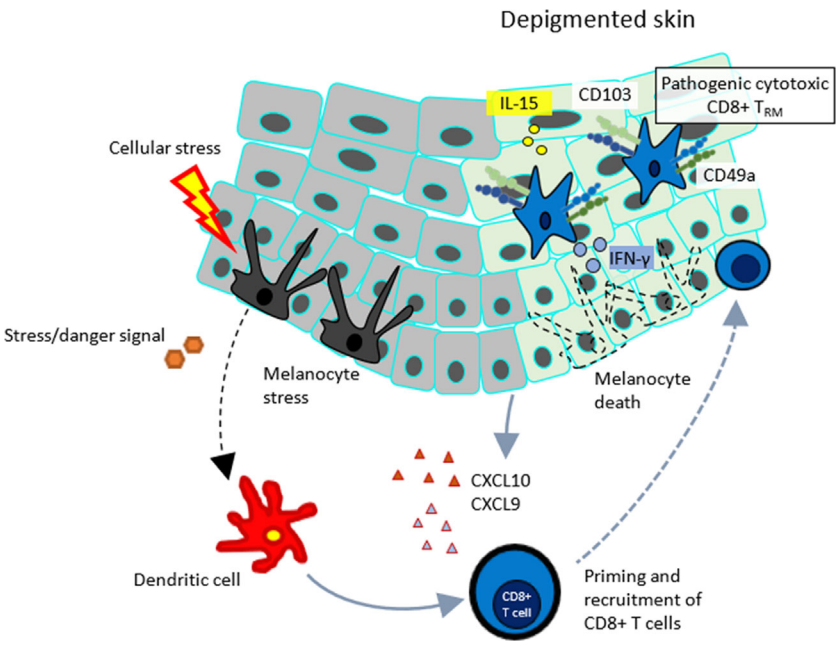

FIGURE 7 | Mechanism of vitiligo.

melanocytes, leading to loss of skin pigmentation (**Figure 7**). The appearance of vitiligo in melanoma patients treated with anti-PD-1 immune checkpoint inhibitors is well known as an immune-related adverse event. Autoreactive cytotoxic lymphocytes (CTLs) against normal melanocytes as well as melanoma tumor cells are activated by the antibody therapy (149).

When aberrantly activated, skin T_{RM} cells have a profound role in vitiligo and melanoma (128). $CD8^+CD103^+CD69^+CD49a^+$ T_{RM} cells serve as CTLs (74, 143). Accordingly, most of CD8 T_{RM} cells express CXCR3 in vitiligo, indicating inclusion of the population of melanocyte-specific CD8 T cells, which display increased production of IFN-γ and tumor necrosis factor-α with moderate cytotoxic activity (143). Autoreactive T_{RM} cells are also present in mouse models of vitiligo. However, it was found that not only skin T_{RM}, but also recirculating memory T cells, plays a role in the development of vitiligo (150). They sense autoantigen in the skin long after stabilization of disease and produce IFN-γ, which further induces CXCL9, and CXCL10 production. Blockade of recirculating memory T cell recruitment to the skin with FTY720 or depletion of them with an antibody reverse disease, indicating that recirculating memory T cells cooperate with T_{CM} to maintain disease (150).

Targeting of T_{RM} cells could become a promising treatment strategy for vitiligo. Moreover, recent evidence demonstrates that induction of melanoma-reactive T_{RM} cells is needed to effectively control tumor growth (9). In a murine model, IL-15 is essential for T_{RM} formation and functions. Both human and mouse T_{RM} cells express IL-15Rβ subunit CD122, and that keratinocytes or other antigen presenting cells up-regulate the expression of IL-15Rα subunit CD215, thereby promoting activation of T cells. Blocking the IL-15 signaling with an anti-CD122 antibody improves the skin depigmentation in mice with established vitiligo. Although prolongation of treatment with anti-CD122 antibody depletes T_{RM} cells from the skin lesion, and the short-term treatment with systemic or local anti-CD122 antibody inhibits IFN-γ production from T_{RM} cells and promotes skin repigmentation (151). Thus, targeting IL-15 signaling *via* CD122 may be a promising therapy for vitiligo.

SKIN T_{RM} CELLS IN CUTANEOUS LYMPHOMAS

Cutaneous T-cell lymphoma (CTCL), encompassing mycosis fungoides (MF), Sézary syndrome (SS) and other variants, is a mature T-cell lymphoma, which is currently thought to develop primarily in the skin by a clonal expansion of a transformed, T_{RM} cell (14, 112, 152, 153).

In the epidermis, both $CD8^+CD103^+$ and $CD4^+CD103^+$ T_{RM} are present and have potent effector functions (14), although the former $CD8^+$ population is present at a higher frequency in the normal and psoriatic lesional skin (70, 138, 142). Skin T_{RM} in the dermis are $CD4^+CD69^+CD103^-$. In recirculating T cells, there are $CCR7^+$L-selectin$^+$ central memory T cells (T_{CM}) and $CCR7^+$L-selectin$^-$ skin-tropic migratory memory T cells (T_{MM}). Clonal malignant T cells from the blood of Sézary syndrome (SS) patients universally coexpress CCR7 and L-selectin as well as the differentiation marker CD27, a phenotype consistent with T_{CM} cells (14). CCR4 is also universally expressed at high levels, and there is variable expression of other skin addressins (CCR6, CCR10, and CLA). In contrast, T cells isolated from MF skin lesions lack CCR7/L-selectin and CD27 but strongly express

CCR4 and CLA, a phenotype suggestive of skin T_{RM} cells (152). $CD4^+$ and $CD8^+$ skin T_{RM} cells reside predominantly within the hair follicle epithelium. Hair follicle expression of IL-15 is required for $CD8^+$ skin T_{RM} cells, and IL-7 for $CD8^+$ and $CD4^+$ skin T_{RM} cells, to exert epidermotropism (112).

However, the skin T_{RM} origin concept for the development of MF does not explain the occurrence of multiple, widespread skin lesions. A whole-exome sequencing approach to detect and quantify TCR-α, β, and γ clonotypes in tumor cell clusters suggests the existence of multiple T-cell clones within the tumor cell fraction, with a considerable variation between patients and between lesions from the same patient (153). Thus, circulating neoplastic T-cell clones may continuously replenish the lesions of MF, thus increasing their heterogeneity by a mechanism analogous to the consecutive tumor seeding.

Adult T-cell leukemia/lymphoma (ATLL) is a malignancy of mature T cells caused by human T-cell leukemia virus type I. Approximately 50% of ATLL patients exhibit skin lesions where malignant $CD4^+CD25^+$ T cells histologically show epidermotropism (154). We documented a case of adult T-cell leukemia/lymphoma (chronic type), which had a phenotype of $CD4^+CD25^+CD69^+CD103^+$ T_{RM} cells (155), indicating the T_{RM} property of this case and the presence of T_{RM} malignancy in cutaneous lymphomas other than MF. Taken together these observations in CTCL and ATLL, the vast majority of cutaneous lymphomas are derived from skin $CD4^+$ T_{RM} cells.

It has been reported that some patients with MF have malignant $CD8^+$ T cells instead of $CD4^+$ T cells. Accordingly, a case of $CD8^+$ primary cutaneous peripheral T-cell lymphoma arising from skin T_{RM} cells was also reported (156). Pagetoid reticulosis is histologically characterized by dense infiltration of atypical mononuclear cells in the epidermis that produce a pagetoid appearance. This unique disease is historically divided into the localized type (Woringer-Kolopp disease) and the disseminated type (Ketron-Goodmann disease). However, a case showing progression from the former to the latter was documented (157), and currently, pagetoid reticulosis is regarded as a subtype of MF. In the immunohistochemical phenotype, cases of pagetoid reticulosis can be divided into three subtypes: $CD4^+$ (37.5%), $CD8^+$ (29.2%), and $CD4^-CD8^-$ (33.3%) types (157). While the single positive types are derived from αβ T cells, the double negative type originates from γδ T cells. It should be noted that one third of pagetoid reticulosis cases are $CD8^+$, suggesting that this subtype is an epidermal $CD8^+$ T_{RM} cell tumor (**Figure 8**). The pagetoid fashion of this tumor may reflect the nature of skin T_{RM} cells.

SKIN T_{RM} CELLS IN FIXED DRUG ERUPTION

Fixed drug eruption is induced by skin T_{RM} cells (**Figure 9**). $CD8^+$ T_{RM} cells in the epidermis possess an effector-memory phenotype and play a role in development of localized tissue damage in fixed drug eruption (7). These epidermal $CD8^+$ T cells constitutively express an early activation marker CD69 even before challenge. A large proportion of these $CD8^+$ T cells exhibit immediate effector function as proven by the rapidly increased IFN-γ production after challenge, resulting in localized epidermal injury. In addition, the intracellular cytokine assay *ex vivo* supports the great capability of these T cells to produce IFN-γ (158).

Although reactivation of these $CD8^+$ T_{RM} cells is sufficient to initiate the lesion, the recruitment of circulating $CD4^+$ and $CD8^+$ T cells is necessary to cause extensive tissue damage observed in the fully evolved lesions. The abundance of regulatory T cells in the epidermis of fully evolved lesions would serve to limit aberrant immune reactions. Local IL-15 production from lesional epidermis could maintain the survival of the epidermal $CD8^+$ T_{RM} cells even without antigen stimulation over a prolonged period of time (159).

The presence of T_{RM} cells in the epidermis and ocular surface may also play a key role in immune activation and antigen recognition. Some evidence supports the role of T_{RM} cells in Stevens-Johnson syndrome and Toxic epidermal necrolysis, and disease distribution may relate to their site-predominance (160).

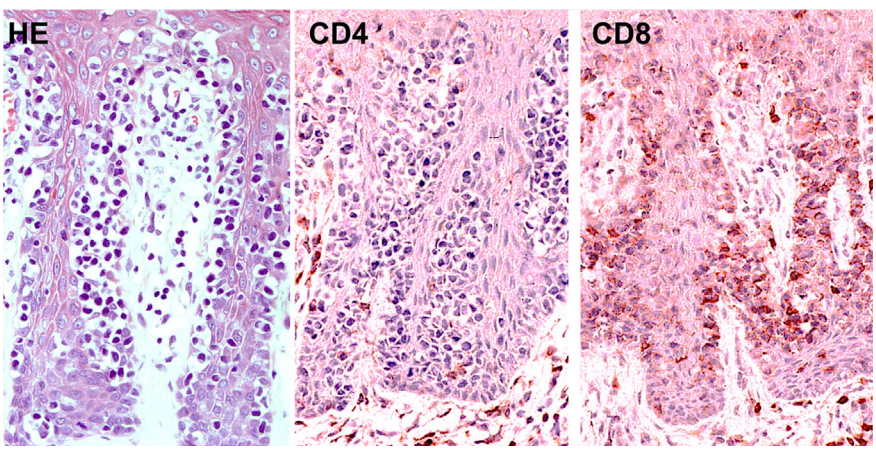

FIGURE 8 | Histopathology (left; hematoxylin and eosin, HE) and immunostaining for CD4 (middle) and CD8 (right) in $CD8^+$ pagetoid reticulosis.

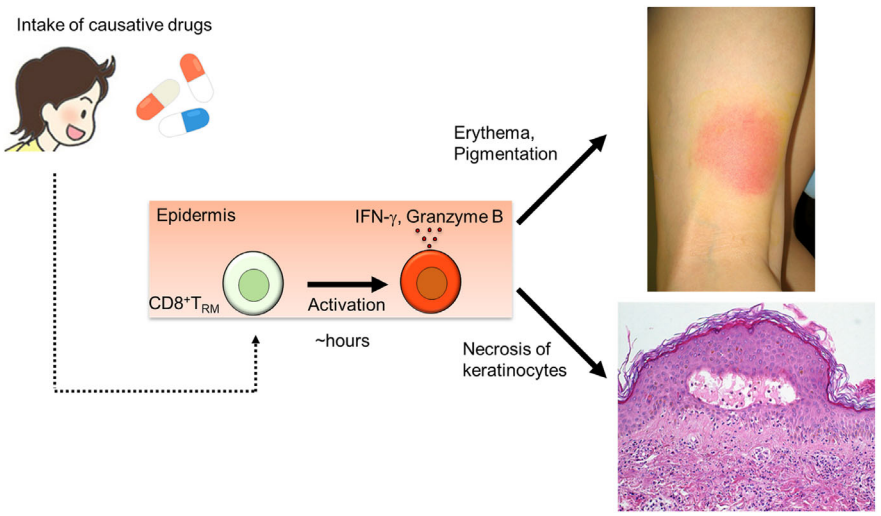

FIGURE 9 | Mechanism of fixed drug eruption.

DISCUSSION

One of the important issues on the residency status of skin T_{RM} cells in which what conditions allow T_{RM} cells to emigrate from the tissue is under debate. Skin T_{RM} fate decision seems to be established prior to antigens recognition. Once these naïve T cells encounter with cognate antigen presented by DCs, these pre-conditioned T cells will be ready to become a skin-homing T_{RM} precursor, implying that preconditioned naïve T_{RM} cells are prepared during homeostasis, and skin-homing molecules are imprinted during T cell priming (89). Inflammatory signals from inflamed skin attract these skin-homing cells to the local inflammation site. After entering the skin, local signals induce T_{RM} precursors to differentiate into mature skin T_{RM} cells. The non-differentiated T_{RM} precursors may recirculate between the skin, blood and LNs, where these cells possibly represent circulating memory T cells that have been described as skin recirculating memory T cells in mice (67) or skin-tropic migratory memory T cells in human (14). Interestingly, skin recirculating memory T cells are induced greatly by skin infection but not by intravenous infection (67). Moreover, a very recent study reported that skin T_{RM} could exit their residential skin and rejoin the circulating pool of memory T cells (97). In human *ex vivo* skin experiments, using the nanobody labeling technique also demonstrated that $CD8^+$ T_{RM} cells can migrate from the epidermis to the papillary dermis (161). However, whether T_{RM} cells that migrate out of the skin are authentic T_{RM} cells or these cells are skin recirculating memory T cells that intermittently present in skin remains to be elucidated.

Memory T cell populations are more diverse and heterogeneous than initial expectation, and tissue memory responses may be involved beyond the T_{RM} cell population. Recently, a novel concept of tissue memory beyond the role of adaptive immune memory has emerged. The inflammatory memory can be exerted by various cell types and the interaction among these memories across cell lineages and may impact on tissue adaptation and maladaptation (162). It should be noted that the characteristics and behavior of T_{RM} cells are different among barrier tissues, as each barrier tissue has specialized cells residing in each location, as exemplified by keratinocytes in the skin. A chemical allergen like DNFB can persist in the skin for several weeks, especially in keratinocytes around hair follicles, a part of which are slow-cycling epidermal stem cells (99). This remaining allergen in keratinocytes correlate with the number of antigen-specific $CD8^+$ T_{RM} cells (99). This epithelial memory may contribute to or instruct immune memory cells, and they coordinate each other to maximize the protection. $CD8^+$ T_{RM} cells that we have observed may just only a tip of the iceberg in the process of tissue memory responses.

In several cutaneous diseases, the presence of skin T_{RM} cells has been investigated in the active lesional skin and resolved lesional skin along with non-lesional, normal appearing skin. Unexpectedly, in the active lesion, it is no easy task to identify and enumerate T_{RM} cells, because many T cell populations are intermingled with each other and their activity, residency, and fate cannot be easily expected. For example, the involvement of T_{RM} cells in the recurrent lesions of psoriasis and fixed drug eruption are well known. However, it remains a matter of debate whether the cells with T_{RM} markers in the active lesions belong to T_{RM} cells. We have only limited information on the activity and residency of these cells in relation to the clinical significance.

In our clinical study in psoriasis patients, the cells with T_{RM} markers were increased in the active skin lesion and decreased after the systemic treatment with anti-IL-17A mAb, although they were relatively resistance to the treatment compared to the non-T_{RM} cells (142). In addition, T cells bearing T_{RM} markers in the active lesion were capable of producing pathogenic cytokines, such as IL-17A, and were possibly related to the unfavorable disease course (70). In active skin lesion, $CD8^+CD103^+$ cells tended to be present in the middle to upper epidermis, while they were located at the basal layer in the resolved skin and non-lesional skin of

psoriasis. Therefore, T_{RM} cells or T_{RM} marker-bearing cells behave as effector cells and likely serve as crucial effectors in psoriasis pathology. Further investigations on their dynamics, detailed functions, and residency are required. Furthermore, to see the disease specificity of these T_{RM} cells, T_{RM} characterization in atopic dermatitis is in progress in our laboratory.

AUTHOR CONTRIBUTIONS

Concepts: YT. Wrote the paper: YT, PP, TH, and TF. Designed the figures: PP, YT, and TH. Reviewed manuscript: TH and KK. All authors commented on the manuscript. All authors contributed to the article and approved the submitted version.

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Organ-Specific Surveillance and Long-Term Residency Strategies Adapted by Tissue-Resident Memory CD8$^+$ T Cells

Jens V. Stein, Nora Ruef and Stefanie Wissmann*

Department of Oncology, Microbiology and Immunology, University of Fribourg, Fribourg, Switzerland

Tissue-resident CD8$^+$ T cells (CD8$^+$ T_{RM}) populate lymphoid and non-lymphoid tissues after infections as first line of defense against re-emerging pathogens. To achieve host protection, CD8$^+$ T_{RM} have developed surveillance strategies that combine dynamic interrogation of pMHC complexes on local stromal and hematopoietic cells with long-term residency. Factors mediating CD8$^+$ T_{RM} residency include CD69, a surface receptor opposing the egress-promoting S1P1, CD49a, a collagen-binding integrin, and CD103, which binds E-cadherin on epithelial cells. Moreover, the topography of the tissues of residency may influence T_{RM} retention and surveillance strategies. Here, we provide a brief summary of these factors to examine how CD8$^+$ T_{RM} reconcile constant migratory behavior with their long-term commitment to local microenvironments, with a focus on epithelial barrier organs and exocrine glands with mixed connective—epithelial tissue composition.

Keywords: tissue-resident T cells, epidermal barrier, salivary gland, chemokine, integrin

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****Correspondence:***
Jens V. Stein
jens.stein@unifr.ch

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INTRODUCTION

During viral infections, Ag-specific naïve CD8$^+$ T cells (T_N) become activated in reactive secondary lymphoid organs (SLOs), and change their gene expression pattern and metabolism to differentiate into proliferating cytotoxic effector T cells (T_{EFF}) (1, 2). During the effector phase, T_{EFF} are subdivided into KLRG1$^+$ CD127$^-$ short-lived effector T cells and KLRG1$^-$ CD127$^+$ memory precursor effector cells, with a larger potential to generate long-lived memory cells in the latter compartment (3). T_{EFF} killing of infected cells in inflamed tissue requires direct cell-to-cell contact to identify cognate peptide major histocompatibility complexes (pMHC) on target cells, which leads to release of granzymes and perforin for induction of apoptosis (4, 5). Once intracellular infections have been cleared, memory CD8$^+$ T cells patrol the body for rapid protective recall responses upon secondary pathogen encounter. Depending on their surface marker expression and trafficking patterns, distinct subsets of memory CD8$^+$ T cells are classified (6). Central memory T cells (T_{CM}) maintain the ability to recirculate through SLOs through expression of the homing receptors L-selectin (CD62L) and the chemokine receptor CCR7, a characteristic shared with T_N. Recent work has shown that T_{CM} can also be rapidly recruited to sites of inflammation outside lymphoid tissue (7). Effector memory T cells (T_{EM}) lack CD62L and CCR7 expression and are thought to patrol non-lymphoid tissues (NLTs), although their precise functions are still not well-defined (8). Peripheral memory CD8$^+$ T cells (T_{PM}) have been recently described based on intermediate expression of the chemokine receptor CX3CR1 as predominant subset surveying NLTs (9).

Finally, self-renewing, non-recirculating tissue-resident memory T cells (T_{RM}) populate barrier organs after clearing of an infection as first line of defense, both in mice and humans (10–17). In contrast to circulating memory T cell subsets, T_{RM} are in a disequilibrium with blood as they are retained for months or years within their tissue of residency. Recent data suggest that tissue-residency vs. circulating memory potential is already imprinted during priming in lymphoid tissue. Migratory dendritic cells (DCs) from skin and gut epithelium present active transforming growth factor (TGF)-β to recirculating $CD8^+$ T_N, which preconditions these cells to form T_{RM} in a skin vaccination model (18). Such conditioning is another example of lymphoid tissue-directed steering of ensuing immune responses, such as reported for differential homing receptor induction in skin-vs. gut-draining lymphoid tissue (19). In line with this observation, a tissue-resident gene expression signature is readily detectable in early circulating T_{EFF} cells prior to entry into NLTs (20). Notably, presence of cognate antigen at infiltrated target sites is not a prerequisite for T_{RM} formation, although it increases their local abundance (21). Finally, in addition to sites of microbial infection, $CD8^+$ T cells with a T_{RM} signature are also detectable in tumors and in autoimmune inflammatory conditions, where these cells exert protective and detrimental effects, respectively (17).

Studies following the development of epidermal $CD8^+$ T_{RM} have shown that $KLRG1^-$ precursor cells enter the dermis during the early effector response and that their entry into the epidermis involves the action of keratinocyte-secreted chemokines that bind to CXCR3 and CCR10 expressed on skin-homing T cells (22, 23). The cytokines IL-15 and TGF-β are involved in the formation and survival of epidermal T_{RM}. In particular, TGF-β transactivation by keratinocytes increases expression of the integrin chain CD103, which plays a role in tissue retention of epidermal T_{RM} (see below) (22, 24, 25). T_{RM} are characterized by a core transcriptional program mediated by the transcription factors Hobit and Blimp1, as well as Runx3 and Notch (26–28). As a local adaptation to the lipid-rich skin environment, fatty acid metabolism, and mitochondrial functions regulate epidermal T_{RM} development and survival (29). In addition to epithelial barriers, T_{RM} have been identified in virtually all organs including central nervous system (CNS), exocrine glands, lungs, liver, kidney, bone marrow, reproductive tract, as well as tumors (10, 17, 30–36). Notably, far from being a homogeneous population, T_{RM} display considerable heterogeneity (37–39) and interact with diverse, undefined non-hematopoietic cells during local reactivation (40). Furthermore, a recent report using a Hobit expression/fate reporter mouse line has uncovered that T_{RM} have the capacity to de-differentiate to T_{EFF}, which occurs in parallel to Hobit downregulation after TCR activation (41).

The localization of T_{RM} to sites of previous pathogen infection poise them to rapidly respond to secondary infections. Accordingly, T_{RM} release cytokines after activation and express high levels of effector molecules such as granzyme B for target cell killing. The protective role for T_{RM} is exemplified by studies in barrier sites of the skin and mucosal surfaces such as the female reproductive tract, where these cells lodge within the epithelium. Antigen re-challenge experiments have shown that T_{RM} act as first-line defense by inducing a tissue-wide alert state, in part via IFN-γ secretion (42–48). These signals relay to innate immune cells for additional cytokine release that results in recruitment of immune cells to the site of pathogen re-emergence, essentially reversing the paradigm that activation of the innate immune system always precedes the adaptive immunity activation. Thus, while T_{RM} also undergo bystander activation through inflammatory cytokines (49, 50), local immune surveillance for cognate pMHC presented on host cells is a key feature of $CD8^+$ T_{RM} cells to provide pathogen-specific, long-lasting host protection. To achieve this extraordinary feat, $CD8^+$ T_{RM} acquire the ability to infiltrate and physically scan their environment for infected cells within virtually any host organ, while avoiding inadvertent tissue exit via blood or lymphatic vessels or out of an epithelial barrier. Accordingly, $CD8^+$ T_{RM} have been found to be patrolling vascular compartments, such as liver sinusoids (51), as well as neuronal and muscle tissue (32, 52). Other anatomical locations surveilled by T_{RM} vary in their content of epithelial and connective tissue: (i) predominantly epithelial (e.g., epidermis and mucosal epithelium), (ii) mixed epithelial—connective (e.g., exocrine and endocrine glands), and (iii) predominantly connective tissue (e.g., lymph nodes and spleen) (**Figure 1**). Here, we will provide a brief overview on tissue retention and surveillance strategies focusing on data gained in mouse models of skin vs. salivary glands as prototypical epithelial barrier site vs. exocrine gland.

MULTIPLE LAYERS OF TISSUE RETENTION COOPERATE FOR LONG-TERM T_{RM} SURVEILLANCE OF EPITHELIAL BARRIER TISSUE

Expression of CD69 is the most commonly employed marker to define T_{RM} in all locations, although it is not an exclusive T_{RM} marker and its expression does not necessarily correlate with establishment of long-term resident T_{RM} populations (53, 54). CD69 is a cis-antagonist of the sphingosine-1-phosphate receptor 1 (S1P1) required for egress via lymphatic vessels, which drain interstitial fluid from organs and which contain higher amounts of S1P than tissue (55, 56). T_{RM} also reduce S1P1 production on a transcriptional level, which is prerequisite for establishing long-term residency (57). In epithelial tissues, most T_{RM} express CD103, which is the α_E chain of the E-cadherin receptor $\alpha_E\beta_7$ (6, 58). E-cadherin is expressed by epithelial cells, where it promotes their homotypic adhesion. In line with this, CD103 promotes the long-term persistence of T_{RM} in skin, presumably by retaining these cells within the keratinocyte layer (22). Epidermal $CD8^+$ T_{RM} further upregulate the collagen receptor $\alpha_1\beta_1$, which also contributes to their long-term permanence (59, 60). Finally, T_{RM} increase expression of the negative regulator of chemoattractant receptor signaling, regulator of G-protein-coupled signaling 1 (RGS1) (61, 62). RGS1 and related members of the RGS family activate the GTPase activity of GTP-bound $G\alpha_i$, which leads to a cessation of $G\alpha_i$-coupled receptors signaling (63). RGS-mediated blunted responsiveness to chemoattractants, such as S1P, likely contributes to long-term residency, although experimental

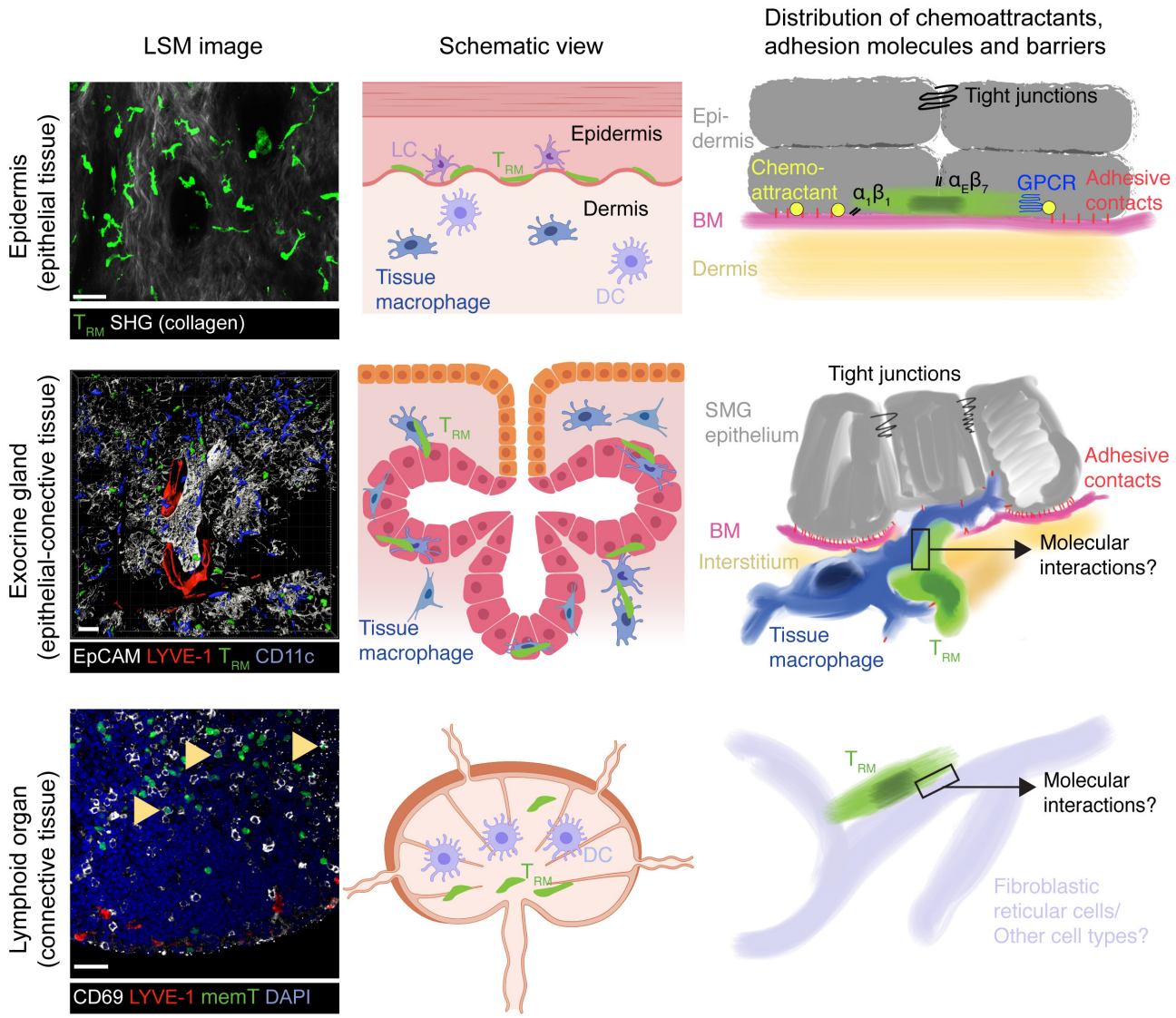

FIGURE 1 | Model of T_{RM} surveillance strategies according to organ topography. In epithelial barrier tissues such as epidermis, T_{RM} mainly locate on top of the basement membrane (BM) separating connective tissue from the epithelium, which themselves are connected by adherens and tight junctions. Both BM and tight junctions serve as physical boundaries to T_{RM} foraging, essentially restricting their motility to a 2D-like surface. Chemoattractants, either constitutively expressed or induced by microbial presence, together with α1 and αE integrins further re-enforce this restricted migration pattern to ensure long-term retention by preventing inadvertent loss of scanning T_{RM} outside the epithelial barrier. In exocrine glands such as the SMG (mixed arborized epithelial—connective tissue), tight junctions between secretory epithelial cells may constitute a similar barrier to prevent loss of T_{RM} into the acini or duct lumen. Yet, the BM separating secretory epithelium from supporting interstitium remains permissive for two-way traffic into and out of epithelial cell layers, which is facilitated in SMG by tissue macrophages. Accordingly, non-inflamed secretory epithelial cells presumably secrete only low levels of chemoattractants that would otherwise retain T_{RM} in this site. This mode of tissue scanning permits rapid accumulation of T_{RM} to sites of secondary pathogen encounters, which would be hampered if T_{RM} were confined exclusively to the epithelial cell layer. While $CD69^+$ memory $CD8^+$ cells also locate to lymphoid tissue following a viral infection (arrowheads), their function and dynamic interactions with local cells enabling their long-term retention and host protective capacity remain unknown. Similarly, it remains unclear whether SLO T_{RM} retain responsiveness to inflammatory chemokines as their counterparts in epithelial layers and exocrine glands. All confocal images show GFP^+ OT-I $CD8^+$ TCR transgenic T cells at >30 days following systemic or local (skin) virus infections. LSM, laser scanning microscope; SHG, second harmonic generation; LC, Langerhans cells; DC, Dendritic cells; BM, basement membrane, memT, $CD8^+$ memory T cells. Scale bar LSM images, 30 μm. Middle panels created with https://biorender.com/.

evidence is still lacking. Taken together, $CD8^+$ T_{RM} have multiple molecular modules at their disposal that in combination reduce the probability to accidentally exit their tissue of residency during homeostatic surveillance. Moreover, the structure of the epithelial microenvironment likely contributes to long-term retention of T_{RM}. Epidermal T_{RM} lodge on top of a dense basement membrane (BM) separating underlying connective tissue from the overlying epithelium, and such BM form physical

barriers that limit leukocyte dissemination (64). At their apical border, epithelial cells are attached via tight junctions that form a barrier for T cell exit out of the epidermis or into the gut lumen, respectively (65, 66). These factors likely help epithelial T_{RM} to establish long-term tissue-residency as a prerequisite for life-long protection at previously infected sites (**Figure 1**).

Within their tissue of residency, epidermal T_{RM} physically scan the local cell neighborhood for cognate pMHC. During this process, they display characteristic elongated shapes with numerous dendrites that constantly extend and contract and move in a $G\alpha_i$-dependent manner with speeds of 1–2 μm/min along the bottom keratinocyte layer, resembling motility on a 2D layer (23, 67, 68). Reconstruction of T_{RM} motility in human skin biopsies revealed that these cells occasionally traversed the papillary dermis, and are therefore less strictly confined to the epidermis as observed in mouse skin (69). Both T_{RM} dendricity and motility contribute to efficient scanning of the epidermis (67). Lack of neither the skin-selective chemokine receptors CCR8 or CCR10 (70), nor CXCR3 or CXCR6 affect baseline motility of epidermal T_{RM}, although lack of CXCR6 reduces T_{RM} dendricity (23). During secondary viral spread, epidermal $CD8^+$ T cells use CXCR3 to follow local chemokine signals and accumulate around infected cells (4, 48). In sum, epidermal T_{RM} maintain responsiveness to inflammatory chemokines despite their $G\alpha_i$-dependent basal motility, suggesting that these chemoattractants override their homeostatic, as yet undefined GPCR input.

Lack of the $\alpha_1\beta_1$ integrin but not CD103 leads to a loss of the dendrite-shaped T_{RM} morphology (23, 60), suggesting that these cells form transient anchors with their protrusions interacting with extracellular matrix. The precise molecular composition of these transient $\alpha_1\beta_1$-mediated adhesions remains to be characterized but they likely differ from the more long-lasting anchoring of tissue macrophage protrusions (71). Furthermore, *ex vivo* migration analysis of lung T_{RM} uncovered a role for CD49a in facilitating T_{RM} translocation, whereas CD103 did not promote motility (72). Instead, lack of CD103 leads to an increase in epidermal T_{RM} speeds *in vivo*, suggesting a primary role for this integrin in tissue retention (23). The impact of CD49a on *in vivo* T_{RM} motility parameters has not been determined yet.

Similar to CD49a deficiency, microtubule network depolymerization following nocodazole treatment leads to a loss of the characteristic T_{RM} dendricity (23). This phenomenon is likely due to global release of Rho-activating factor ArhGEF2 otherwise trapped in microtubules (73). Controlled release of ArhGEF2 from depolymerizing microtubules has been recently shown to play an important role in retracting protrusions that are not following the nuclear translocation path during amoeboid cell displacement (74). This pathway serves therefore as a proprioceptive mechanism to control amoeboid cell shape in complex environments such as formed by the tightly packed keratinocyte layer, and is essential to avoid accidental cell rupture. A role for ArhGEF2 in facilitating epidermal T_{RM} motility has thus far not been experimentally addressed. Taken together, continuous retention of epithelial T_{RM} is mediated by multiple integrin receptor interactions and homeostatic GPCR signaling. Long-term T_{RM} colonization may be further facilitated by "layered" architecture of epidermis with a BM separating the underlying connective tissue and the tight junction seal on the apical part of the epithelial layer (**Figure 1**).

T_{RM} LODGING AND SURVEILLANCE OF "NON-BARRIER" NLTs

In addition to the well-studied epidermis and small intestinal epithelium that are constitutively exposed to microbes, T_{RM} lodge to organs that are less subjected to constant microbial challenge and contain few or no E-cadherin-expressing epithelial layers. These organs include CNS, kidney, submandibular salivary glands (SMG), liver, and bone marrow (10, 16, 75, 76). In contrast to epidermis where $CD8^+$ T_{RM} are embedded between non-vascularized epithelial cells, these complex organs contain extensive blood and lymphatic vascular systems, innervation, fibroblasts, tissue-resident macrophages, and innate immune cells, as well as in some cases arborized secretory epithelium. In addition to distinct tissue-specific cellular composition (e.g., kidney tubular cells, hepatocytes, CXCL12-abundant reticular cells of the bone marrow) and receptor-ligand expression patterns, these organs differ in their metabolic activity (e.g., liver) or immunosuppressive environment (e.g., reproductive tract) (77, 78). Furthermore, beyond the biochemical and cellular properties of individual tissues, physical parameters such as topography, substrate stiffness, and confinement influence cell-based immune responses and cross-talk with their environment (79, 80). To date, little is known about how the local microenvironment in these organs affects the phenotype and mechanism of surveillance of T_{RM} during homeostasis and recall responses. While the high expression of CD69, CD49a, and RGS1 on a majority of non-barrier NLT T_{RM} suggests similar roles as in epithelial barrier tissues, CD103 expression is not required for long-term retention of T_{RM} in SMG, in contrast to skin (81, 82). Another key issue is whether memory T cells from distinct anatomical locations employ tissue-specific mechanisms of host surveillance.

In a recent study, we have found that T_{RM} lodging in SMG acquire a motility program distinct from T_{CM} and epidermal T_{RM} (83). In contrast to memory T cells isolated from lymphoid tissue or epidermis, *in vivo* observations suggested SMG $CD8^+$ T_{RM} were largely refractory to pharmacological inhibition of $G\alpha_i$-protein-coupled receptors or integrin adhesion molecules during homeostatic tissue surveillance, although they retained the ability to respond to inflammatory chemokines and expressed high levels of the CD103, CD49a, CD49d, and CD11a integrins (83). While integrin-independent migration in 3D matrices has become a widely accepted concept in cell biology based on studies with cell lines and DCs (84), several studies demonstrated integrin involvement during immune surveillance of skin T cells (23, 85). As direct evidence for specific adhesion-independent motility, T_{RM} isolated from salivary glands displayed spontaneous motility under 2D confinement in the absence of integrin ligands or chemoattractants. Adhesion-free motility in 2D conditions was reported for large, blebbing carcinoma cells, based on non-specific friction mediated by

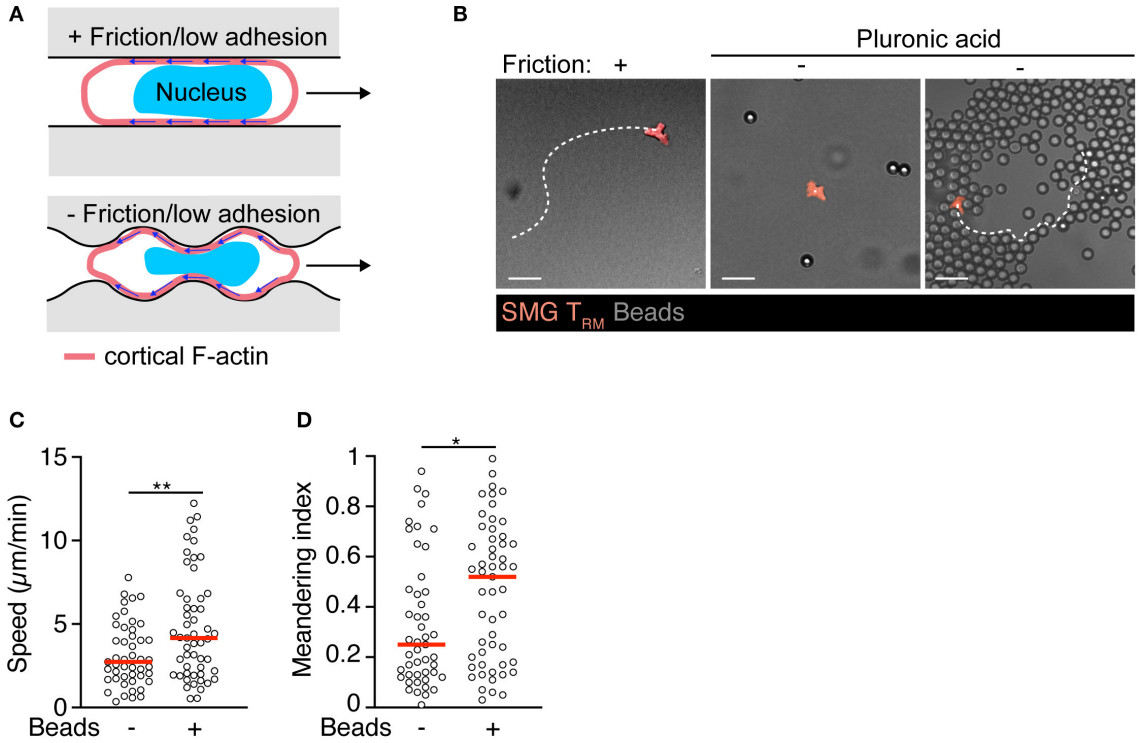

FIGURE 2 | Intrinsic motility of SMG T_{RM} triggered by environmental topography. **(A)** Model for autonomous exocrine gland T_{RM} motility generated by baseline retrograde F-actin flow coupled via non-specific substrate friction or low adhesiveness under physical confinement. In addition, under completely non-adhesive conditions, cell propulsion can be generated through bending of the retrograde cortical actin flow by the environmental topography. Adapted from Reversat et al. (88). **(B)** Exemplary track of isolated SMG T_{RM} in "under agarose" confinement on human serum albumin with and without pluronic acid (PA) passivation to abolish residual friction or lodged between 7 μm-polystyrene bead clusters. Scale bar, 20 μm. **(C)** T_{RM} speeds within or outside of polystyrene bead clusters in presence of PA. **(D)** T_{RM} meandering index within or outside of polystyrene bead clusters in presence of PA. Data were analyzed by unpaired *t*-test **(C)** or Mann–Whitney test **(D)**. $^{*}p < 0.05$; $^{**}p < 0.01$.

a large interface between migrating cells and substrates (**Figure 2A**) (86, 87). Similarly, we observed that non-specific substrate friction is sufficient to trigger intrinsic SMG T_{RM} motility in 2D confinement (83). In turn, T_{RM} isolated from salivary glands did not show displacement on "slippery surfaces," i.e., in presence of EDTA or when surfaces were passivated with pluronic acid, which reduces friction below a threshold for cell translocation (**Figures 2B–D**). Notably, these cells regained the capability to translocate in absence of substantial friction when a 3D geometry was created by immotile neighboring objects (**Figures 2B–D**). This motility mode correlated with continuous changes in cell shapes during migration through microchannels formed by the microenvironment. In this setting, SMG T_{RM} continuously form multiple simultaneous protrusions that probe the environmental geometry, leading to their insertion into permissive gaps and subsequent cell body translocation (83). In the complex 3D exocrine organ architecture, tissue macrophages embedded within the epithelial and connective tissue compartments contributed to generate available extracellular space for protrusion-forming T_{RM} (83).

How do T_{RM} shape changes generate tractive force for cell translocation under these conditions? A recent study has identified adhesion-free cellular locomotion driven by microenvironmental architecture (**Figure 2A**) (88). Thus, a permissive local topography facilitates cell motility by adapting the cell shape to features of the environment such as crevices and serrated surfaces. At these non-smooth surfaces, rearward cortical F-actin flow generates non-normal forces that results in forward cell motility, rendering cellular translocation autonomous from external influences (**Figure 2A**). These data provide a model for adhesion-free T_{RM} motility in the absence of friction, and highlight the multiple ways T_{RM} are able to integrate chemical signals (e.g., chemoattractants) and tissue architecture to patrol complex 3D structures such as secretory glands.

What may be the advantages of such a non-canonical migration mode for immune surveillance of mixed connective—epithelial tissues? In contrast to the epidermally restricted migratory behavior of $CD8^{+}$ skin T_{RM} (89), exocrine gland T_{RM} display a bidirectional trafficking pattern into and out of epithelial layers, a process facilitated by tissue macrophages (**Figure 1**) (83). Such bidirectional trafficking would be perturbed by epithelial chemokine secretion, which could furthermore lead to continuous leukocyte influx and exacerbated inflammation after clearance of infection. Instead, this modus allows T_{RM} to remain responsive to inflammatory chemokines that are locally secreted at sites of pathogen re-emergence. In this context, not

being confined to arborized secretory epithelium shortens the pathlength that T_{RM} need to travel in order to accumulate at local sites of inflammation. Furthermore, as ECM proteins and other integrin ligands differ in distinct NLTs (90, 91), integrin-independent motility may endow T_{RM} subsets with flexible topography-driven organ surveillance in non-epithelial barrier sites. A non-proteolytic pathway is beneficial to preserve the integrity of the target tissue, as it does not require constant repair of newly generated discontinuities in the ECM matrix (92). The scanning strategy adopted by homeostatic SMG T_{RM} resembles the migration pattern of T cell blasts in 3D collagen networks, where these cells routinely bypass dense collagen areas, while probing the environment for permissive gaps for cell body translocation (93). In sum, these observations are consistent with a model where certain NLT T_{RM} switch during homeostatic immune surveillance to a self-motile "autopilot" mode supported by tissue macrophage topography, while remaining susceptible to locally produced inflammatory signals for concerted cytotoxic activity. Whether $CD8^+$ T_{RM} have adapted a comparable mode for other non-barrier NLTs and whether autonomous motility is shared by other tissue-resident leukocytes, such as $CD4^+$ T_{RM}, NK or innate lymphoid cells, remains unknown.

DISCUSSION

Here, we put the general tissue architecture of epidermis and salivary glands as prototype epithelial vs. mixed epithelial—connective tissues into context with published observations on the dynamic surveillance strategies adapted by T_{RM}. Reflecting the acknowledged heterogeneity, T_{RM} develop distinct tissue-specific scanning modalities, i.e., chemokine- and integrin-dependent and -independent in epidermis and exocrine glands, respectively, to balance retention and local pMHC interrogation. Independent of their baseline homeostatic migration mode, T_{RM} remain susceptible to inflammatory chemokines produced during pathogen re-encounter, which facilitates their clustering at target sites, perhaps reflecting the low killing rate of cytotoxic $CD8^+$ T cells against stromal cell targets (94). Furthermore, certain organs such as epithelial barrier sites might have a higher abundance of promigratory factors in steady state owing to their continuous exposure to microbes. In contrast, non-barrier NLTs may generally express low amounts of chemoattractants in absence of inflammation that demand an adaptation of local immune cells. Recent data suggest that nuclear sensing of confinement may contribute to generate cellular translocation in the absence of external factors (95, 96). Yet, it remains unclear whether or in which NLTs this contributes to T_{RM} surveillance patterns.

A recent observation made by Masopust and colleagues was the presence of *bona fide* $CD69^+$ T_{RM} in the red pulp (RP) of spleen and medullary area of LNs (97) (**Figure 1**), which are at least in part derived from NLT T_{RM} precursors (53). In contrast to $CD62L^+$ $CCR7^+$ T_{CM} (98), the physiological role of T_{RM} in SLO remains essentially unknown to date. Notably, recent data suggest that in humans a large proportion of memory $CD4^+$ and $CD8^+$ T cells are $CD69^+$ *bona fide* T_{RM}, including in LNs and spleen (99). While some of these cells may retain the capacity to recirculate (53), these observations suggest the presence of specific T_{RM} niches with a potential role during re-infection, e.g., via cytokine secretion and/or de-differentiation into T_{EFF} (41). At the same time, the close spatial proximity of spleen T_{RM} to vascular sinuses in the RP (97) raises the question how these cells reconcile dynamic tissue surveillance with long-term retention in a connective tissue with few major tissue barriers such as extensive tight junctions and basement membranes as compared to epithelial barrier sites (**Figure 1**) (100). Taken together, many incognita remain on the organ-specific T_{RM} cross-talk with the local microenvironment. Combining *in vivo* analysis with high resolution single cell technologies to take into account cell heterogeneity will shed light on these open points.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Discipline in Stages: Regulating CD8$^+$ Resident Memory T Cells

Rut Mora-Buch[†] *and Shannon K. Bromley*[*]

Center for Immunology and Inflammatory Diseases, Division of Rheumatology, Allergy and Immunology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States

Resident memory CD8$^+$ T (T_{RM}) cells are a lymphocyte lineage distinct from circulating memory CD8$^+$ T cells. T_{RM} lodge within peripheral tissues and secondary lymphoid organs where they provide rapid, local protection from pathogens and control tumor growth. However, dysregulation of CD8$^+$ T_{RM} formation and/or activation may contribute to the pathogenesis of autoimmune diseases. Intrinsic mechanisms, including transcriptional networks and inhibitory checkpoint receptors control T_{RM} differentiation and response. Additionally, extrinsic stimuli such as cytokines, cognate antigen, fatty acids, and damage signals regulate T_{RM} formation, maintenance, and expansion. In this review, we will summarize knowledge of CD8$^+$ T_{RM} generation and highlight mechanisms that regulate the persistence and responses of heterogeneous T_{RM} populations in different tissues and distinct microenvironments.

Keywords: tissue resident memory T cell, T cell differentiation, recall response, microenvironment, transcriptional regulation

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***Correspondence:**
Shannon K. Bromley
sbromley@mgh.harvard.edu

[†]***Present address:***
Rut Mora-Buch, Cell Therapy Services, Blood and Tissue Bank, Barcelona, Spain

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INTRODUCTION

Long-term memory to pathogens is a key feature of the adaptive immune system. The ability of memory T cells to mount rapid and potent responses against previously encountered antigens maintains human health by controlling infections and tumor growth; it also provides the rationale for designing vaccines against pathogens and immune therapies to treat cancer. By recirculating through blood and lymph, circulating memory T cells may provide broad tissue immune surveillance. However, recent findings demonstrated that long after the resolution of infection, the majority of memory CD8$^+$ T cells are non-circulating (1). Rather, most CD8$^+$ memory T cells are stably maintained in tissues as tissue resident memory T cells (T_{RM}) that exhibit transcriptional and phenotypic characteristics distinct from circulating memory CD8$^+$ T cells (2). Early studies identified T_{RM} within the epithelial compartment of barrier tissues including skin, lung, and intestine (3–8). Later, T_{RM} were identified in the tissue stroma as well as in non-barrier tissues such as liver, brain, and secondary lymphoid organs including spleen and lymph nodes (LN) (9–12). CD8$^+$ T_{RM} deliver highly effective, localized responses to pathogen challenge (4, 8). Additionally, CD8$^+$ T cells with a T_{RM} phenotype are a target candidate for anti-tumor immunotherapy (13–15) and predict an improved prognosis in several different cancers (16–23). Although T_{RM} provide potent protection against pathogens and tumors, T_{RM} dysregulation has been linked to immune-mediated diseases including psoriasis (24), vitiligo (24), and alopecia areata in the skin (25), and inflammatory bowel disease in the intestine (26). Additionally, T_{RM} develop following sensitization to allergens and play a role in hypersensitivity reactions in allergic contact dermatitis (27, 28) and asthma (29). Finally, T_{RM} have been linked to fixed drug eruptions (30), as well as rejection of solid

organ transplants (31). This review will discuss intrinsic and extrinsic mechanisms that promote $CD8^+$ T_{RM} formation, maintenance and function for defense against invading pathogens, as well as mechanisms that limit T_{RM} formation and effector response to prevent excessive inflammation and tissue damage (**Figure 1**).

STAGE 1: PRIMING AND PRECURSOR FORMATION: $CD8^+$ T CELLS, BORN OR TRAINED TO BE T_{RM}?

Following cognate antigen recognition, naïve $CD8^+$ T cells become activated, proliferate and give rise to heterogeneous progeny with distinct effector and memory cell fates. Recent experimental evidence suggests that extrinsic signals can influence $CD8^+$ T cell fate even before antigen recognition (32) (**Box 1**). After antigen activation, the majority of activated T cells die by apoptosis during the contraction phase of the immune response, but a small minority survive to become memory $CD8^+$ T cells. Whether activated T cells survive may depend on external signals, including growth factor availability, antigen, and inflammation, as well as internal signals such as transcription factor and growth factor receptor expression. Multiple, non-mutually exclusive models have been proposed to explain the development of diverse populations of effector and memory $CD8^+$ T cells (34). For example, the fixed lineage model proposes that commitment to effector or memory T cell lineages occurs soon after T cell stimulation, as early as the

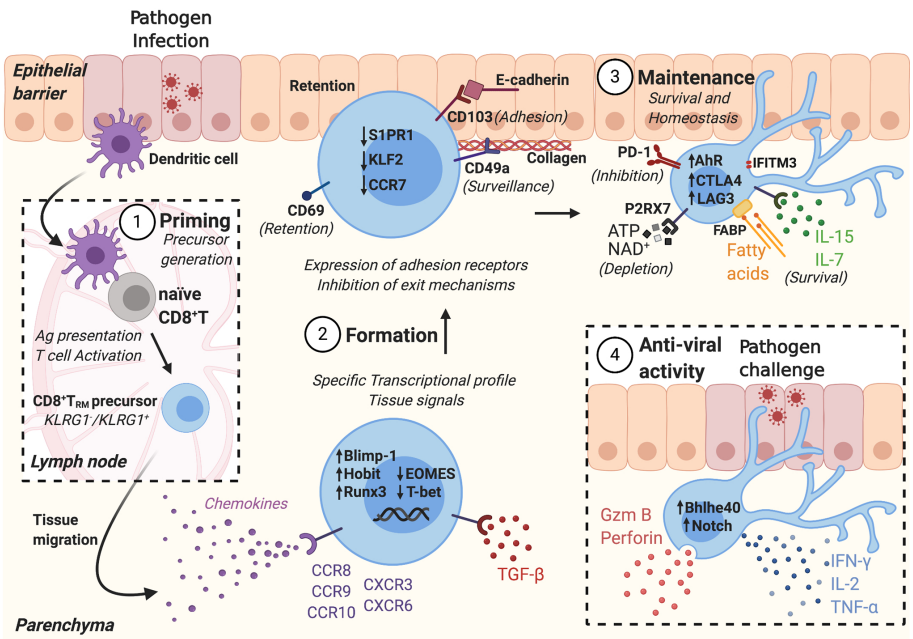

FIGURE 1 | $CD8^+$ T_{RM} formation and anti-viral activity is tightly regulated in different stages. 1) Following pathogen infection, tissue dendritic cells (DCs) migrate to the draining lymph nodes and present antigens to naïve T cells. Antigen-specific naïve T cells are activated, generating $CD8^+$ T_{RM} precursors. 2) $CD8^+$ T_{RM} precursors migrate into peripheral tissues, following chemotactic signals. $CD8^+$ T_{RM} formation depends on tissue signals that activate a T_{RM} transcriptional profile, including the expression of adhesion receptors and inhibition of exit mechanisms. 3) $CD8^+$ T_{RM} are maintained in the tissue where they receive survival signals and express inhibitory receptors to maintain tissue homeostasis. 4) During secondary infection, $CD8^+$ T_{RM} are activated, secrete effector molecules, and amplify the immune response.

BOX 1 | Pre-Programmed Naïve $CD8^+$ T Cells: The Existence of a Stage 0.

Although current models suggest that a single naïve T cell has the potential to differentiate into all effector and memory subsets depending on the antigen, costimulatory, and cytokine stimulation they receive, recent experimental evidence suggests that extrinsic signals influence $CD8^+$ T cell fate even before antigen recognition. Recent work by Mani et al. demonstrated that extrinsic cytokine signaling can imprint naïve $CD8^+$ T cells for subsequent T_{RM} formation. Migratory DCs expressing TGF-β-activating integrins in the LN activate TGF-β and epigenetically condition naïve $CD8^+$ T cells, even before antigen stimulation, to form epithelial $CD8^+$ T_{RM} in the skin (32). These results suggest that during immune homeostasis, the LN environment affects future T cell fate. In addition, research using a tamoxifen-inducible fate-mapping mouse model to mark $CD8^+$ T cells made in the thymus during fetal, neonatal, and adult stages, Smith et al. demonstrated that naïve $CD8^+$ T cells generated during different developmental stages, fetal vs. adult, acquire different phenotypes upon antigen encounter. These results suggest that $CD8^+$ T cell fate may be controlled by the timing of naïve precursor cell maturation in the thymus (33). These studies open the possibility of additional regulatory mechanisms and signals that impact future $CD8^+$ T_{RM} generation even before inflammatory or antigen insult. Future studies are needed to better understand how intrinsic and extrinsic signals during naïve $CD8^+$ T cell generation and homeostasis influence $CD8^+$ T cell fate.

first cell division and may result from the asymmetric division of effector fate-associated factors. On the other hand, the decreasing potential model posits that early effector cells have memory potential that is lost with increased or prolonged stimulation with antigen or cytokines. More recently, Rosato et al., have proposed an expanded model of decreasing potential to include $CD8^+$ T_{RM}. They propose that the differentiation of $CD8^+$ T cells along a continuous axis of decreasing memory potential is irreversible. However, they also divide cells based on parallel paths of migration status-stationary or migratory, that may be altered by extrinsic stimuli including TCR signaling and inflammation (35), reflecting the cells' plasticity.

$CD8^+$ T_{RM} Precursor Differentiation

Expression of KLRG1 and CD127 has been used to define the memory potential of effector $CD8^+$ T cells around the peak of the immune response. Adoptive transfer studies suggest that $KLRG1^+$ $CD127^-$ short-lived effector cells (SLEC) tend to die following clearance of antigen, whereas $KLRG1^-$ $CD127^+$ memory precursor effector cells (MPEC) preferentially survive to give rise to memory $CD8^+$ T cells (36). Using a single cell adoptive transfer approach, Stemberger et al. tracked the progeny of individual naïve $CD8^+$ T cells. Using CD62L and CD127 as phenotypic markers, and IL-2, TNF-α, IFN-γ and CD107a expression as functional readouts, they demonstrated that diverse effector and memory $CD8^+$ T cells can arise from the same naïve precursor T cell (37). Additionally, single cell tracing experiments using adoptive transfer of barcode labeled OT-I T cells and systemic or local infection models, confirmed that both effector and memory $CD8^+$ T cell subsets derive from the same precursors in the naïve T cell pool (38). Moreover, TCR repertoire analysis of antigen-activated $CD8^+$ T cells demonstrated that 35 days post-immunization, $CD8^+$ memory T cells recovered from the skin share a common clonal origin with memory $CD8^+$ T cells isolated from draining and distant LNs, suggesting that T_{RM} and circulating memory T cells can develop from an individual naïve T cell (39). Together, these results suggest that memory T cell fate is not imprinted on naïve T cells, but rather that individual naïve T cells can give rise to all effector and memory $CD8^+$ T cell subsets. However, recent data suggest that although the majority of naïve T cells contribute to both circulating memory and $CD69^+$ $CD103^+$ T_{RM} cell populations, the contribution of individual clones to each memory pool varies (40). Additionally, analysis of individual T cell families (a naïve T cell and its progeny) demonstrated that clonal expansion and differentiation of T cells bearing the same TCR are heterogeneous, and so the contribution of the progeny of individual naïve T cells varies between primary versus recall responses (41).

Substantial effort has focused on identifying $CD8^+$ T_{RM} precursor cells and defining when $CD8^+$ T cells commit to a T_{RM} fate (**Supplementary Table 1**). Like circulating memory $CD8^+$ T cells, $CD8^+$ T_{RM} can also differentiate from $KLRG1^-$ precursor cells. Mackay et al. demonstrated that $KLRG1^-$, but not $KLRG1^+$, HSV-specific gBT-I effector T cells sorted from the spleens of mice 6 days post-HSV infection, generated cutaneous $CD103^+$ T_{RM} cells following their adoptive transfer into HSV-infected recipient mice (42). Subsequent studies suggested that $CD8^+$ T_{RM} are derived from MPEC after their entry into peripheral tissues. For example, following infection with *Listeria monocytogenes* (LM), splenic MPEC and SLEC lack expression of the T_{RM} receptors, CD69 and CD103. However, MPEC but not SLEC recovered from the intestine express CD103 and CD69 (43). Additionally, elegant work performed by Kurd et al. used single-cell RNA sequencing to define the gene expression patterns of individual $CD8^+$ T cells in the spleen and small intestine intraepithelial lymphocyte (siIEL) compartments over the course of lymphocytic choriomeningitis virus (LCMV) infection. Four days post-infection, the earliest time-point that virus specific $CD8^+$ T cells are detected within intestinal tissue, activated $CD44^{hi}$ small intestinal $CD8^+$ T cells display a transcriptional profile distinct from splenic $CD44^{hi}$ $CD8^+$ T cells. Even at day 3 following infection, splenic $CD8^+$ T cells do not resemble siIEL, suggesting that circulating precursors are not committed to a T_{RM} fate until after entry into the tissue (44). In contrast, using lineage tracing and single-cell transcriptome analysis, Kok et al. identified a subset of circulating effector $CD8^+$ T cells at the peak of effector T cell expansion after skin DNA vaccination that are enriched for T_{RM} fate-associated gene expression and have a higher propensity to form T_{RM} (40). Because the clonal composition of T_{RM} recovered from anatomically separate skin immunization sites is similar, they proposed that a committed T_{RM} precursor pool exists in the circulation, before entry into the tissue. Although the nature, timing or location of the early signals that imprint the ability to form T_{RM} before tissue entry were not defined by this study, work by Mani et al. suggests that during immune homeostasis, naïve $CD8^+$ T cells are epigenetically preconditioned for T_{RM} formation through their interaction with migratory dendritic cells (DCs) expressing TGF-β-activating integrins (32).

Recent studies suggest that effector cells may maintain plasticity to dedifferentiate and seed the memory pool. Using a $KLRG1^{Cre}$ reporter system that allows tracking of $KLRG1^+$ T cells over time, Herndler-Brandstetter et al. demonstrated that early post infection, $KLRG1^+$ effector $CD8^+$ T cells can downregulate KLRG1 and differentiate into all memory T cell lineages, including $CD8^+$ T_{RM} in the lung, intestine, and skin, and mediate effective protective immunity (45). Additionally, work by Youngblood et al. examined the transcriptional and epigenetic changes in naïve $CD8^+$ T cells during differentiation to effector and memory cells over the course of an acute LCMV infection. Whole genome bisulfite sequencing analysis demonstrated that epigenetic repression of naïve-associated genes in effector $CD8^+$ T cells can be reversed in cells that develop into long-lived memory $CD8^+$ T cells, while key effector genes including *Gzmb* and *Prf1* remain demethylated (46). These studies suggest that effector $CD8^+$ T cells may not have a fixed fate and contribute to the diversity of the memory T cell pool.

Intrinsic Control of CD8$^+$ T_{RM} Precursor Generation: TCR Affinity and Signal Strength

The finding that CD8$^+$ T_{RM} and circulating memory CD8$^+$ T cells can express identical TCR sequences (37) counters the hypothesis that TCR affinity or signal strength determines CD8$^+$ T_{RM} differentiation. However, intrinsic signals, including TCR signal strength and antigen affinity can influence CD8$^+$ memory T cell development. For example, a study using OT-I TCR transgenic mice with a point mutation in the conserved antigen receptor transmembrane (CART) motif suggests that effector and memory T cell differentiation require different signals. Both WT and mutant T cells differentiate comparably into effector T cells. However, mutant cells fail to polarize TCR to the immunological synapse, have decreased NFKB induction, and this impaired TCR signaling is correlated with decreased memory CD8$^+$ T cell differentiation (47). Additionally, studies have demonstrated that higher affinity TCR interactions direct CD8$^+$ T cells to a CD62L$^-$ T_{EM} fate, whereas lower TCR affinities promote CD62L$^+$ T_{CM} formation (48). Several studies also support the idea that TCR affinity and signal strength have a direct and unique impact on CD8$^+$ T_{RM} formation. For example, in a mouse model of persistent polyomavirus (MPyV) infection, high-affinity CD8$^+$ CD69$^+$ T_{RM} cells in the brain originate from high-affinity CD62L$^-$ effector cells present in the tissue during acute infection (49). In contrast, in a separate study again using a model of MPyV, the data instead suggested that lower TCR stimulation strength improves memory potential and generates functional brain CD62L$^-$ CD69$^+$ T_{RM} cells (50). Similarly, in an acute influenza infection model, lower affinity TCR stimulation is more likely than higher affinity interactions to induce T_{RM} formation, suggesting that TCR affinity can influence T_{RM} differentiation (51) and may provide a mechanism to regulate the diversity of antigen-specific T_{RM} within tissues.

Additional intrinsic CD8$^+$ T cell characteristics may also affect CD8$^+$ T cell fate. For example, variation in expression levels of signaling proteins including CD8, ERK-1 and SHP-1 generates a range of CD8$^+$ T cell responsiveness to antigen stimulation. However, co-regulation of signaling proteins limits this variability, potentially providing a mechanism to diversify cell fate, but control self-reactivity (52). Similarly, Marchingo et al. used a high-throughput clonal assay to simultaneously measure the expansion fate of multiple clonal families expressing identical TCR in a single culture well. Their results demonstrate that following stimulation, progeny from clonal families stop dividing and return to quiescence at or near the same generation, suggesting that regulation of CD8$^+$ T cell expansion fate is at the level of the individual clone (53). Stochastic variation in costimulatory and cytokine receptor expression by naïve CD8$^+$ T cells, for example differences in CD28 receptor expression, influences the generation at which an initial individual activated cell reverts to a quiescent state (53). Future *in vivo* research is required to determine whether stochastic variation in protein expression by naïve T cells, either before or during early priming, has an effect on subsequent T cell fate, including CD8$^+$ T_{RM} differentiation.

Extrinsic Control of CD8$^+$ T_{RM} Precursor Generation

Antigen and Antigen Presentation During Priming

Contact between DCs and antigen-specific CD8$^+$ T cells can influence the fate of responding T cells (54–57). DCs carrying pathogen-derived antigens migrate to draining LN and prime naïve CD8$^+$ T cells. The interaction between DCs and T cells within the LN occurs in three stages initiated by brief encounters, followed by more stable contacts and concludes with a return to brief contacts and rapid T cell migration, accompanied by the commencement of T cell proliferation (58). Multiphoton intravital microscopy (MP-IVM) allowed for the analysis of how and when the interactions between naïve CD8$^+$ T cells and DCs determine effector and memory CD8$^+$ T cell differentiation, and suggested that stable contacts and a high antigen concentration are critical to induce memory T cell generation (59). Additionally, Ballesteros-Tato et al. showed that more abundant influenza epitopes are preferentially cross-presented at late times in the primary response, and responding T cells are favorably programmed toward a memory cell fate (60). More recently, studies have identified specific cross-priming DC populations that favor CD8$^+$ T_{RM} precursor differentiation. In a mouse model of vaccinia virus (VACV) infection, DNGR-1$^+$ Batf3-dependent DCs prime naïve CD8$^+$ T cells within the LN to form T_{RM} within skin or lung (61). Further, human studies and experiments using a humanized mouse metastatic lung model identified a subset of activated CD88$^-$CD1c$^+$CD163$^+$CD14$^{+/-}$ DCs, or DC3s, that prime naïve CD8$^+$ T cells and induce TGF-β-triggered CD103 expression (62).

Route of Entry and Inflammatory Milieu

The gene expression profile and half-life of activated CD8$^+$ T cells are determined by many signals during pathogen invasion, such as antigen presentation by mature DCs, T cell stimulation by receptor ligands and inflammatory cytokines (63). During T cell priming, different LN environments direct expression of distinct T cell homing receptors (5, 64, 65). For example, oral, but not intranasal mouse infection with LM induces efficient homing and precursor development of CD8$^+$ T_{RM} in the intestinal epithelium (43). In contrast, CD8$^+$ T cells lodge within the skin following infection with herpes simplex virus (HSV) *via* either skin scarification or subcutaneous injection after controlling for priming efficiency (66).

Distinct patterns of cytokine expression within the LN environment during priming also modulate precursor formation and program CD8$^+$ T cell fate (67, 68). For instance, IL-12 produced during LCMV infection induces T-bet expression in CD8$^+$ T cells in a dose-dependent manner, and favors the development of SLEC over MPEC (69, 70). On the other hand, IL-10 plasma levels early following immunization with peptide antigen and adjuvant strongly correlates with the frequencies of antigen specific T_{RM} in the lung of mice and non-human primates at a memory time point. Production of IL-10 by monocytes acts in an autocrine manner to release TGF-β during

priming, increasing $CD8^+$ T cell responsiveness to subsequent TGF-β stimulation, and thereby favors the formation of $CD8^+$ $CD103^+$ T_{RM} (71).

STAGE 2: MECHANISMS THAT ENCOURAGE $CD8^+$ T_{RM} TO SETTLE IN PERIPHERAL TISSUES

$CD8^+$ T_{RM} Phenotype and Transcriptional Regulation

Following $CD8^+$ T cell activation and clonal expansion within draining LN, T_{RM} precursors migrate to non-lymphoid tissues. Entry into peripheral tissues induces a unique T_{RM} phenotype that promotes $CD8^+$ T cell retention and prevents egress (**Supplementary Table 2**). More than a decade ago, Masopust et al. demonstrated that as early as 7 days following intestinal LCMV infection, the gut microenvironment induces a unique $CD8^+$ T cell differentiation program; $CD8^+$ IELs express both CD69 and CD103, while splenic circulating memory $CD8^+$ T cells do not (72). Similarly, Ray and colleagues found that within 8 days following influenza infection, flu-specific $CD8^+$ T cells recovered from the lung were predominantly $CD49a^+$, while those recovered from the mediastinal LN were $CD49a^-$ (7). This phenotype persisted at memory timepoints. More recently, Mackay et al. performed microarray analysis of $CD103^+$ $CD8^+$ T_{RM} isolated from the skin, gut, and lungs of mice and determined that $CD8^+$ T_{RM} express a unique T_{RM} transcriptional signature that is distinct from circulating memory $CD8^+$ T cells. This analysis identified 37 transcripts commonly regulated by T_{RM} from all three tissues, including *S1pr1*, *Itga1* and *Itgae*, encoding sphingosine 1-phosphate receptor-1 (S1P1), CD49a and CD103, respectively (42). A similar human $CD8^+$ T_{RM} core transcriptional profile was also later defined (73, 74).

CD69 is perhaps the most ubiquitous marker for $CD8^+$ T_{RM} cells in mouse and human tissues (74, 75). CD69 forms a complex with the chemoattractant receptor S1P1, inducing S1P1 internalization and thereby impairing S1P-directed lymphocyte exit *via* afferent lymphatic vessels (42, 75, 76). In parallel, downregulation of kruppel-like factor 2 (KLF2), the transcription factor that drives S1P1 gene expression, is necessary for the establishment of $CD8^+$ T_{RM} in tissues (77, 78). CD69 expression by $CD8^+$ T cells is necessary for the generation of $CD8^+$ T_{RM} in the kidney (79) and skin (75). However, recent work demonstrated that CD69 expression is dispensable for the formation of $CD8^+$ T_{RM} in small intestine, lung, and female reproductive tract (79). Like CD69, the integrin, CD103 has also been used extensively as a marker for $CD8^+$ T_{RM}. CD103 is expressed by $CD8^+$ T_{RM} in the epithelial compartment of multiple tissues (4, 42, 80, 81) and is thought to mediate T_{RM} retention through its interaction with e-cadherin. However, although CD103 is necessary for $CD8^+$ T_{RM} accumulation within epithelium, it is dispensable for T_{RM} persistence in other tissue compartments (42, 43). For instance, Bergsbaken et al. demonstrated that following *Yersinia pseudotuberculosis* (Yptb) infection, a $CD103^-$ $CD8^+$ T_{RM} cell population persists long-term in the intestinal lamina propria (82). Additionally, CD49a, the α chain of integrin α1β1, is expressed by $CD8^+$ T_{RM} and promotes their accumulation within multiple mouse and human tissues (4, 7, 24, 74, 83, 84).

Comparison of $CD8^+$ T_{RM} and circulating memory $CD8^+$ T cells transcriptomes has identified several transcription factors that are differentially expressed between memory $CD8^+$ T cells subsets. Expression of *Zfp683*, encoding homolog of Blimp1 in T cells (Hobit) is upregulated in $CD8^+$ T_{RM} and is necessary for $CD8^+$ T_{RM} cell development in the skin, gut, liver and kidney of mice (83). Interestingly, Hobit has been described in several other cell lineages, including $CD4^+$ T, Natural killer (NK), NKT, and Mucosal-associated invariant T (MAIT) cells, and acts as a transcriptional regulator of residency (83, 85–87). Hobit, together with the transcription factor Blimp1 coregulate genes required for tissue egress (83). In the absence of Hobit and Blimp1, Klf2, S1p1, and CCR7 are de-repressed. However, although human lung and liver $CD69^+$ $CD8^+$ T cells express Hobit, so do human circulating $CD45RA^+$ $CD27^-$ and $CD45RA^-CD27^-$ $CD8^+$ T cells, suggesting that Hobit may not specifically promote human $CD8^+$ T_{RM} differentiation (88). Additionally, the requirement of Hobit for T_{RM} differentiation may be tissue-specific. In the lung, Blimp1, but not Hobit, is required for the formation of virus-specific $CD8^+$ T_{RM} in a mouse influenza infection model (89). Moreover, Milner et al. used single-cell RNA sequencing (scRNA-seq) analysis to characterize $CD8^+$ siIEL populations over time following LCMV infection. They demonstrated heterogeneity in the $CD8^+$ siIEL T_{RM} and identified distinct resident memory $CD8^+$ T cell populations based on their expression of the transcription factors Blimp1 and Id3. Previous studies demonstrated that $Blimp1^{hi}$ expression favors an effector T cell fate (90). Accordingly, Milner et al. showed that compared to $Blimp1^{lo}$ $Id3^{hi}$ siIEL, $Blimp1^{hi}$ $Id3^{lo}$ siIEL $CD8^+$ T cells dominate the early response and express increased effector-associated genes. Nonetheless, lower numbers of $Blimp1^{hi}$ $Id3^{lo}$ siIEL $CD8^+$ T cells are still present in the tissue at memory timepoints. Although Blimp1 was expressed by a subset of $CD8^+$ T cells across multiple non-lymphoid tissues, expression of Id3 was more restricted, raising the possibility that T_{RM} transcriptional programs may be regulated by the local tissue microenvironment (91).

Two T-box transcription factors, Eomesodermin (Eomes) and T-bet, control $CD8^+$ $CD103^+$ T_{RM} cell formation in lung, skin, and brain. Although T_{CM} express both Eomes and T-bet (92), expression of these transcription factors must be downregulated for $CD8^+$ T_{RM} development. While extinguishment of Eomes expression is required for $CD8^+$ $CD103^+$ T_{RM} cell formation (93, 94), residual T-bet expression maintains $CD8^+$ T cell IL-15Rβ expression and IL-15 responsiveness for long-term T_{RM} survival within lung and skin (94, 95). Additionally, recent data generated using ATAC-seq and transcriptional profiling identified the transcription

factor, Runx3 as a central regulator of $CD8^+$ T_{RM} differentiation (32, 44, 73, 96). Runx3, previously described as a transcriptional regulator of $CD8^+$ effector T cells (97), promotes expression of tissue residency genes while suppressing genes involved in tissue egress. Runx3$^{-/-}$ $CD8^+$ T cells have elevated T-bet levels, suggesting that Runx3 represses T-bet expression; knockdown of T-bet expression in Runx3$^{-/-}$ $CD8^+$ T cells increases $CD8^+$ T_{RM} numbers and restores CD69 and CD103 expression. Runx3 deficiency results in loss of $CD8^+$ T_{RM} in barrier (skin and lung) as well as non-barrier (salivary gland and kidney) tissues, suggesting that Runx3 may regulate $CD8^+$ T_{RM} formation independent of the local tissue milieu (96).

$CD8^+$ T_{RM} generation and long-term maintenance are also regulated by nuclear receptor subfamily 4 group A member 1 (NR4A1) (44, 98). Nr4a1, also known as Nur77, is rapidly induced following TCR stimulation and regulates $CD8^+$ T cell proliferation and effector function (99). In a mouse model of influenza infection, similar numbers of co-adoptively transferred *Nr4a1*$^{-/-}$ and wild-type antigen-specific $CD8^+$ T_{RM} are recovered at the effector phase. However, fewer *Nr4a1*$^{-/-}$ $CD8^+$ T cells are recovered from the liver and intestine at a memory time point, although similar numbers are recovered from lung (98). Finally, scRNA-seq analysis of siIEL and splenic $CD8^+$ T cells over the course of LCMV infection demonstrated increased expression of *Nr4a2*, Junb proto-oncogene (*Junb*) and FOS-like 2 (*Fosl2*) in siIEL relative to splenic $CD8^+$ T cells. Knockdown of these genes results in impaired formation of siIEL $CD8^+$ T_{RM} compared to circulating memory $CD8^+$ T cells, although the mechanisms were not determined (44).

In Situ Antigen Dependence

Following vesicular stomatitis virus (VSV) infection, local antigen presentation is required to drive CD103 expression by infiltrating $CD8^+$ T cells that promotes their persistence within brain (9). Similarly, local antigen recognition is required for T_{RM} formation in the lung (100, 101). Following influenza infection, viral antigen-bearing pulmonary monocytes interact with influenza-specific $CD8^+$ T cells *in vivo* and can induce CD103 expression by $CD8^+$ T cells *in vitro* (102). While localized inflammation can recruit $CD8^+$ T cells into the lung, in the absence of local antigen recognition, memory $CD8^+$ T cells fail to express the retention receptors CD69, CD103, and CD49a or persist long-term (103). However, the requirement of antigen recognition within peripheral tissues for $CD8^+$ T_{RM} formation is not absolute. $CD8^+$ $CD103^+$ T_{RM} can be generated in the absence of antigen recognition in barrier tissues, including skin, intestine, and female reproductive tract (104–106). Nonetheless, subsequent studies demonstrated that local recognition of antigen dramatically increases the formation of $CD8^+$ T_{RM} in VACV-infected skin (107, 108). Moreover, local competition between $CD8^+$ T cells of different specificities for different viral epitopes shapes the repertoire of cutaneous $CD8^+$ T_{RM} cells following VACV infection (107), underlining the importance of local antigen recognition in regulating the establishment of $CD8^+$ T_{RM}.

Tissue-Derived Signals: Cytokines, Inflammatory Molecules, and Other Immune Cells Signals

The local tissue cytokine microenvironment influences $CD8^+$ T_{RM} phenotype. TGF-β is critical for the formation of $CD103^+$ $CD8^+$ T_{RM} in several tissues, including the siIEL compartment, skin epidermis, lung, and kidney (105, 109–111). $CD8^+$ T cells expressing mutant TGF-β receptors fail to express CD103 or persist within multiple peripheral tissues (42, 43, 81, 105, 109). Recent data suggest that epidermal $CD8^+$ T_{RM} cells require transactivation of autocrine TGF-β for their long-term persistence, and competition for limited TGF-β influences which clones persist within the epidermis (112). $CD8^+$ T cell TGF-β responsiveness is controlled by the transcription factors EOMES and T-bet, and downregulation of Eomes and T-bet is required for $CD8^+$ T cell TGF-β responsiveness and $CD8^+$ T_{RM} formation (94). Additionally, recent research has identified a role for the transcriptional cofactor, SKI, in regulating $CD8^+$ T cell CD103 expression. Using an LCMV infection model, Wu et al. demonstrated that ectopic expression of SKI proto-oncogene restricts CD103 expression by $CD8^+$ T cells *in vitro* and *in vivo*. SKI is recruited to the *Itgae* locus to suppress CD103 transcription by preventing histone acetylation in a Smad4-dependent manner. Moreover, in the absence of Smad4, CD103 is constitutively expressed by $CD8^+$ T cells even in the absence of TGF-β signaling, suggesting that modulation of TGF-β-SKI-Smad4 pathway could determine $CD8^+$ $CD103^+$ T_{RM} generation (111).

Inflammatory cytokines produced in response to local infection, and the chemokines they induce also regulate T_{RM} formation and phenotype. IFN-γ and the IFN-γ-induced chemokines, CXCL9 and CXCL10 have been shown to orchestrate $CD8^+$ T_{RM} precursor migration and localization within tissues in multiple infection models. For example, following influenza infection, IFN-γ produced by $CD4^+$ T cells promotes the localization of $CD8^+$ T cells to the airways, thereby controlling their exposure to TGF-β (95). Similarly, following genital HSV-2 infection, IFN-γ induces local expression of the CXCR3 ligands, CXCL9 and CXCL10 that promotes $CD8^+$ T cell localization and long-term persistence within the tissue (113). Furthermore, local application of these chemokines is sufficient to recruit $CD8^+$ T cells into the genital tract where they are retained long-term and enhance memory response to reinfection (106). Similarly, keratinocytes express CXCL9 and CXCL10 during HSV skin infection. $KLRG1^-$ $CD8^+$ T_{RM} precursors show preferential migration to these chemokines *ex vivo* compared to $KLRG1^+$ effector $CD8^+$ T cells. Moreover, following intradermal injection, CXCR3$^{-/-}$ $CD8^+$ T cells generate fewer $CD103^+$ T_{RM} than adoptively transferred WT $CD8^+$ T cells, suggesting that CXCR3 mediates T_{RM} precursor entry into the epidermis where locally activated TGF-β may promote subsequent epidermal $CD8^+$ $CD103^+$ T_{RM} generation (42, 114). Additionally, CXCR3-directed localization of type I Treg expressing the TGF-β activating integrin, αvβ8, within local inflammatory sites promotes $CD8^+$ T_{RM} generation in the intestine, liver, and lung. Positioning of these Treg adjacent to

effector $CD8^+$ T cells promotes $CD8^+$ T_{RM} generation *via* activated TGF-β availability (115). In contrast, generation of $CD8^+$ $CD103^-$ T_{RM} following oral Yptb infection is independent of TGF-β signaling, but requires CXCR3-dependent clustering of effector $CD8^+$ T cells with CXCL10-producing $CX3CR1^+$ intestinal cells in areas of inflammation within the intestinal lamina propria, suggesting that the microenvironment formed by immune cell aggregates supports $CD8^+$ T_{RM} formation (116). Indeed, IL-12 and IFN-β produced by intestinal macrophages during Yptb infection prevents TGF-β-induced CD103 expression by $CD8^+$ T cells, favoring the differentiation of $CD8^+$ $CD103^-$ T_{RM} cells (82). Thus, inflammatory cytokines not only function to induce local chemokine expression to promote the recruitment of T_{RM} precursors into tissues, but also influence the differentiation of $CD8^+$ T cells within the tissue, providing a mechanism to promote T_{RM} phenotypic diversity.

Several additional chemokine receptors may also participate in the formation of $CD8^+$ T_{RM} within peripheral tissues. For example, expression of the intestinal homing chemokine receptor CCR9 by $CD8^+$ siIEL is increased compared to their circulating counterparts throughout their differentiation (5, 44). Additionally, expression of CXCR6 and CCR10 by mouse $CD8^+$ T cells are required for optimal $CD8^+$ T_{RM} formation in the skin (117). Although $CD8^+$ T_{RM} formation in mouse skin appears to be CCR8-independent (117), human cutaneous $CD69^+$ $CD103^+$ T_{RM} express CCR8, raising the possibility that CCR8 and its ligands may regulate human cutaneous $CD8^+$ T_{RM} generation or function (118, 119).

Competition for survival cytokines may also impact $CD8^+$ T_{RM} accumulation within tissues. A recent report using an LCMV infection model demonstrated that $NK1.1^+$ innate lymphoid cells (ILCs) control the accumulation of memory $CD8^+$ T cells in salivary glands. Specifically, establishment of $CD8^+$ T_{RM} is enhanced in anti-$NK1.1^+$ antibody pretreated mice. The authors propose that ILCs might compete for survival signals such as IL-7, although no specific mechanism was determined (120). Similarly, following HSV skin infection, $CD8^+$ T_{RM} formation is accompanied by a concomitant local decrease in dendritic epidermal γδ T cells, suggesting possible competition for survival cytokines within the epidermal niche.

Costimulatory signals also play a role in the establishment of $CD8^+$ T_{RM} within tissues. During influenza infection, Zhou et al. showed that interaction of the costimulatory molecule, 4-1BB with its ligand 4-1BBL is necessary for the induction of long-lived lung-resident $CD103^+$ and $CD103^-$ memory $CD8^+$ T cell populations (121). In addition, glucocorticoid-induced TNFR-related protein ligand (GITRL), expressed by lung monocyte-derived inflammatory antigen presenting cells, provides a costimulatory signal for lung $CD8^+$ T cells expressing GITR during influenza infection. GITRL/GITR interaction in the LN and lung is required for the differentiation of $CD8^+$ T_{RM} precursors and the formation of $CD8^+$ T_{RM} within the lung parenchyma (122).

Additional microenvironmental cues may also regulate the generation of $CD8^+$ T_{RM}. For example, microRNA-155 is upregulated during infection in response to TLR signaling and inflammatory cytokines (123). $CD8^+$ T_{RM} are established in the brain following infection of mice with neuroinvasive LM, and their accumulation is decreased in the absence of miR-155 (124). Also, $CD8^+$ T cells require P2RX7 expression for $CD8^+$ T_{RM} formation in the siIEL, female reproductive tract, kidney, salivary glands, and liver. Extracellular ATP is released during inflammation and injury, and is sensed by the purinergic receptor, P2RX7. Upon $CD8^+$ T cell activation, expression of TGF-β receptors is transiently down-regulated. Extracellular ATP derived from intestinal microbiota, activated cells and/or damaged tissue restores TGF-βRII expression and TGF-β responsiveness, resulting in $CD8^+$ T cell CD103 upregulation, KLF2 downregulation, enhanced mitochondrial function and T_{RM} formation (125). On the other hand, microbiota depletion by antibiotic treatment increases the antigen load following LM infection and promotes CXCR3-directed $CD8^+$ T cell accumulation within the large intestinal lamina propria, resulting in increased mucosal $CD8^+$ T_{RM} accumulation and response (126).

STAGE 3: $CD8^+$ T_{RM} MAINTENANCE IN PERIPHERAL TISSUES

In Situ Antigen Dependence

$CD8^+$ T_{RM} persist long-term within several tissues, including intestinal IEL (105), vaginal mucosa (106), and skin (104, 127) independent of cognate antigen recognition. In contrast, lung $CD8^+$ T_{RM} are rapidly lost from the tissue. Several studies suggest that cognate antigen recognition is required for the persistence of lung $CD8^+$ T_{RM}. Residual local antigen persistence may promote continuous development of lung T_{RM} and allow for the maintenance of $CD8^+$ T_{RM} within the tissue (128). Following influenza infection, $CD8^+$ T_{RM} receive chronic local TCR stimulation even weeks after the clearance of infectious influenza virus. Furthermore, tamoxifen-inducible $H\text{-}2D^b$ depletion or B7-CD28 blockade starting at least three weeks post-infection results in impaired maintenance of $CD8^+$ T_{RM} cells within the lung (129). Based on these findings, novel methods are being developed in attempt to prolong the persistence of $CD8^+$ T_{RM} within the lung. Combined subcutaneous and intranasal vaccination of mice with an adenovirus vector expressing influenza antigen is reported to induce persistent antigen expression in the lungs and maintains T_{RM} within the lung for at least one year post-vaccination (130). Continual recruitment of circulating $CD8^+$ T_{EM} may convert into T_{RM} following antigen recognition and help to sustain T_{RM} within the interstitium.

However, the requirement of circulating memory $CD8^+$ T cell recruitment for the long-term maintenance of lung $CD8^+$ T_{RM} has been questioned by a recent study using parabiosis and intravascular staining to exclude analysis of $CD8^+$ T cells within the circulation. Takamura et al. demonstrated that $CD8^+$ T_{RM} can be retained in specific niches created at sites of tissue regeneration within the lung parenchyma, distant from lymph vessels, and independent of $CD8^+$ T cell recruitment from the

circulation (100). Still, the half-life of $CD8^+$ T_{RM} within lung airways is less than 14 days (131), and so they propose that maintenance of airway memory $CD8^+$ T cells may require residual antigen-driven reactivation of $CD8^+$ T_{RM} in the lung parenchyma and recruitment into the airways (100, 132). More recently, an additional mechanism has been proposed to maintain regional immune memory specific for lung pathogens. Stolley et al. demonstrated that following influenza infection, $CD8^+$ T cells migrate to draining mediastinal LN *via* lymphatic vessels. These cells express CD103 and CD69, are maintained long-term within the LN in an antigen-independent manner and maintain effector molecule expression. As such, repositioning and persistence of $CD8^+$ T_{RM} within the draining mediastinal LN may provide a means to maintain regional immune memory despite rapid attrition of lung $CD8^+$ T_{RM} (133).

$CD8^+$ T_{RM} Receptors and Transcriptional Regulators

Maintenance of $CD8^+$ T_{RM} is thought to require expression of retention receptors that act as adhesive anchors (Formation markers and transcriptional regulators in stage 2, **Supplementary Table 2**, and **Supplementary Table 3**). CD103 binds to E-cadherin, which is expressed in skin epidermis (134) and intestinal epithelium (5, 105). This interaction is thought to anchor $CD8^+$ T_{RM} within the epithelial compartment of tissues and facilitate their long-term residence (135). Similarly, CD49a binds collagen type I and IV, and also facilitates $CD8^+$ T_{RM} persistence within skin, lung, and intestine (7, 84, 136). In addition to its adhesive function, CD49a may also provide a pro-survival signal, limiting $CD8^+$ memory T cell apoptosis (7).

Although CD69 is required for $CD8^+$ T_{RM} establishment in several tissues, it may not be required for their long-term maintenance. Following mouse influenza infection, $CD8^+$ T_{RM} are retained long-term within the lung independent of CD69 expression. Early after infection, CD69 is important for the accumulation of $CD8^+$ T cells within the airways to inhibit strong S1P1-mediated exit signals. However, once $CD8^+$ T_{RM} are established, CD69 is dispensable even though the cells maintain residual S1P1 reactivity (100). Downregulation of KLF2, the transcription factor that drives S1P1 expression, may preclude the need for continued CD69 expression in T_{RM} to inhibit any S1P-mediated exit signal. Moreover, physical separation of T_{RM} from lymphatic vessels by their positioning within lung niches or within the epidermis may also facilitate their retention within tissues independent of CD69.

The expression patterns of several transcription factors that regulate $CD8^+$ T_{RM} formation are maintained long-term in established T_{RM} (Transcriptional regulators in stage 2, **Supplementary Table 2** and **Supplementary Table 3**). However, Milner et al. found divergent transcription factor expression patterns in $CD8^+$ T cells with distinct phenotypic properties during different stages of T_{RM} formation and maintenance. Specifically, while $Blimp1^{hi}$ $Id3^{lo}$ siIEL $CD8^+$ T cells are abundant at the effector phase of the immune response, $Blimp1^{lo}$ $Id3^{hi}$ siIEL $CD8^+$ T cells progressively accumulate over time, and are more abundant at the memory phase of the response. Moreover $Blimp1^{lo}$ $Id3^{hi}$ siIEL $CD8^+$ T cells have higher recall proliferative capacity and multipotency than $Blimp1^{hi}$ siIEL $CD8^+$ T cells (91). Additionally, Aryl hydrocarbon receptor (AhR) also regulates $CD8^+$ T_{RM} maintenance. Expression of AhR is increased in skin $CD8^+$ T_{RM} compared to naïve or circulating memory T cells. While $Ahr^{-/-}$ $CD8^+$ T cells initially enter into sites of DNFB-induced skin inflammation, over time, they disappear from the skin but not spleen (134), suggesting that AhR is required for the long-term persistence of cutaneous $CD8^+$ T_{RM}. Accordingly, AhR expression is increased in mouse intestinal T_{RM} compared to circulating memory $CD8^+$ T cells following LCMV infection (44), as well as in human lung $CD8^+$ $CD103^+$ T_{RM} compared to circulating memory T cells (73). Finally, Notch signaling regulates the maintenance of $CD8^+$ $CD103^+$ T_{RM} in the lung by regulating both CD103 expression and $CD8^+$ T_{RM} metabolism (73).

Tissue-Derived Signals: Cytokines, Inflammatory Molecules, and Other Immune Signals.

TGF-β is not only required for the establishment of $CD8^+$ T_{RM} in multiple barrier tissues, but also to preserve their phenotype and long-term persistence in the intestine (109). Similarly, after cutaneous $CD103^+$ $CD8^+$ T_{RM} have been established, neutralization of the TGF-β-activating integrin, αvβ6, results in reduced numbers of T_{RM} in the epidermis but not LN or spleen over time (114). These results suggest that continuous TGF-β signaling is required for the long-term persistence of epidermal $CD8^+$ T_{RM}.

Survival cytokines also provide for the long-term sustenance of tissue-resident $CD8^+$ T cells. Both IL-7 and IL-15 are required for the persistence of $CD8^+$ T_{RM} in the skin (94, 137). In contrast, maintenance of T_{RM} in the lung and intestine is IL-15-independent (138, 139). On the other hand, IL-12 regulates Bcl-2 expression to promote the survival of $CD8^+$ $CD103^-$ T_{RM} within the intestinal lamina propria (82).

Although P2RX7 promotes $CD8^+$ T_{RM} formation within the intestine (125), Stark et al. demonstrated that sterile tissue damage led to loss of established WT, but not $P2rx7^{-/-}$ $CD8^+$ T_{RM} from the liver (140). They found that TCR triggering downregulates P2RX7 expression, and so proposed that tissue damage-induced depletion of established T_{RM} might free space for the formation of new $CD8^+$ T_{RM} with infection-relevant specificities. In contrast, Wakim et al. determined that persistent expression of the anti-viral transmembrane protein, IFITM3 by lung $CD103^+$ $CD8^+$ T cells promotes the survival and maintenance of $CD8^+$ T_{RM} at sites of viral infection. Following influenza infection, cognate antigen induces persistent IFITM3 expression preferentially by lung $CD8^+$ T_{RM} compared to splenic memory $CD8^+$ T cells. $CD8^+$ T_{RM} that lack IFITM3 expression exhibit increased susceptibility to influenza infection compared to $IFITM3^+$ $CD8^+$ T_{RM}, and are selectively lost following virus challenge (141).

Finally, $CD8^+$ T_{RM} long-term survival and protective function require lipid uptake and oxidative metabolism.

Fatty-acid-binding proteins 4 and 5 (FABP4 and FABP5) are required for the long-term maintenance of $CD8^+$ T_{RM} within the skin following VACV infection, and for $CD8^+$ T_{RM}-mediated protection from viral challenge (142). However, $CD8^+$ T_{RM} exhibit distinct patterns of FABP gene expression depending on their tissue of residence. An additional study demonstrated that following HSV infection, skin $CD8^+$ T_{RM} express *Fabp4* and *Fabp5*, but lack expression of other FABP isoforms. However, following LCMV infection, liver $CD8^+$ T_{RM} highly express *Fabp1*, some *Fabp4*, but no *Fabp5*. In contrast, siIEL $CD8^+$ T_{RM} express *Fabp1*, *Fabp2*, and *Fabp6*, but negligible *Fabp4* and *Fabp5*. These differences in FABP expression are determined by tissue-derived signals, and by altering FABP expression, $CD8^+$ T cells can adapt to different host tissues (143).

STAGE 4: PATHOGEN CHALLENGE

Location and Relocation

$CD8^+$ T_{RM} are positioned to provide a first line of host defense in response to pathogen challenge. Recognition of cognate antigen stimulates $CD8^+$ T_{RM} to rapidly secrete cytokines that induce expression of anti-viral and anti-bacterial genes, activate innate immune cells, and enhance chemokine and adhesion receptor expression for increased recruitment of circulating immune cells (144–146). Following tissue entry, circulating memory $CD8^+$ T cells can undergo antigen-dependent $CD69^+$ $CD103^-$ T_{RM} differentiation (147) as well as antigen-independent $CD69^{+/-}$ $CD103^{+/-}$ T_{RM} differentiation (148, 149) *in situ*. Additionally, intravital microscopy studies revealed that established $CD8^+$ T_{RM} proliferate within the female reproductive tract and skin upon cognate antigen encounter. These cells dominate the recall response and contribute more than circulating memory $CD8^+$ T cells to the pool of secondary T_{RM} cells (148, 149).

At homeostasis, $CD8^+$ T_{RM} persist long-term within peripheral tissues, separate from the circulation. However, following antigen reencounter, $CD8^+$ T_{RM} exhibit plasticity. Beura et al. determined that $CD8^+$ $CD69^+$ T_{RM} in the draining LNs derive from cells present in the upstream nonlymphoid tissue (11). Complementary studies by Behr et al. used Hobit reporter mice to demonstrate that $CD69^{lo}$ $Hobit^+$ antigen specific T cells accumulate in the draining LNs in the effector phase after reinfection, and upregulate CD69 expression in the secondary memory phase, forming LN T_{RM}. Virus challenge not only induces local proliferation of $CD8^+$ T_{RM} cells in peripheral tissues that can participate in the accumulation of secondary T_{RM} in the draining LN, but also, formation of circulating memory $CD8^+$ T cells downstream of $CD8^+$ T_{RM}. Studies using Hobit lineage tracer mice revealed that $Hobit^+$ $CD8^+$ T_{RM} can downregulate Hobit expression upon antigen encounter and form $KLRG1^+$ $CXC3CR1^+$ circulating T_{EM} with enhanced capacity to protect against reinfection (150). Similarly, Fonesca et al. demonstrated that following challenge, small intestinal iEL T_{RM} give rise to circulating T_{CM} and T_{EM}. These ex-T_{RM} cells are epigenetically poised for migration back to the tissue of origin and T_{RM} re-differentiation (151).

$CD8^+$ T_{RM} Antigen Reencounter: Dependence on $CD11c^+$ DCs

Intravital confocal microscopy illustrated that $CD8^+$ T_{RM} actively patrol skin epithelium in search of cognate antigen, raising the possibility that T_{RM} within barrier tissues do not depend on antigen delivery by professional APCs (152). In line with this hypothesis, Masopust et al. demonstrated that following depletion of ~90% of host DC in CD11c-DTR bone marrow chimeric mice, T_{RM} still proliferate in response to challenge with cognate peptide antigen (149). In contrast, in the vaginal mucosa, T_{RM} reactivation following HSV-2 challenge depends on $CD301b^+$ DCs (153). In addition, transplantation of the dorsal root ganglia of HSV-infected mice under the kidney capsule of naive mice induces viral reactivation. Here, the $CD8^+$ T_{RM} proliferative response is initiated by recruitment of $CD11b^+$ $CD11c^+$ DCs. Together, these results suggest that the DC requirement for $CD8^+$ T_{RM} response to antigen challenge may be context dependent. Indeed, in models of LCMV and influenza infection, cDCs are dispensable for lung $CD8^+$ T_{RM} reactivation. Rather either hematopoietic or non-hematopoietic antigen presenting cells are sufficient, but they induce different T_{RM} functional outputs. Whereas antigen presentation by hematopoietic cells reduces gene transcription of chemokines and cytokines such as *Ccl1*, *Ccl3*, *Ccl9*, and *Ifng*, activation by nonhematopoietic cells promote transcription of genes involved in cell cycle and proliferation but curbs type I interferon stimulated genes (154).

Patrolling the Tissue: Surveillance and Motility

Although T_{RM} remain resident long-term in peripheral tissues, they are not sessile cells; T_{RM} continuously patrol the local area for invading pathogens. Upon cognate antigen recognition, $CD8^+$ T_{RM} become rounded and arrest their migration before undergoing proliferation *in situ* (148, 149). However, intravital microscopy studies demonstrated that depending on their tissue of residence, T_{RM} display different migration speeds and morphologies. T_{RM} migrate within skin epidermis, albeit slowly at a rate of ~1.3 μm/min, and extend dendrites laterally to probe their surroundings for cognate antigen (134). Imaging of the mouse uterus after acute LCMV infection revealed that $CD8^+$ T_{RM} migrate at different rates within the stroma of the female reproductive tract and this migratory speed correlates with collagen density. T_{RM} within the collagen-rich perimetrium migrate more slowly than in the less collagen-rich myometrium where T_{RM} exhibit motility rates that are similar to those of circulating lymphocytes in LNs (149). Interestingly, a recent study in influenza-infected mice suggests that the collagen receptor, CD49a promotes $CD8^+$ T cell motility within the trachea to facilitate tissue surveillance (155). In contrast, CD103 restrains T_{RM} motility in both trachea and skin (117, 155). How changes in the local microenvironment following

challenge with distinct pathogens might affect $CD8^+$ T_{RM} phenotype and migratory behavior requires additional study.

Antiviral Activity: Effector Molecule Expression

$CD8^+$ T_{RM} provide immediate effector functions against secondary infections (**Supplementary Table 4**). The transcriptional profiles of both mouse and human $CD8^+$ T_{RM} exhibit higher expression of effector molecules compared to circulating memory $CD8^+$ T cells (73, 74, 93, 105, 156). Constitutive expression of mRNAs encoding effector molecules may facilitate rapid T_{RM} response. For example, notch signaling contributes to the maintenance of constitutive *Ifng* expression by lung T_{RM} (73). Notch signaling transactivates *Ifng*, increasing *Ifng* expression by T_{RM} independent of TCR stimulation. Following recognition of cognate antigen, $CD8^+$ T_{RM} secrete IFN-γ, IL-2 and TFN-α, inducing a rapid recall response at the site of pathogen invasion (146, 156–158). IFN-γ induces vascular cell adhesion molecule 1 (VCAM-1) expression by endothelial cells, as well as production of inflammatory chemokines that recruit circulating immune cells, resulting in amplification of the memory response (146). Additionally, resting lung $CD8^+$ T_{RM} constitutively express *CCL3*, *CCL4*, *CCL20* and *XCL1* (73), and intestinal $CD8^+$ T_{RM} express *Ccl3* and *Ccl4* (44), suggesting that $CD8^+$ T_{RM} themselves express genes to rapidly amplify the memory immune response.

$CD8^+$ T_{RM} targeted secretion of the cytotoxic proteins, perforin and granzyme B, destroy target cells. While circulating memory $CD8^+$ T cells lack cytotoxic protein expression, T_{RM} that form within intestinal IEL, liver, and brain following LCMV infection express granzyme B during quiescence (72, 156, 159). Constitutive expression of granzyme B might promote rapid control of pathogen infection. In contrast, airway $CD8^+$ T_{RM} are reported to be poorly cytolytic, even in the presence of antigen stimulation (157). The nutrient-poor airway environment induces cellular stress, limiting T_{RM} effector function and survival at homeostasis, and perhaps providing a mechanism to prevent unnecessary epithelial damage (160).

Controlling T_{RM} Activity: Inhibitory Molecules and Metabolic Arrest

Inhibitory molecule expression may be critical to prevent T_{RM}-mediated damage in barrier tissues. The inhibitory surface protein programmed death protein 1 (PD-1), upregulated by exhausted T cells and tumor infiltrating lymphocytes (TILs), is also expressed by $CD8^+$ T_{RM} in mouse and human tissues (74, 161). Multiple studies suggest that PD-1 may provide T_{RM} functional restraint. For example, PD-1 expression by T cells correlates with response to anti-PD-1 blockade treatment in patients with cancer (162). Additionally, $CD8^+$ $PD\text{-}1^{hi}$ T_{RM} cells in human pancreas may maintain immune homeostasis through interactions with resident macrophages; in samples from chronic pancreatitis, $CD8^+$ T cells exhibit reduced PD-1 expression (163). Moreover, following influenza infection, antigen specific $CD8^+$ T cells in the lung acquire both a memory and exhausted phenotype, including PD-1 surface expression. Blocking PD-1 ligand (PD-L1) promotes exhausted-like T_{RM} cell expansion, and augments T_{RM} cell function, enhancing T_{RM}-mediated protection from reinfection. However, anti-PD-L1 treatment also causes chronic tissue fibrotic sequelae, suggesting that inhibitory receptors are important for balancing immune protection and fibrotic processes (129). Similarly, $CD8^+$ T_{RM} that form in the epidermis following acute contact hypersensitivity reaction express inhibitory checkpoint receptors that limit T_{RM} reactivation. Treatment with inhibitory molecule antagonists increases the magnitude and severity of eczema exacerbations (27).

Human lung $CD8^+$ T_{RM} express not only PD-1, but also genes encoding inhibitory molecules such as CTLA4, BTLA, LAG3, SPRY1, and the adenosine receptor A2AR (73). Similarly, a recent study using sc-RNA seq demonstrated that inhibitory receptors including *Ctla4*, *Lag3*, *Cd101*, and *Tigit*, are upregulated early during formation of intestinal IEL $CD8^+$ T cells in an acute LCMV infection model, suggesting a possible role in T_{RM} differentiation (44). Moreover, following influenza infection, differences in T_{RM} inhibitory molecule expression are observed depending on the T cell epitope, suggesting that initial TCR-MHCp interactions may determine not only T cell activation, but also inhibitory programs (161).

The balance between $CD8^+$ T_{RM}-mediated immune response and immune pathology may also be regulated by alterations in mitochondrial membrane composition. $CD8^+$ T_{RM} express early activation markers, contain cytolytic proteins, and have the capacity to release cytokines. However, epithelial T_{RM} are metabolically arrested in a semi-activated state. Alterations in the mitochondrial membrane, including the cardiolipin composition, regulate IEL proliferation, and effector functions (164).

Finally, $CD8^+$ T_{RM} adaptation to the environment is regulated by mitochondrial gene expression. The transcription factor, Bhlhe40 is highly expressed in mouse and human $CD8^+$ T_{RM} compared to circulating memory $CD8^+$ T cells (44, 165), and promotes T_{RM} mitochondrial gene expression. $Bhlhe40^{-/-}$ $CD8^+$ T_{RM} exhibit decreased oxygen consumption and enhanced mitochondrial damage. Additionally, Bhlhe40 deficiency results in reduced acetyl-CoA and histone acetylation of T_{RM} effector loci. Lack of Bhlhe40 reduces the production of IFN-γ, granzyme B and TNF by $CD8^+$ T_{RM}, suggesting that Bhlhe40 promotes epigenetic programs permissive for effector gene expression. PD-1 signaling inhibits Bhlhe40 expression. Importantly, however, targeting downstream epigenetic machinery rescues $CD8^+$ T_{RM} mitochondrial function and cytokine production in the absence of Bhlhe40, suggesting a possible mechanism for improved immunotherapy (165).

DISCUSSION

Over the last decade, scientists around the globe have contributed to the study of $CD8^+$ T_{RM}. Rapid progress has been achieved in understanding the generation, regulation, and

protective or pathogenic functions of T cells that reside within tissues. Since the discovery of $CD8^+$ T_{RM}, much effort has focused on elucidating the transcriptional networks and mechanisms that regulate these cells. These studies have identified core transcriptional signatures for both mouse and human $CD8^+$ T_{RM} that promote their long-term retention and maintenance. However, with increasing data examining T_{RM} formation and function in multiple tissues and infection models, it has become increasingly clear that T_{RM} are a heterogeneous pool of cells with plastic properties. T_{RM} formation and phenotype are influenced by extrinsic signals such as antigen, cytokines, nutrients, costimulatory, and inhibitory signals within the LN and tissue microenvironments, as well as by intrinsic receptor and signaling protein expression. These factors can shape T_{RM} differentiation, maintenance and response, and their variability in different tissues and inflammatory settings promotes T_{RM} diversity between organs, and even within the same tissue. Although a great deal has already been learned, an improved understanding of the mechanisms that regulate T_{RM} formation and/or function in varied tissue environments is necessary not only to prevent autoimmune diseases, but also to improve cancer treatments and vaccine strategies.

AUTHOR CONTRIBUTIONS

RM-B drafted and edited the manuscript and figures. SB edited and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Rapid Isolation of Functional *ex vivo* Human Skin Tissue-Resident Memory T Lymphocytes

*Weijie Du[1†], Daniel Lenz[1†‡], Ralf Köhler[2‡], Erping Zhang[3], Carla Cendon[1], Jinchan Li[1], Mona Massoud[1], Joachim Wachtlin[3,4], Juliane Bodo[5], Anja E. Hauser[2,6], Andreas Radbruch[1] and Jun Dong[1]**

[1] Cell Biology, Deutsches Rheuma-Forschungszentrum Berlin, Institute of the Leibniz Association, Berlin, Germany, [2] Central Lab for Microscopy, Deutsches Rheuma-Forschungszentrum Berlin, Institute of the Leibniz Association, Berlin, Germany, [3] Sankt Gertrauden Krankenhaus, Berlin, Germany, [4] Medizinische Hochschule Brandenburg, Neurrupin, Germany, [5] Plastische und Ästhetische Chirurgie, Berlin, Germany, [6] Immune Dynamics, Rheumatology and Clinical Immunology, Charité Universitätsmedizin Berlin, Berlin, Germany

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Edited by:
Toshinori Nakayama,
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Craig Michael Walsh,
University of California, Irvine, United States
Akihiko Murata,
Tottori University, Japan

***Correspondence:**
Jun Dong
dong@drfz.de

†Present address:
Weijie Du,
BIH Center for Regenerative Therapies, Berlin, Germany
Daniel Lenz,
Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

‡These authors have contributed equally to this work

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Studies in animal models have shown that skin tissue-resident memory T (T_{RM}) cells provide enhanced and immediate effector function at the site of infection. However, analyses of skin T_{RM} cells in humans have been hindered by the lack of an optimized isolation protocol. Here, we present a combinatorial strategy-the 6-h collagenase IV digestion and gentle tissue dissociation – for rapid and efficient isolation of skin T_{RM} cells with skin tissue-specific immune features. In comparison with paired blood circulating memory T cells, these *ex vivo* isolated skin T cells express typical T_{RM} cell markers and display higher polyfunctional properties. Moreover, these isolated cells can also be assessed for longer periods of time in *ex vivo* cultures. Thus, the optimized isolation protocol provides a valuable tool for further understanding of human skin T_{RM} cells, especially for direct comparison with peripheral blood T cells at the same sample collection time.

Keywords: human skin, tissue-resident memory T cells, yield, epitope, collagenase IV, gentle tissue dissociation, cell isolation

INTRODUCTION

Recent research has provided compelling evidence that, in addition to circulating memory T cells, there are also significant non-circulating tissue-resident memory T (T_{RM}) cells residing in many tissues, such as in the skin, lungs, gut, liver (1–5), and bone marrow (6–10). Most but not all these T_{RM} cells express CD69 (7, 11–13), which probably contributes to their retention in tissues (14–16). Similarly, most T_{RM} cells do not express the chemokine receptor CCR7 (3, 7). Animal models showed that skin T_{RM} cells mediate first lines of defense against previously encountered pathogens (1, 2, 17, 18). Approximately 2 × 10e10 resident T cells have been estimated to be present in normal human skin. This number doubles that of circulating T cells in the peripheral blood (19). However, present understanding of human skin T_{RM} cells has been challenged by the lack of an optimized isolation protocol. In this regard, various approaches have been utilized to isolate skin T_{RM} cells, such as EDTA isolation (19), collagenase P (20), collagenase IV digestion (19, 21), and skin explants (19). Nevertheless, these methods either suffer from low yield or require long-term *in vitro* culture periods.

To establish an optimized protocol for rapid and efficient isolation of skin T_{RM} cells, we have evaluated six different protocols in terms of the preservation of epitopes of interest, cell viability, and yield. Among these six approaches, the modified collagenase IV (M.CoIV) protocol, i.e., the combination of 6-h collagenase IV digestion and gentle tissue dissociation, outperformed other protocols and resulted in the highest viable cell number while robustly preserving critical surface marker expressions (such as CD4, CD8, and CD69). Importantly, the M.CoIV isolation procedure does not induce skin TRM cell activation and proliferation. Cytokine profiles of isolated skin memory T cells stimulated by SEB and anti-CD3/CD28 revealed functional capacities, to which the successfully isolated various types of antigen-presenting cells (APCs), such as dermal dendritic cells (DDCs) and Langerhans cells (LCs), may contribute.

RESULTS

Characterization of Human Skin T Cells *in situ*

To characterize the human skin T cells *in situ*, we performed immunofluorescence histology on 6 μm sections of eyelid and abdominal skin samples from healthy donors (**Supplementary Table 1**). Sections without antibody staining (**Supplementary Figure 1A**) or only with secondary antibody staining (**Supplementary Figure 1B**) were used as background controls. As shown in a large tile scan and the regions of interest (ROI) 1 and 2 in **Figure 1A**, $CD8^+$ T cells localized in both the epidermis and dermis layers, while $CD4^+$ T cells were mainly detected in the dermis and clustered around the hair follicles, with only few $CD4^+$ T cells detected in the epidermis. Most $CD3^+$ T cells, ($CD4^+$ and $CD8^+$), expressed CD69, indicating a tissue residency status of these T cells (**Figure 1A**). Skin $CD3^+$ T cells expressed the skin homing markers, such as CLA (cutaneous lymphocyte-associated antigen) (**Figure 1B**) and did not express the proliferation marker Ki-67 (**Figure 1C**) or lymph node homing markers, such as CCR7 (**Figure 1D**). Quantitative analysis of immune cells present in the skin sections (**Supplementary Figure 2**) showed that, 14.4% (± 10.8) of skin cells were $CD3^+$ T cells and among them 68.97% (± 8.06) and 24.56% (± 13.81) were $CD4^+$ and $CD8^+$ T cells, respectively, resulting in the ratio of $CD4^+$ to $CD8^+$ T cells of ∼3:1 (**Figure 1E**). Additionally, while more than 65% of $CD3^+$ T cells co-expressed CD69 and 75% co-expressed CLA, there were only 16% of $CD3^+$ T cells co-expressing CCR7 (**Figure 1E**). The variation in frequencies especially of $CD3^+$ T cells may reflect their uneven distribution in the skin. To identify the spatial distribution between T cells and dendritic cells, CD1a was concomitantly used with CD3 in the immunofluorescence staining. We observed that $CD1a^+$ dendritic cells mainly resided in the epidermis layer and were close to $CD3^+$ T cells (**Figure 1F**). Similarly, T cells expressing CD69 were also identified in the dermis of abdominal skin samples (**Supplementary Figure 3A**), although a strong autofluorescence signal in the FITC channel was detected (**Supplementary Figure 3B**), likely due to the intensive collagen fiber structures present in the abdominal skin. Together, these results suggest that normal human skin T cells are resting and qualify as T_{RM} cells.

The Modified Collagenase IV Protocol Best Preserves Cell Surface Markers of Interest With High Cell Viability and Yield

To optimize the protocol for isolating human skin T cells, skin samples were minced and subjected to six reasonable protocols, each including a 3-, 6-, or 12-h enzymatic digestion (**Figure 2A**). These protocols are: combination of 1) a 12-hour collagenase IV digestion, i.e. modified collagenase IV digestion (M.CoIV)_12h; 2) M.CoIV_6h; 3) whole skin dissociation plus enzyme P digestion (WSD+EnzP_12h); 4) WSD-EnzP_12h (without enzyme P digestion); 5) CoP+CoIV_12h; or 6) cocktail of enzymes (collagenase I, elastase, hyaluronidase, and trypsin inhibitor) (Cocktail_3h), with gentle tissue dissociation (**Supplementary Table 2**). Cell isolated using these protocols were compared for expressions of CD45, CD3, CD4, CD8, CD69, CLA, and CCR7 among viable cells by flow cytometry. Notably, the modified collagenase IV (either 6- and 12-h digestion time) and cocktail protocols were the best to preserve the epitopes of antigens, such as CD4 (**Figure 2B**), CD8 (**Figure 2C**), and CD69 (**Figure 2D**). In terms of cell viability, significantly higher percentages of viable cells were isolated when using the M.CoIV_12 h and M.CoIV_6 h protocols (42.30 ± 5.01% and 42.36% ± 3.31%, respectively) than the cocktail_3 h protocol (26.33 ± 5.14%) (**Figure 2E**). In terms of viable T cell number, the M.CoIV_6 h protocol isolates more cells (28.73 ± 7.68 × 10^4 live T cells per cm^2) than the M.CoIV_12 h and cocktail_3 h protocols (19.29 ± 3.25 × 10^4 and 10.81 ± 5.29 × 10^4 live T cells per cm^2, respectively) (**Figure 2F**). Thus, the M.CoIV_6 h protocol significantly outperformed other isolation protocols, representing an optimized protocol for isolating skin T cells.

Characterization of *ex vivo* Skin T Cells

Using the optimized isolation protocol M.CoIV_6 h, we next characterized cells isolated from 12 (including 8 paired) skin samples in comparison with peripheral blood samples of 50- to 80-year-old individuals by flow cytometry (**Supplementary Figure 4**). Compared to blood, skin contained significantly lower frequencies of CD45 expressing lymphocytes (72 vs. 20%) (**Figure 3A**). However, among $CD45^+$ lymphocytes, frequencies of $CD3^+$ T cells as well as $CD4^+$ and $CD8^+$ T cells were comparable between skin and blood, resulting in the ratio of $CD4^+$ to $CD8^+$ T cells of 3:1 (**Figure 3B**), in line with that of skin T cells *in situ* (**Figure 1E**). The majority of skin T cells expressed CD45RO (87.93%), indicating a memory phenotype, whereas only approximately half of blood T cells (50.90%) expressed CD45RO (**Figure 3C**). Moreover, *ex vivo* skin memory T cells expressed the tissue resident markers such as CD69 (81.86%) and skin homing molecule CLA (75.08%) but rarely tissue egress markers, such as CCR7 (10.91%), in contrast to blood T cells (57.37%) (**Figure 3D**). Among $CD3^+$ T cells, except for CCR7

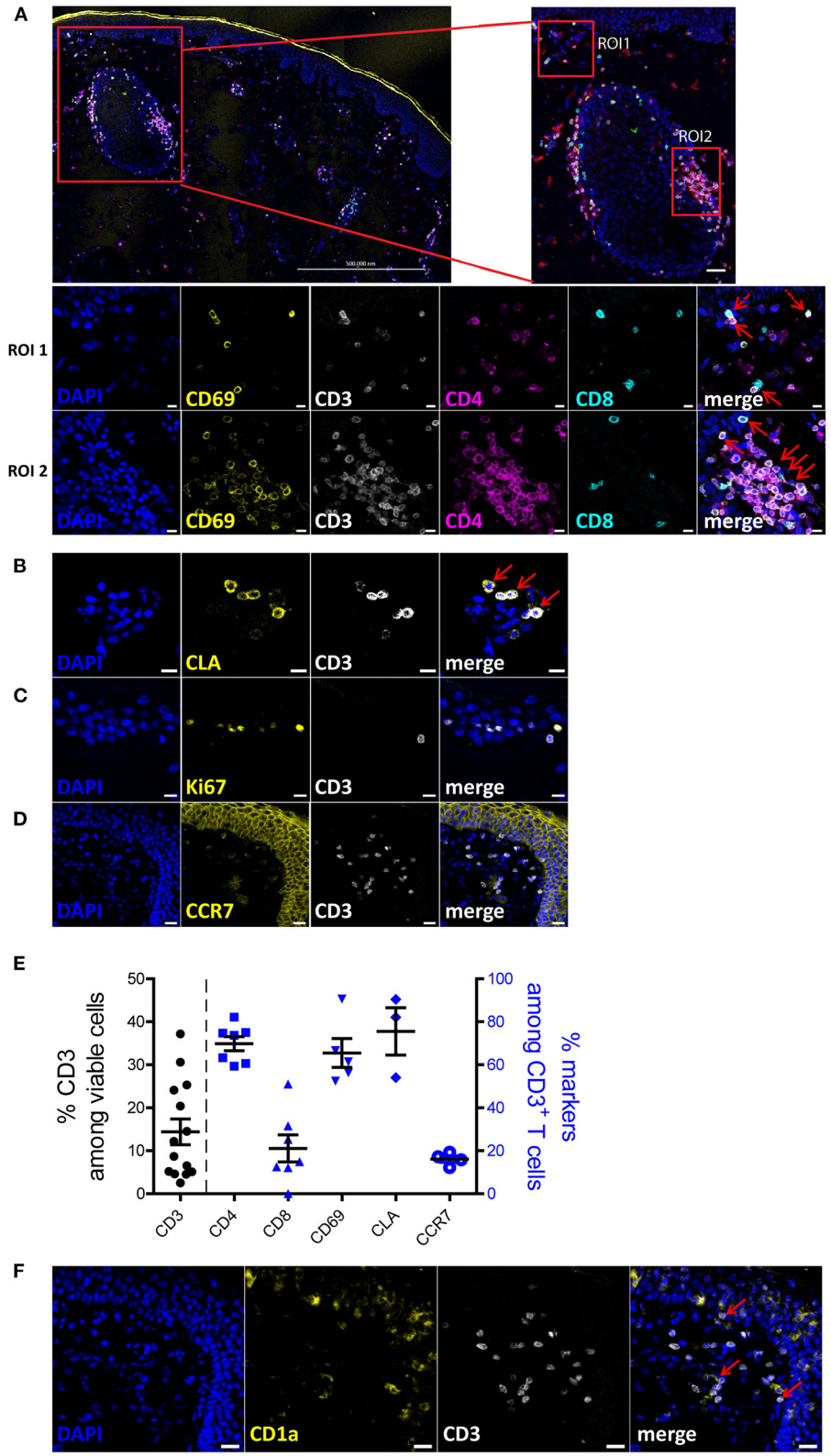

FIGURE 1 | Phenotypic characterization of human skin cells *in situ*. **(A)** A 3 × 4 tile scan image of skin section stained with DAPI (blue), CD69 (yellow), CD3 (white), CD4 (violet) and CD8 (turquoise). Region of interest (ROI) 1 is a representative image of the cells located in the epidermis and ROI2 is a representative image of the cells around a hair follicle in the dermis. (**B–D,F**) Skin sections were stained with DAPI (blue) and CD3 (white) as well as one of the following: CLA **(B)**, Ki-67 **(C)**, CCR7

(Continued)

FIGURE 1 | (D) or CD1a **(F)**. Scale bar: 500 μm for **(A)** upper left, 20 μm for **(A)** upper right, **(D,F)**; 10 μm for A-ROI1, A-ROI2, **(B,C)**. Co-expression of $CD3^+CD4^+/CD8^+$ and $CD69^+$ cells, and CLA^+ and $CD3^+$ cells are indicated by red arrows. Representative image sets from three independent experiments are shown. Scale bar: 20 μm for **(A,D,F)**; 10 μm for **(A–C)**. **(E)** Frequencies of $CD3^+$ T cells among total cells (left y-axis) and frequencies of indicated subpopulations of T cells among $CD3^+$ T cells (right y-axis), according to image cell quantification ($n = 3$; 14 fields).

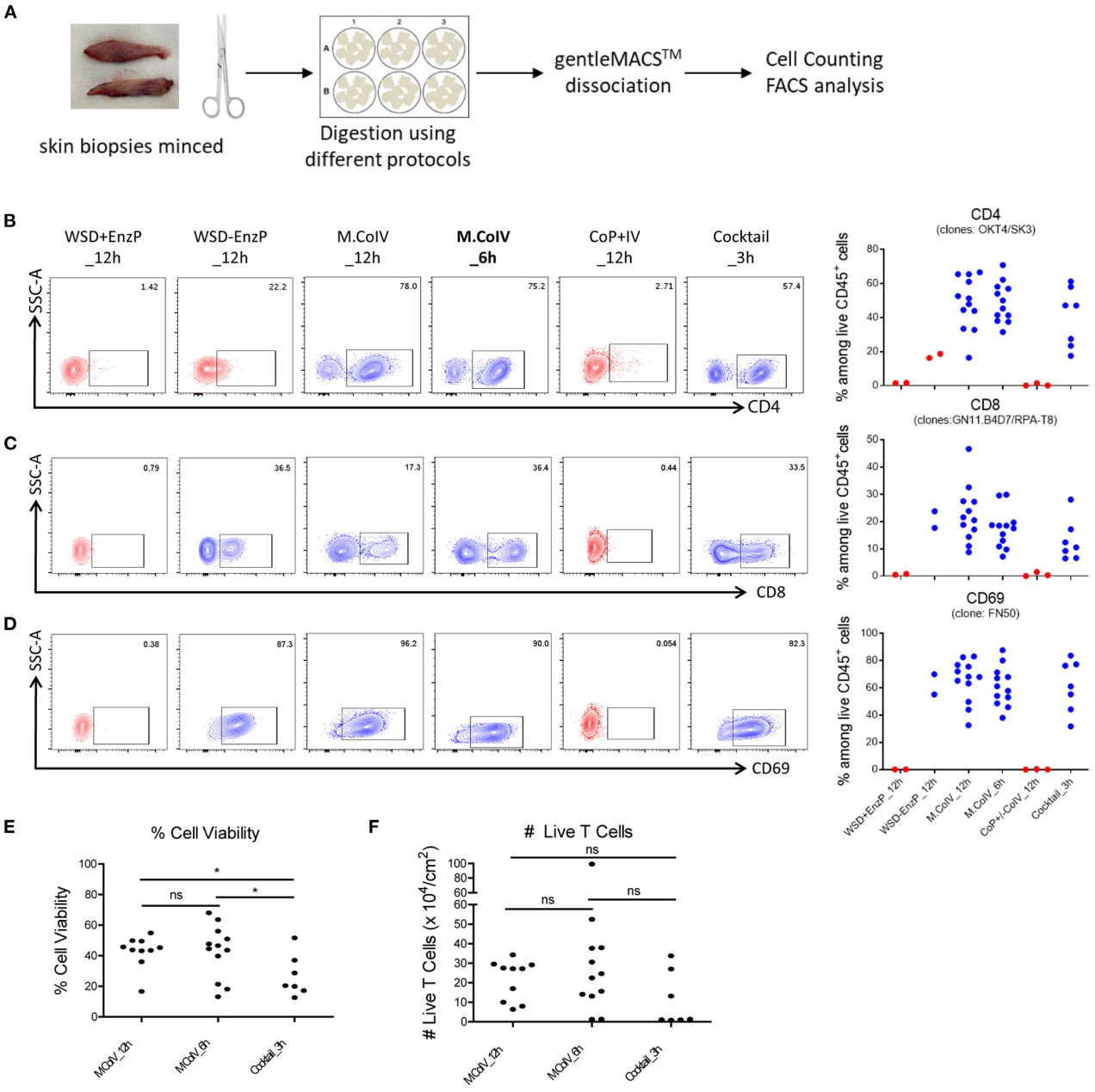

FIGURE 2 | Modified Collagenase IV protocol best preserves the epitopes of surface antigens with high cell viability and yield. **(A)** Schematic workflow of isolating cells from human skin samples. **(B–D)** Frequencies of $CD4^+$ **(B)**, $CD8^+$ **(C)** and $CD69^+$ **(D)** T cells among live $CD45^+$ lymphocytes isolated by six different isolation protocols: (1) WSD+EnzP_12 h ($n = 2$), (2) WSD-EnzP_12 h ($n = 2$), (3) M.ColV_12 h ($n = 12$), (4) M.ColV_6 h ($n = 12$), (5) CoP+/-ColV_12 h ($n = 3$), and (6) Cocktail_3 h ($n = 7$). Each dot represents data obtained from one donor. Red dots showing cells isolated from skin samples using protocols that did not preserve the CD4 epitope. **(E)** Frequencies of viable cells and **(F)** the total number of viable T cells isolated by using the M.ColV_12 h, M.ColV_6 h and cocktail_3 h isolation protocols. Statistical significance was calculated by two-tailed, unpaired *t*-test with Welch's correction. $p < 0.05$ (*).

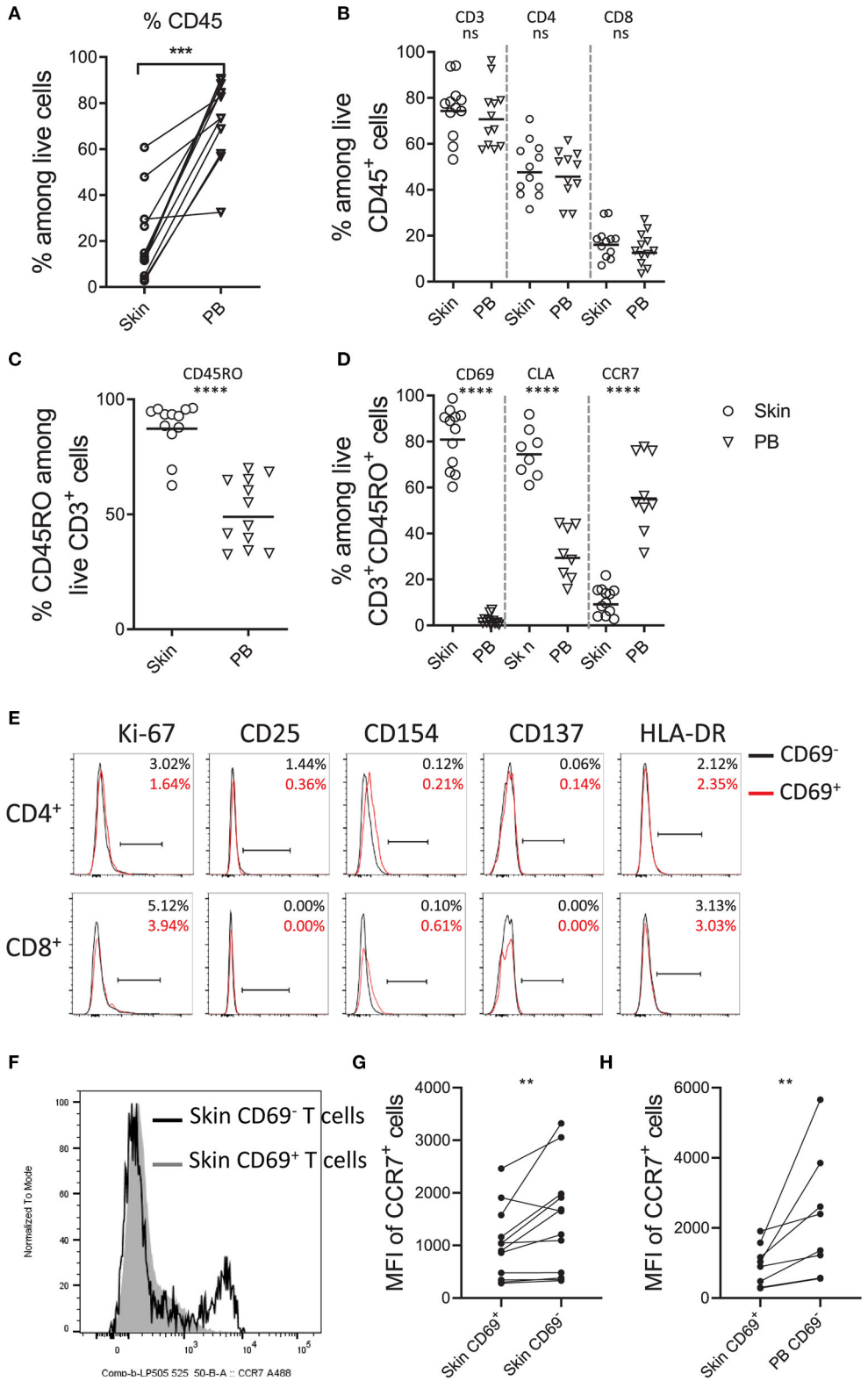

FIGURE 3 | Phenotypic characterization of skin T cells by flow cytometry. Frequencies of CD45$^+$ lymphocytes **(A)**, CD3$^+$, CD4$^+$, and CD8$^+$ T cells **(B)**, CD45RO$^+$ memory T cells **(C)**, and CD69$^+$, CLA$^+$ and CCR7$^+$ cells among memory CD3$^+$ T lymphocytes **(D)** in paired **(A)** and unpaired human skin and peripheral blood samples **(B–D)**. **(E)** Overlay of histograms showing the percentages of cells expressing proliferating and putative activation markers (Ki-67, CD25, CD154, CD137

(Continued)

FIGURE 3 | and HLA-DR) on $CD69^-$ (black line) and $CD69^+$ (red line) memory $CD4^+$ and $CD8^+$ T cells. Percentages are shown in the upper right of each plot. Representative data from more than 10 independent experiments are shown. **(F)** Overlay of histograms showing the expression of CCR7 on $CD69^+$ (filled gray area) and $CD69^-$ (black line) skin T cells. Comparison of MFI (Mean Fluorescence Intensity) of $CCR7^+$ cells between skin $CD69^+$ and $CD69^-$ T cells **(G)** and between skin $CD69^+$ and blood $CD69^-$ T cells. In **(A,G,H)**, Wilcoxon matched-pairs signed rank test, two-tailed; in **(B–D)**, unpaired *T*-test with Welch's correction, two-tailed. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.005$, ns.

(16 vs. 10%), the frequencies of these markers by *ex vivo* skin $CD3^+$ T cells were similar to those by *in situ* skin $CD3^+$ T cells (**Figure 1E**), suggesting that the M.CoIV_6 h protocol enables isolation of proportional skin cells.

Studies have shown that steady-state $CD69^+$ T_{RM} cells from other tissues, such as the bone marrow (7), are resting in terms of activation. To test whether that would be also the case for normal skin T cells, we analyzed the expressions of proliferation marker Ki-67 and putative activation markers CD25, CD154, CD137 and HLA-DR on *ex vivo* skin T cell subsets isolated using the M.CoIV_6 h isolation protocol. Similar to $CD69^-$ memory $CD4^+$ and $CD8^+$ T cells, $CD69^+$ memory $CD4^+$ and $CD8^+$ T cells did not express these analyzed proliferation or activation markers (**Figure 3E**). This was not due to downregulation of these markers that might be potentially induced by the isolation procedure, in control experiments where T cells expressing these markers there was no downregulation of their expression following the isolation procedure (**Supplementary Figures 5A,B**). In agreement with T_{RM} features described from other tissues (3, 7), $CD69^+$ skin T cells significantly downregulated CCR7 both in frequency (**Figure 3F**) and expression levels (**Figures 3G,H**), in comparison with their $CD69^-$ counterparts in the skin (**Figures 3F,G**) or paired blood (**Figure 3H**). Together, these results describe a steady-state, memory T cell population as resident in the normal human adult skin. Furthermore, they demonstrate that the optimized M.CoIV_6 h isolation protocol does not activate skin T cells.

Various Types of Antigen-Presenting Cells Can Be Isolated From the Human Skin by the M.CoIV Protocol

APCs mediate cellular immune responses by processing and presenting antigens for the recognition by T cells. We next analyzed whether the M.CoIV_6 h protocol enables the isolation of major types of human skin APCs. The following five major described types of APCs in the human skin (24) were characterized, namely, (1) plasmacytoid dendritic cells (pDCs), (2) conventional dendritic cells (cDCs), (3) $CD14^+$ dermal dendritic cells ($CD14^+$ DDCs), (4) $CD1a^+$ dermal dendritic cells ($CD1a^+$ DDCs), and (5) Langerhans cells (LCs) (**Figures 4A,B**). Among *ex vivo* lineage negative human skin lymphocytes ($CD45^+HLA\text{-}DR^+DUMP^-$), pDCs were rare while cDCs were relatively abundant (0.27 vs. 11.75%), which is consistent with previous findings (22, 23), that the low levels of CD303 expression by skin pDCs were not due to the downregulation that might be potentially induced by the isolation procedure (**Supplementary Figures 5C,D**). Additionally, $CD1a^+$DDCs (37.91%), $CD14^+$ DDCs (3.00%), and LCs (5.00%) could also be identified (**Figure 4C**). Thus, the M.CoIV_6 h protocol is capable of effectively isolating various types of APCs from human skin tissues.

Ex vivo Skin T Cells Exhibit Functional Capacities

To validate whether memory T cells isolated from human skin are functional, cytokine profiles of cells upon *ex vivo* antigenic stimulation were evaluated in comparison with paired blood memory T cells (**Supplementary Figure 6**). Skin and blood mononuclear cells were stimulated with the super antigen SEB and CD28 antibodies for 7 h. Memory $CD4^+$ T cells reacting to the antigen were identified according to the expression of CD154 (25, 26) and one or more of the cytokines TNF-α, IFN-γ, IL-2, or IL-17 as assessed by intracellular immunolfluorescence (7). T cells that have two or more functions, such as the production of cytokines, are polyfunctional. Polyfunctionality of T cells is associated with enhanced protection (27). In response to the stimulation with SEB, $CD154^+$cytokine$^+$ cells were readily detectable both in blood and skin with comparable frequencies (**Figure 5A**) and absolute cell numbers (data not shown). Among four matched samples, the fraction of polyfunctional cytokine-producing ($polyCyt^+$) T cells were higher in memory $CD4^+$ T cells from skin than blood (**Figure 5B**). Likewise, higher frequencies of $polyCyt^+$ memory $CD8^+$ T cells were found from skin than blood in three out of four donors (**Figure 5B**). In terms of the expression of IL-17A, both skin $CD4^+$ and $CD8^+$ T cells secreted more $IL\text{-}17^+CD154^+$ cells than their blood-derived counterparts in two analyzed donors (**Figure 5C**). In addition, on average about 30% of skin memory T cells responded to the stimulation with anti-CD3 and anti-CD28 (**Figure 5D**, **Supplementary Figure 7**).

Finally we evaluated whether memory T cells isolated from human skin could be used for antigen-specific responses and other parameters that may require longer periods of time in *ex vivo* cultures. To this end, skin cells were isolated using the optimized M.CoIV_6 h protocol and further examined after 5-day *ex vivo* cultures for their viability and proliferation potential. When cultured in medium alone, the number of skin T cells on day 5 remained similar to that of day 0 (data not shown). Of note, when cultured in medium supplemented with IL-2, about 30% of skin T cells had proliferated (**Figure 6A**) on day 5, that more than 70% of proliferation was observed in cultures in the presence of additional anti-CD3 and anti-CD28 (**Figure 6B**). Together, these results demonstrate that expanded skin (T) cells can be used for further downstream applications.

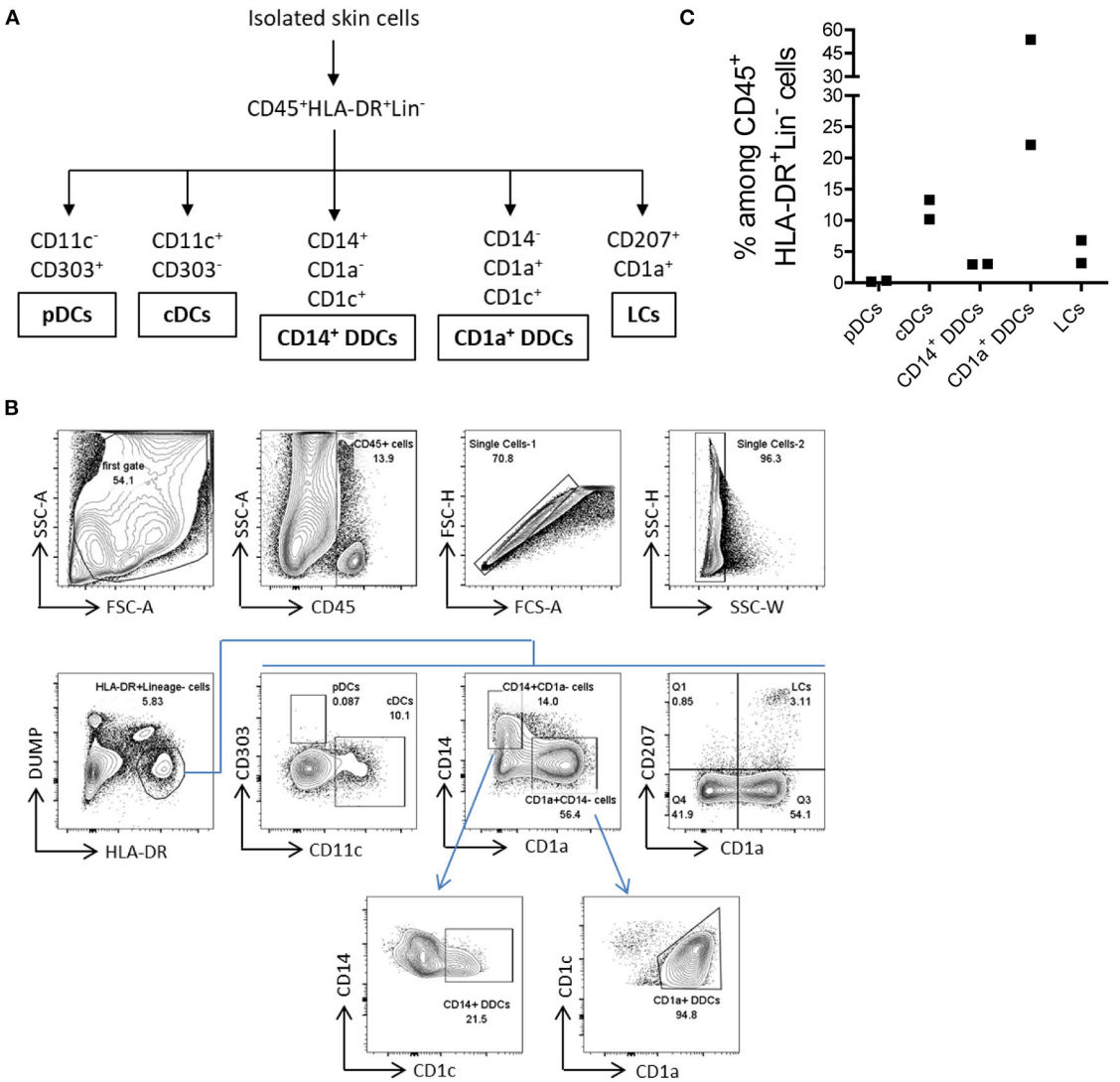

FIGURE 4 | Isolation of five major types of APCs from normal human skin by using the M. CoIV_6 h protocol. **(A)** Classification of five main types of APCs distinguished by their surface markers (22, 23). **(B)** Gating Strategy for analyzing APCs from *ex vivo* human skin cells. Lineage markers included CD3, CD20, CD34, and CD56. Dead cells were excluded by DAPI staining. pDCs and CDCs were distinguished by CD11c against CD303. $CD14^+$ DDCs and $CD1a^+$ DDCs were distinguished by CD1a against CD14 and further based on the CD1C expression. LCs cells were gated based on the expression of CD1a and CD207. **(C)** Frequencies of *ex vivo* pDCs, cDCs, $CD14^+$ DDCs, $CD1a^+$ DDCs and LCs among $CD45^+HLA\text{-}DR^+Lin^-$ viable cells isolated from human eyelid skin samples. Representative data from two independent experiments are shown.

DISCUSSION

We report in this study optimization of rapid and efficient isolation protocols for characterizing human skin T_{RM} cells, in comparison with their matched blood counterparts. To date, human cutaneous $\alpha\beta^+$ T cells *in situ* have been characterized mostly by immunohistochemistry staining (19, 28, 29), which might be biased either by the reaction itself or by incorrect interpretation (30). In the present report, by applying immunofluorescence staining techniques, we showed that $CD4^+$ and $CD8^+$ T cells can be detected both in the epidermis and dermis, with $CD4^+$ T cells predominantly detected in the dermis (especially clustered around hair follicles). Our findings are supported by other studies (31–33) describing the T_{RM} cell tropism to the epidermis and follicles as epidermotropism. Studies in animal models showing that the preferential location of $CD4^+$ and $CD8^+$ T_{RM} cells in skin demonstrate their immediate local immune surveillance and protective responses at the site of antigen exposure (1, 2, 17, 34). The loose spatial structure of the dermis shaped by an abundant extracellular matrix may facilitate the interaction between $CD4^+$ T helper cells with other immune cells and non-immune components, e.g., hair follicles

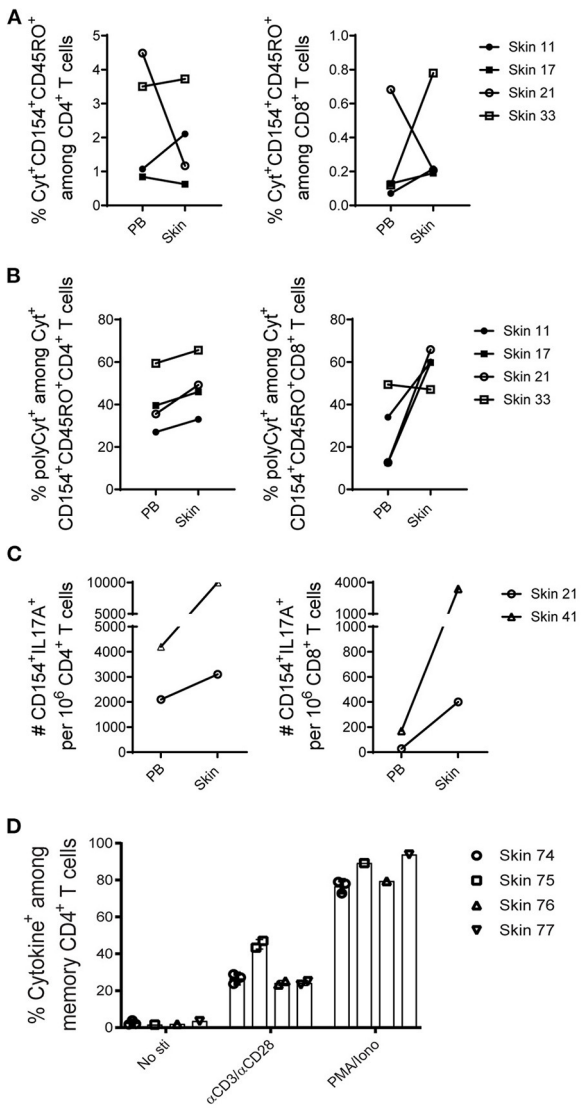

FIGURE 5 | Functional capacities of T cells from skin and paired peripheral blood samples. **(A–C)**, mononuclear cells isolated from five paired skin and PB samples were stimulated with the SEB, and the induced cytokine production (IFN-γ, IL-2, or TNF-α; alternatively IFN-γ, IL-2, TNF-α, or IL-17A) in memory $CD4^+$ and $CD8^+$ T cells was examined according to CD154 expression. For each subpopulation, the background (as detected in the anti-CD28 stimulated but otherwise equally treated control samples) was subtracted. **(A)** Antigen-specific $CD154^+$cytokine$^+$ (total cytokine-producing) memory $CD4^+$ and $CD8^+$ T cells are shown in frequencies among $CD4^+$ and $CD8^+$ T cells. **(B)** The proportions of polyCyt$^+$-producing (more than one of the analyzed three or four cytokines TNF-α, IFN-γ, IL-2, or IL-17A) memory T cells among cytokine$^+$$CD154^+CD45RO^+$ $CD4^+$ and $CD8^+$ T cells. **(C)** The absolute numbers of IL-$17A^+$ cells per million $CD4^+$ and $CD8^+$ T cells isolated from two analyzed paired skin and blood samples upon SEB stimulation are shown. **(D)** Percentage of cytokine$^+$ cells among skin memory $CD4^+$ T cells in response to αCD3/αCD28 stimulation. No antigens and PMA/Ionomycin stimulation were included as controls. Bars with two or three data points are shown as the mean of replicates of cells analyzed from each sample.

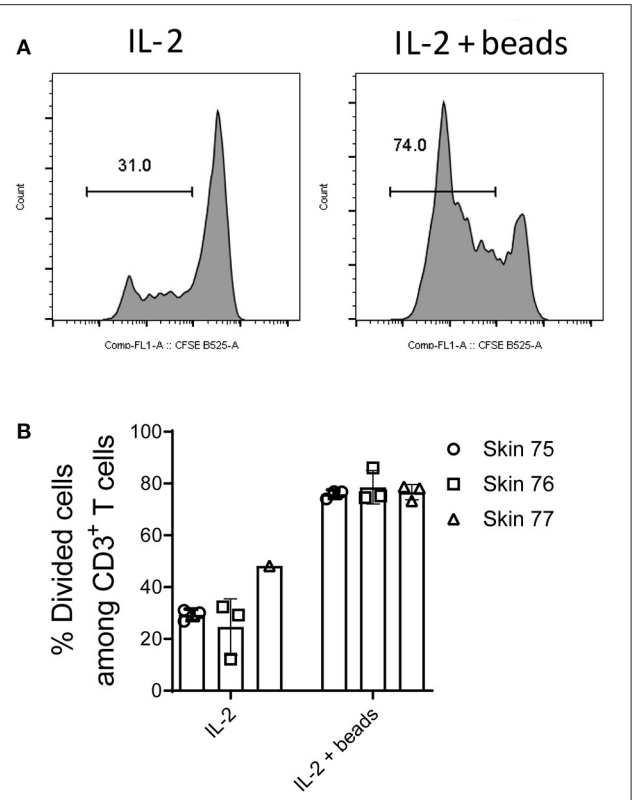

FIGURE 6 | Long-term cultures and expansion of *ex vivo* isolated skin T cells. Skin mononuclear cells were isolated using the optimized M.ColV_6 h isolation protocol from three individual skin samples. **(A)** Representative histograms and **(B)** percentages of divided cells among skin $CD3^+$ T cells cultured in medium supplemented with Proleukin (IL-2) (left) or in the presence of additional T cell expansion beads (right). In **(B)** bars with three data points represent the mean (± SEM) of three replicates of cells analyzed from each sample.

(35). Studies in mice have demonstrated that after HSV infection, memory $CD4^+$ T cells are recruited and formed clusters around hair follicles in a CCL5-dependent manner (32). Moreover, hair follicle keratinocyte-derived IL-15 has been described to be required for the maintenance of $CD8^+$ T_{RM} cells, and IL-7 for $CD8^+$ and $CD4^+$ T_{RM} cells (31). Therefore, hair follicles may be a preferred site of pathogen exposure and thus, for locating T_{RM} cells. On the other hand, our histological data also showed a close proximity of $CD3^+$ T cells to $CD1a^+$ DCs, which may facilitate antigen presentation and provision of other survival signals by $CD1a^+$ DCs to T_{RM} cells. Interestingly, only cells in the epidermis but not T cells express CCR7 and Ki-67, which is a feature of keratinocytes (36).

To study the human skin T_{RM} cells, several isolation methods were reported (20, 21). However, these methods either suffer from low yield or require long-term *in vitro* culture periods. To overcome these challenges, here we have established an optimized protocol for rapidly isolating skin T_{RM} cells by the combinatorial and sequential procedures of a short period of collagenase IV digestion and a gentle mechanical tissue dissociation. As the dermis has an abundant extracellular matrix comprised of collagen and elastin fibers (35), different types of collagenases [I (37), 1A (38), or IV (21)] alone or in combination have been applied to break down these extracellular structures. In particular, type IV collagenase has a lower tryptic activity and

high collagenase activity, which limits the damage to membrane proteins and receptors while effectively breaking down the collagen-rich dermal tissues, resulting in the effective release of intact T_{RM} cells for downstream isolation. However, isolation of skin T cells with collagenase IV (19) or enzyme alone (20, 21) is not effective in isolating large number of T cells. Indeed, among the six analyzed protocols, only the M.CoIV_6 h enabled a high yield of viable total mononuclear cells and T cells, on average 2.8×10^5 cells per cm^2 of skin. Based on the reasonable estimate that the number of T cells in 1 cm^2 of skin is 1.1×10^6 (19), we were able to isolate more than 20% of proportioned skin T cells, which is comparable with skin T cells isolated using the skin explant cultures (19). Additionally, although we observed slightly lower frequencies of $CCR7^+$ T cells from *ex vivo* than from *in situ*, it has been shown that neither collagenase digestion nor mechanical dissociation method modify the expressions of both CLA and chemokine receptors, such as the CCR4, CCR6, CCR8, and CCR10, on isolated *ex vivo* skin T cells (20). Thus, the M.CoIV_6h protocol should not alter features of skin T cells. In fact, the M.CoIV_6 h protocol also best preserved critical expressions of surface markers such as CD4, CD8, and CD69 on skin T cells. In line with their *in situ* status, isolated *ex vivo* skin T cells exhibit a memory phenotype, express the tissue-resident marker CD69 and the skin-homing receptor CLA but lack the expression of CCR7, Ki-67, and other putative activation markers, indicating their non-proliferating, inactive, and tissue resident status in the steady state.

In addition, using the M.CoIV_6 h protocol, not only memory T cells but also major types of APCs could be effectively isolated from fresh human skin tissues, which allowed for a further assessment of the functionalities of skin T cells. In line with our previous findings of preferential enrichment of polyfunctional memory T cells in the human bone marrow (7), polyfunctional memory T cells are also more frequent in the skin than blood. This observation suggests that there is a preferential location of memory T cells in the skin with distinct antigen exposure experience, such as to *Candida albicans* (39), as evidenced by their higher amount of IL-17 production. The *ex vivo* skin T cell responses are likely an attribute of the effective isolation of various types of APCs. Thus, this optimized protocol could help pave the way for research in human skin T_{RM} cells as such and, in particular, in direct comparison with their blood-circulating counterparts at the same sampling time.

MATERIALS AND METHODS

Study Cohort

This study was approved by the ethics committee at the Charité – Universitätsmedizin Berlin, Germany (EA1/290/14). All blood and skin tissue samples were obtained with informed consent from all donors. Samples taken from normal adult skin with paired peripheral blood samples (mean age ± SEM, 67.29 ± 3.55 *y*; $n = 14$) or without (mean age ± SEM, 58.42 ± 2.90 *y*; $n = 24$) were obtained from healthy donors undergoing plastic cutaneous surgeries (**Supplementary Table 1**).

Histological Staining

Skin samples were immediately fixed in 2% paraformaldehyde (Carl Roth) for 4 h at 4°C. Following fixation, samples were sequentially equilibrated in solutions supplemented with 10–30% sucrose (Carl Roth), each for 24 h at 4°C. Samples were then embedded in O.C.T™ media (SAKURA) and stored at −80°C until cryosectioning using Kawamoto's tape method (40) with a microtome MH560 cryostat (Thermo Fisher). Tissue sections in 6 µm were blocked with blocking buffer (PBS with 0.1% Tween 20, and 10% FCS) for 1 h at room temperature and then stained with primary and secondary antibodies as well as DAPI (2 µg/mL) to label cell nuclei. Among the used anti-human antibodies, anti-CD3 Alexa Fluor 594 (UCHT1), anti-CD4 Alexa Fluor 555 (TT1), anti-CD8 Alexa Fluor 647 (GN11/134D7), anti-CD1a Cy5 (OKT6) were conjugated in house. Other antibodies include anti-CD69 Alexa Fluor 488 (FN50; Biolegend), anti-CCR7 Alexa Fluor 555 (Y59; Abcam), Anti- Ki-67 Biotin (SolA15; eBioscience), anti-CLA APC (HECA-452; Miltenyi) and streptavidin Alexa Fluor 488 (Thermofischer).

Following staining, the sections were mounted with fluorescent mounting medium (DAKO). Confocal images were generated using a Zeiss LSM710 (Carl Zeiss). Skin section picture composites were generated by three-dimensional tile scanning using a Plan-Apochromat 20X (0.8 numerical aperture; NA) air objective lens. The displayed overview image was part of 3 × 4 tile scans, with maximum intensity projections of z-stacks each with 1.3 µm z-resolution and x-y resolution of 7,578 × 5,734 pixels. Tiles were recorded with a 10% overlap and projections stitched together by the acquisition software to generate three high-resolution images. Images were analyzed using the ZEN software (blue edition).

For quantification of cells, the segmentation pipeline was designed using a previously described similar approach (41) and performed in Fiji, a distribution of ImageJ/Fiji (1.52p) (42). In every image set nuclei were identified by a plugin called "StarDist" (43). The objects were further used to measure the nuclear area and mean intensity in every staining. Signals above defined intensity thresholds were counted as positive signals. Counting of co-expressing cells was performed by using multiple thresholds for the markers of interest.

Skin and Blood Sample Preparation

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient sedimentation using Ficoll-Paque™ Plus (Sigma-Aldrich). Skin samples were delivered in CUSTODIOL® HTK solution (kindly provided by the Köhler Chemie, Germany) for <24 h until further preparation. In brief, skin samples were rinsed with cold PBS buffer, and the subcutaneous fat and hairs were carefully removed. Skin tissues were minced with sterile scissors into 2–4 mm fragments. About 25–50 fragments were digested in 3 mL digestion media in an incubator at 37°C and 5% CO_2. Various components

were used to digest skin fragments in different protocols (**Supplementary Table 2**), such as 0.8 mg/mL collagenase IV (Worthington), 0.4 mg/mL collagenase P (Roche), 1.25 mg/mL collagenase I (Sigma-Aldrich), 0.5 mg/mL elastase (Worthington), 0.5 mg/mL hyaluronidase (Worthington), 0.02 or 0.1 mg/mL DNAse I (Roche), 0.1 mg/mL trypsin inhibitor (Sigma-Aldrich) and 3.2 mm $CaCl_2 \cdot 2H_2O$. RPMI1640 or DMEM culture medium (Thermo Fisher) was supplemented with 5% human AB serum (Sigma-Aldrich), 1% HEPES, 1% Pen/Strep (100 U/mL penicillin; 100 µg/mL streptomycin). The digestion procedure was terminated by adding an equal volume of PBS consisting of 2 mM EDTA. Skin fragments were then dissociated with a Gentle MACS Dissociator (Miltenyi Biotec). The homogenized tissue samples were further filtered through a 70 µm cell strainer (Miltenyi Biotec). If present, residual fragments were dissociated through a second dissociation step. Upon isolation, viable cells were quantified with DAPI using a MACSQuant. Digestion procedures using the whole skin dissociation kit with or without enzyme P (WSD+/-EnzP) (Miltenyi Biotec) were performed according to manufacturer's recommendation.

Ex vivo Antigen Stimulation

Isolated mononuclear cells from the blood and skin were adjusted to a density of 1×10^7 cells/mL in culture medium. Cells were stimulated with 1 µg/mL Staphylococcus Enterotoxin B (SEB) (Sigma-Aldrich), plate bound αCD3/αCD28 (Thermo Fischer; each 1 µg/mL) or PMA (1ng/mL) plus Ionomycin (1 µg/mL) (Thermo Fischer) for 7 h at 37°C, 5% CO_2, with 5 µg/mL Brefeldin A (Biolegend) added during the last 2 h. Cultured cells without added antigen served as negative controls.

Cell Surface and Intracellular Staining for Flow Cytometry Analysis

Up to 10 million cells were stained with antibodies and Fc Blocking reagent (Miltenyi Biotec) for 10 min in the dark at 4°C. When staining with the anti-CCR7 antibody, cells were stained for 15 min in the dark at 37°C. To detect the intracellular production of cytokines, stimulated cells were fixed with 2% paraformaldehyde followed by permeabilization (Perm 2; BD Biosciences), prior to intracellular CD154 and cytokine staining. The following fluorochrome-conjugated mouse anti-human antibodies were used to stain cells: anti-CD45 PE-vio770 (5B1), anti-CD45 APC-vio770 (5B1), anti-CLA APC (HECA-452), anti-CD25 APC (REA570), anti-CD11c Percp-vio770 (MJ4-27G12), anti-CD207 Pe-vio770 (MB22-9F5) and anti-CD1c FITC (AD5-8E7) (Miltenyi Biotec), anti-CD45 BV785 (HI30), anti-CD3 A700 (HIT3a), anti-CD8 BV785 (RPA-T8), anti-CD69 BV421 (FN50), anti-CD154 BV421 (24-31), anti-HLA-DR APC-Cy7 (L243), anti-CD45RO BV650 (UCHL1), anti-CCR7 A488 (G043H7), CD20 BV510 (2H7), CD34 BV510 (581), CD56 BV510 (HCD56), CD14 BV605 (M5E2), CD303 BV421 (201A), IL-2 FITC (MQ1-17H12), and IFN-γ PE-Cy7 (B27) (Biolegend), anti-CD3 APC-H7 (Sk7), anti-CD3 V500 (UCHT-1), anti-CD19 V500 (HIB19), CD141 BV711 (1A4) and TNF APC (MAb11) (BD Biosciences), anti-CD4 Pe-Cy5.5 (Sk3), anti-Ki-67 PE (20Raj1), anti-CD137 FITC (4B4) and IL-17 PE (eBio64DEC17) (eBioscience), and anti-CD14 Pacific Orange (TM1), anti-CD19 Pacific Orange (BU12) and CD1a Cy5 (OKT6) (house conjugate). Stained cells were acquired using a MACSQuant (Miltenyi Biotec) or a LSRFortessa (BD Biosciences) flow cytometer. At least 1×10^6 lymphocytes were acquired. The data were analyzed with Flowjo V10 (Tree Star).

CFSE Labeling and Long-Term Cell Culture

Freshly isolated skin mononuclear cells using the M.CoIV_6 h isolation protocol were labeled with Carboxyfluorescein succinimidyl ester (CFSE) at the final concentration of 2.5 µM. Briefly, cells were washed twice in PBS and the cell pellet was resuspended in PBS at density of 10×10^6 cells/mL, and then labeled with 2.5 µM CFSE at 37°C for 10 min. The reaction was stopped by adding 5 mL FCS and washed twice. Labeled skin cells were cultured in X-vivo 15 medium (Lonza) containing 10% human AB serum and 500 IU/mL Proleukin (IL-2; Novartis) as well as 1% Pen/Strep for 5 days. Fractions of skin cells were additionally stimulated with T activation/expansion beads (Miltenyi Biotec) at a bead-to-cell ratio of 1:1.

Statistics

Statistical analyses were performed with Graphpad Prism software (version 5.04). For analysis of two groups, two-tailed Wilcoxon matched-pairs signed rank test or unpaired *T*-test with Welch's correction was used, and a *p*-value under 0.05 was considered statistically significant.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee at the Charité University Medicine, Berlin, Germany (EA1/290/14). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JD: conceptualization, writing – review & editing, supervision, and project administration. WD, DL, RK, AEH, and JD: methodology. EZ, JW, and JB: sample resource. WD: investigation and writing – original draft. WD, RK, CC, JL, and MM: analysis. WD and JD interpretation. JD and AR funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.624013/full#supplementary-material

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REVIEW
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Age-Related Dynamics of Lung-Resident Memory CD8$^+$ T Cells in the Age of COVID-19

Nick P. Goplen[1]*, *In Su Cheon*[1] and *Jie Sun*[1,2,3]*

[1] Division of Pulmonary and Critical Medicine, Department of Medicine, Mayo Clinic, Rochester, MN, United States, [2] The Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, United States, [3] Department of Immunology, Mayo Clinic, Rochester, MN, United States

Following respiratory viral infections or local immunizations, lung resident-memory T cells (T_{RM}) of the CD8 lineage provide protection against the same pathogen or related pathogens with cross-reactive T cell epitopes. Yet, it is now clear that, if homeostatic controls are lost following viral pneumonia, CD8 T_{RM} cells can mediate pulmonary pathology. We recently showed that the aging process can result in loss of homeostatic controls on CD8 T_{RM} cells in the respiratory tract. This may be germane to treatment modalities in both influenza and coronavirus disease 2019 (COVID-19) patients, particularly, the portion that present with symptoms linked to long-lasting lung dysfunction. Here, we review the developmental cues and functionalities of CD8 T_{RM} cells in viral pneumonia models with a particular focus on their capacity to mediate heterogeneous responses of immunity and pathology depending on immune status.

Keywords: viral pneumonia, influenza, resident memory, pathology, homeostasis, age

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***Correspondence:**
Nick P. Goplen
goplen.nicholas@mayo.edu
Jie Sun
sun.jie@mayo.edu

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INTRODUCTION

"Infectious diseases are no respecters of wealth, power, or personal merit. Pandemic infectious disease is one situation where we cannot accept Margaret Thatcher's view [there is no such thing as society]. With a fast spreading respiratory virus, for example, everyone is ultimately in the same boat" (Peter C. Doherty concluding remarks in *Pandemics*, 2013). Respiratory viruses that infect the lower airways such as influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) can cause severe acute lung injury (ALI) and are serious public health challenges. A year after the initial outbreak, SARS-CoV2 infection has resulted in more than 95 million cases and 2 million deaths globally (https://coronavirus.jhu.edu). Conventional T cells, particularly CD8 cytotoxic T cells, play important roles in the control of respiratory viral infection (1, 2). Additionally, CD8 T cells can form a long-lived immunological memory that protects from reinfection of the same or related viruses (3). Among the different subsets of memory CD8 T cells, tissue-resident memory T cells (T_{RM}) that reside within the respiratory tract provide superior immunity against viral re-infections (4). Therefore, vaccines that can elicit robust CD8 T_{RM} cells are highly promising for the prevention/amelioration of future pandemics. Conversely, recent studies have suggested that exaggerated CD8 T_{RM} cell presence and/or uncontrolled CD8 T_{RM} cell function could lead to chronic pathogenic sequelae in the lungs (5, 6). Here, we will review recent literature on pulmonary CD8 T_{RM} cell development and maintenance and discuss their roles in immune protection as opposed to how they may provoke pulmonary pathologies when not tightly regulated. We primarily use influenza virus infection studies as the model for this review.

Pulmonary Memories Fade Away

Pulmonary CD8 T_{RM} cells poised for rapid responsiveness, contribute substantially to immune protection of the host against previously encountered viral pathogens (4). As in other organs, pulmonary T_{RM} cell function appears to be dependent on *in situ* proliferation and the production of IFN-γ which activates the vasculature enabling recruitment of innate and adaptive responses (4, 7–10). Compared to T effector (T_{EM}), T central (T_{CM}), and T peripheral (T_{PM}) memory cells that collectively circulate through blood, lymph, peripheral and secondary lymphoid organs, T_{RM} cells are transcriptionally and functionally distinct (11–16). The lung is one of few sites where CD8 T_{RM} cells are relatively short-lived and not permanently lodged in tissues compared to the limited number of organs investigated (17–19). Their loss over time has been attributed to migration from the parenchyma to the airways where they encounter a hostile environment eventually leading to their apoptosis (19). Additionally, pulmonary T_{RM} cells can re-enter the circulation and migrate to the draining lymph nodes where they re-establish residency, contributing to their loss from lung tissue (18). Of note, lung T_{RM} cell loss can be mitigated by local prime-boost strategies and/or repeated antigen exposure (20). Given the potential for their short life-span and their importance in clearing subsequent respiratory viral infections, it is critical to understand the environmental and immune-status cues that regulate T_{RM} cell differentiation, maintenance, and function in the lung in order to exploit their benefits through immunotherapies such as vaccines.

Pulmonary T_{RM} Cells—the Human Experience

Counterparts to T_{RM} cells discovered in mice exist in all organs investigated in humans (11, 21). The lung faces constant microbial exposure, yet histology snapshots suggest the distal airways are remarkably sterile environments in the absence of acute infection. Accordingly, *in situ* estimates suggest human lung explants contain as many as 10 billion memory T cells (22). There is a diverse antigen-specific CD4 and CD8 T cell presence in most lungs including up to 10% of T cells that respond to influenza virus challenge with proliferation (22). Like CD8 T cells, CD4 T cells in the human lung appear transcriptionally primed for response (23, 24). While the resident CD4:CD8 memory T cell ratios vary by compartment (airway vs. parenchyma), 20–50% of pulmonary CD8 T cells expected to be critical for anti-viral memory responses, display a recently activated phenotype indicated by HLA-DR antigen on their surface (22, 25, 26), suggesting active vigilance.

Tracking of donor lung T cells following pulmonary transplantation, indicates T_{RM} cells are found sparsely in the blood at any given time, similar to what is observed in mouse studies (6, 26, 27). Further, donor and recipient airway T_{RM} cell transcriptional profiles overlap indicating a shared signature imparted by the lung microenvironment despite disparate HLA matches (26). As in mouse studies, a substantial fraction of human lung CD8 T_{RM} cells express multiple inhibitory receptors, suggesting a strong stimulus may be needed for their re-activation (24). Relative to peripheral blood memory T cells, human $CD69^+$ pulmonary CD8 T_{RM} cells almost universally express CD29, CD49a, CXCR6, and PSGL-1 with heterogenous expression of CD103 and CD101. Despite this heterogeneity, strong stimulation through the T Cell Receptor (TCR) results in proliferation of the majority of human T_{RM} cells with their progeny exhibiting enhanced polyfunctional capacity relative to their parents (28). This suggests T_{RM} cells act as sentinels in human lung mucosa and are important for maintaining sterility of alveolar spaces.

What Makes a Pulmonary T_{RM} a Pulmonary T_{RM}?

Recent barcode lineage-tracing and single-cell transcriptome analyses found that a subset of T cell clones possesses a heightened capacity to form T_{RM} cells, as enriched expression of T_{RM}-fate-associated genes is already apparent in circulating effector T cell clones (13). Consistently, following initial trafficking to the lung, T_{RM}-like phenotypes are observed as early as 2 weeks following influenza infection and these phenotypes, but not numbers, are stable in the airways, lung parenchyma, and trachea for up to 3 months (17, 29). Pulmonary T_{RM} cells have been defined inconsistently throughout the literature, as warranting caution when comparing studies.

While pulmonary CD8 T_{RM} cell definition(s), differentiation, maintenance, and functions have largely been established from monoclonal T cell receptor (TCR) transgenic models, polyclonal experiments give a more heterogeneous and physiological relevant picture of T_{RM} cells coexisting within the same tissue, but have not been widely reviewed. Markers (e.g., CD69, CD103, CD49a, CXCR6, and PD-1) typically used to identify pulmonary CD8 T_{RM} cells in mice are heterogeneously co-expressed within T_{RM} populations (5, 6, 27, 29–32). For example, E-cadherin in the lung is expressed in the cell-cell junctions between bronchiole epithelium (33). Although E-cadherin-binding CD103 is intrinsically important for cytotoxic capacity (34) and is expressed on nearly 100% of T_{RM} in the skin, CD103 is heterogeneously expressed in lung T_{RM} cells, inhibits T_{RM} cell motility, and is not required for heterosubtypic protection against influenza. Conversely, although the collagen IV-binding integrin CD49a is a less common marker used for the identification than CD103, it is required for the heterosubtypic immunity against influenza infection (28, 29).

Furthermore, CD103 is expressed at a substantially lower frequency on the T_{RM} cells that form the bulk of the protective response vs. influenza nucleoprotein (D^b-$NP_{366-374}$) in C57BL/6 mice compared to another immune-dominant epitope from viral polymerase peptide (D^b-$PA_{224-233}$) (5). Nonetheless, parabiosis studies indicate both of these phenotypically different populations exhibit similar degrees of tissue residency 2 months following infection (6). Though the significance is unclear, this immunodominant population (responding to D^b-$NP_{366-374}$) in a secondary response that mostly lacks CD103 expression, abundantly expresses classic exhaustion markers (PD-1, TIM-3, LAG-3, and TIGIT) relative to D^b-$PA_{224-233}$ and K^b-$OVA_{SIINFEKL}$ –specific T_{RM} and memory CD8 T cells in the circulation (5, 6). These insights from various studies highlight

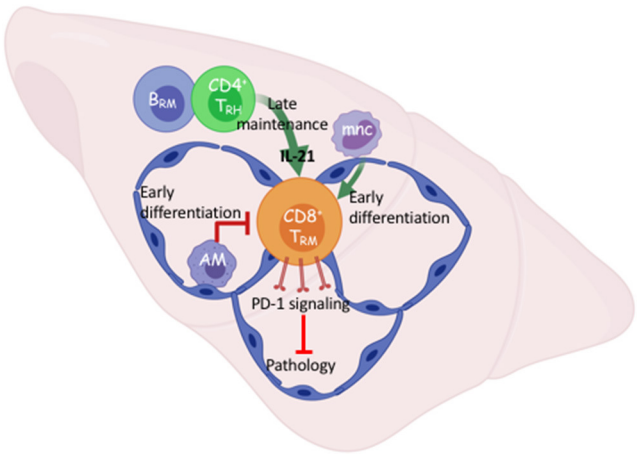

FIGURE 1 | Early and late cellular networks involved in Trm cell differentiation and maintenance. After viral respiratory pneumonia, early pulmonary CD8 T_{RM} cell differentiation is driven by re-encounter with antigen presented *via* interstitial classic monocytes (mnc) and opposed by alveolar macrophages that maintain lung homeostasis. B cell-dependent tissue-resident CD4 helper T cells (T_{RH}) support T_{RM} cell maintenance through IL-21 dependent survival. T_{RM} intrinsic PD-1 signaling prevents pathology in the absence of infectious virus. Created with BioRender.

the marked epitope-specific CD8 T_{RM} cell heterogeneity within the pool of polyclonal T_{RM} cells directed against the same pathogen. Indeed, data from organ donors indicates a diverse TCR repertoire against influenza virus, suggesting that heterogeneity is quintessential in the local pulmonary response (28).

Cellular and Molecular Networks Involved in the Control of Pulmonary CD8 T_{RM} Cell Density

It is becoming clearer that local immune interactions influence CD8 T_{RM} cell numbers without affecting the circulating memory pool. Alveolar macrophages (AMs) are a self-renewing population of airway-resident cells seeded early in embryonic development (35). AMs maintain lung homeostasis and respond to inflammatory cues. Absence or dysfunction of AMs in severe influenza infection leads to exacerbated pulmonary pathology and enhanced mortality (36, 37). In studies where we were investigating the effects of PPAR-γ in the macrophage compartment on influenza severity, intrinsic absence increased the density of pulmonary T_{RM} cells and long-term stromal disrepair indicated by persistent inflammation and collagen deposition (38, 39). We subsequently found that depletion of AMs prior to influenza infection, but not during the CD8 T cell contraction phase, enhanced T_{RM} cell density without affecting the circulatory memory compartment (**Figure 1**) (38). This suggests AMs have an early influence on the lung microenvironment that governs *in situ* T_{RM} cell differentiation. It is not currently clear what subtype of $CD169^+$ AMs are responsible for limiting the T_{RM} cell compartment nor by what means. Conversely, bone-marrow derived monocytes trafficking to the site of infection enhance the early antigen-presentation required for T_{RM} cell differentiation in the lung (40). Yet, inflammatory macrophages in the gut mediate heterogeneous T_{RM} cell differentiation by contributing to the pro-inflammatory milieu (41).

In contrast to the limiting of the T_{RM} cell compartment by innate resident macrophages, we and others have recently shown that a population of CD4 tissue-resident helper T (T_{RH}) cells aid the persistence of pulmonary CD8 T_{RM} cells following influenza infection (42, 43). This novel population of T_{RH} cells simultaneously exhibits T follicular helper (T_{FH})-like properties that enhance the local B cell response and tissue-resident memory T cell features. CD4 T_{RH} cells are the major cellular sources of IL-21 in the tissue, and blockade of IL-21 signaling at the memory stage diminished CD8 T_{RM} cell survival specifically in the D^b-$NP_{366-374}$ population.

While the influenza response in the lung is not an active chronic infection, viral RNA remnants may cause persistent pathology (44). In persistent viral infection in the brain, provision of IL-21 by T follicular-like tissue-resident CD4 T cells likely promotes ATP production in local CD8 T cells through enhancing electron transport chain efficiency (45). Our data suggests this could be a means by which local CD8 T cells differentiate and persist in response to IL-21. Nonetheless, a local interaction between CD8 and CD4 T cells is required for optimal T_{RM} cell responses following both acute and persistent viral infections (**Figure 1**). Importantly, this cellular network was responsible for local secondary protection against heterologous infection mediated by the influenza-specific CD8 T_{RM} cells. Interestingly, T_{RH} cell development requires the presence of B cells (43); thus there exists a local interplay among adaptive immune cells for the maintenance of pulmonary lymphocyte memory following viral pneumonia. Understanding how the local cellular networks modulate immune protection may aid the development of mucosal vaccines. Additionally, understanding the molecular cues governing their persistence will likely be important to elicit proper T_{RM} cell responses through immunotherapies.

Unlike the majority of inflamed organs investigated, where it merely enhances T_{RM} cell differentiation, local antigen signals are required for the establishment of pulmonary CD8 T_{RM} cell (17, 46). As briefly mentioned above, T_{RM} cells with TCRs of different specificities against influenza epitopes, exhibit different phenotypes and have distinct requirements for their maintenance (5). At the transcriptional level, polyclonal CD8 T_{RM} cells also vary in their programs between T_{RM} cells of different specificities (5, 6). The TCR is likely playing an active role in these differences. Just as the quality of TCR signals can determine CD8 T cell fate in the circulation, lower affinity TCR signals enhance the potential to differentiate into pulmonary T_{RM} cells (47–49).

Furthermore, the duration and amount of antigenic signals seem important for establishing the diversity of the T_{RM} cell pool against a given respiratory pathogen. For instance, the differential persistence of influenza NP vs. PA antigen at the memory phase clearly dictates the distinct phenotypes of the T_{RM} cells against the two antigens (5). Influenza virion contains many more NP molecules than PA molecules and NP proteins

and/or $NP_{366-374}$ peptide-MHC-I complex are present for a longer period and potentially in a much higher amount than PA proteins or PA peptide-MHC-I complex at the memory phase (50). In agreement, influenza NP-specific (D^b-$NP_{366-374}$), but not PA-specific (D^b-$PA_{224-233}$), T_{RM} cells receive chronic TCR signaling at the memory phase, leading to the development of an "exhausted-like" phenotype (characterized by the high expression of co-inhibitory molecules including PD-1 and Tim-3) in D^b-$NP_{366-374}$ T_{RM} cells (5). Interestingly, like the persistence of true exhausted CD8 T cells during chronic viral infection, the persistence of "exhausted-like" D^b-$NP_{366-374}$ T_{RM} cells is also dependent on the continuous presence of pMHC-I and co-stimulatory signaling as the induced depletion of MHC-I or the late blockade of CD28 diminished D^b-$NP_{366-374}$ T_{RM} cell magnitude (5). How these antigenic signals in the lung work in concert with the main cytokine (TGF-β) responsible for T_{RM} cell differentiation across a breadth of tissues is unclear.

TGF-β is an integrin-activated cytokine with widely varying effects on white blood cells from the hematopoietic stem cell (HSC) stage through to terminal differentiation (51). TGF-β mediates the fine line between immune-tolerance and appropriate activation of both the innate and adaptive immune systems (52–58). As with most of its cell-type dependent functions, effects of TGF-β on CD8 T cells can be stimulatory or inhibitory, depending on the state of differentiation (57, 59). TGF-β can raise the threshold of TCR-induced activation on naïve CD8 T cells, whereas it can induce either T_{CM}-like or T_{RM}-like differentiation in recently activated CD8 T cells (57, 60–62). TGF-β mediates T_{RM} cell differentiation by imparting a partially shared transcriptional footprint across a breadth of organs, however, it is the tissues themselves that govern the uniqueness of the footprint such as what metabolites T_{RM} cells use to persist (61, 63, 64). Similar to most peripheral sites, TGF-β is essential for differentiation of pulmonary T_{RM} cells of numerous antigen specificities (5, 41, 65). Interestingly, low affinity TCR-pMHC interactions leave CD8 T cells more susceptible to TGF-βR signaling which could explain their proclivity toward T_{RM} cell differentiation (47, 49). For respiratory viral infections, the effects of TGF-β signaling on T_{RM} cell generation is Smad4-independent, which may suggest non-canonical TGF-β R signaling pathways are vital for pulmonary T_{RM} cell differentiation (65, 66). Thus, it is likely the context and tissue dependent circumstances of T cell activation may govern how TGF-β contributes to T_{RM} cell heterogeneity.

Pulmonary T_{RM} Cells Balance Immune Protection and Local Pathology

As mentioned previously, a subset of influenza-specific T_{RM} cells display an exhausted-like phenotype including high expression of PD-1. When PD-L1-PD-1 signaling in influenza infected mice is blocked at the memory stage, the magnitude of the D^b-$NP_{366-374}$, but not D^b-$PA_{224-233}$, T_{RM} cell responses was augmented (5). Furthermore, late PD-L1 blockade increases effector cytokine, particularly TNF, production by D^b-$NP_{366-374}$ T_{RM} cells, indicating targeting the checkpoint molecule PD-1 "rejuvenates" the exhausted-like T_{RM} cells following influenza infection. Consequently, T_{RM} cell-mediated protective immunity was enhanced upon secondary heterologous viral challenge (5). Unexpectedly, pulmonary inflammation and fibrosis were drastically exacerbated following PD-L1 blockade in a CD8 T cell-dependent manner. It is possible that enhanced production of effector molecules from an increased number of CD8 T_{RM}, mediates diffuse alveolar damage in the absence of molecular regulation such as PD-1 signaling (67–69) (**Figure 1**). Failure to acutely repair this CD8-dependent airway damage, could result in exacerbated collagen deposition or impaired degradation suggesting macrophage and/or fibroblast involvement (5, 6). These results suggest that there is a fine balance on T_{RM} cell-mediated protective immunity and lung pathology following viral pneumonia. These data also indicate that the gradual T_{RM} cell loss in the respiratory tract is perhaps a host-protective mechanism to avoid potential collateral damage to a vital organ. There are also examples of CD8 T_{RM} cells causing pathology in the skin and intestine when homeostatic controls are lost and diseases like vitiligo, psoriasis, or celiac may emerge following destruction of melanocytes, epidermal or mucosal barrier tissues, respectively (70–73). Collectively, these data indicate that one's immune-status is an important regulator of the potential harm to local tissue brought on by unruly T_{RM} cell activation.

Altered Immune Homeostasis in Advanced Age

Many hurdles exist with regards to provoking efficacious adaptive immune responses in those of advanced age (>70 years)—the demographic that may benefit most from vaccines for emerging pathogens. To understand how immune responses in aged and young hosts proceed differently, we need to understand how the innate and adaptive systems differ globally during the natural aging process. Low-grade systemic inflammation under homeostatic conditions is a hallmark signature of aging, but to what degree it impairs protective immune responses is unclear. This so-called "inflamm-aging" may in-part, be mediated by enhanced myelopoiesis during aging, another hallmark of aging (74). Interestingly plasma cell accumulation in the bone marrow has been shown to drive the myeloid bias with age. Plasma cells remodel bone marrow stroma that govern hematopoiesis, *via* provision of tumor necrosis factor (TNF), a principle "inflamm-aging" cytokine (75). The skewing of hematopoietic output leads to an age-related decline of naive lymphocytes in the circulation (74–76). Aside from decreased B cell numbers, there is a wide range of age-related functional changes in peripheral B cells that could affect antibody responses to vaccines in the elderly (77–79). Bone marrow is not the only primary lymphoid tissue that suffers age-related output predicaments that might influence vaccine efficacy in the elderly.

Thymic involution starts in the earliest years of life and drops output of naive T cells ∼10-fold past the age of 40 (80). This impacts the circulatory T cell compartment as there are fewer recent thymic emigrants seeding secondary lymphoid tissue. For unknown reasons, this affects the diversity of the naïve CD8 compartment more than the CD4 T cell compartment (81). Thus, with age, CD8 memory T cells are enriched and TCR repertoires

are likely narrowed across tissues (80–85). Notably, if memory CD8 T cells are formed early in life, they likely provide life-long diverse secondary responses (86, 87).

However, the ability to generate new memory is dependent on naive CD8 T cells, which in our later years (mouse and human), skew to a more differentiated state with the majority exhibiting immuno-senescence, characterized by high signaling thresholds for activation and proliferation (88–91). Moreover, once lymphocytes exit their developmental sites and emigrate to secondary lymph tissue, they encounter age-related stromal deterioration influencing their organization within lymph nodes (92). The above confounders likely affect naïve lymphocyte generation, maintenance, activation and in sum, negatively impact formation of protective immunity toward pathogens and vaccines (85, 93).

The Aged Environment Provokes Malfunctional CD8 T_{RM} Cell Accumulation

One of the first clinical observations in the current pandemic was that mortality and severe morbidity in COVID-19 disproportionately affects those of advanced age (94). This is also true of most severe influenza seasons (95). Severe influenza-like illness are associated with delayed, but prolonged innate and adaptive responses during the effector phase (96). We have recently examined pulmonary CD8 T_{RM} cell responses in young (2 months) and aged (20–22 months) C57BL/6 mice following influenza infection. Aging is associated with the decreased potential of circulating memory T cell generation (97). In sharp contrast, lungs from aged mice have 40-fold more CD8 T_{RM} cells compared to those of young lungs (6). Transfer of CD8 T cells from young mice into the aged hosts results in increased accumulation of memory T cells derived from young mice in the aged lungs following influenza infection. This indicates that the aged environment provokes exaggerated accumulation of T_{RM} cells (6). We found higher levels of *Tgfb*1 transcript in the aged lungs and the accumulation of T_{RM} cells in aged hosts was largely TGF-β dependent (**Figure 2**). Relatedly, Chikungunya virus infection in aged mice leads to heightened and dysregulated TGF-β production that exacerbates pathology (98).

Of note, alveolar macrophage numbers and function dwindle with age (99). Given the suppressive roles of alveolar macrophages in T_{RM} cell generation (38), it could be possible that diminished alveolar macrophage function may aid the exaggerated development of T_{RM} cells during aging. Notably, many factors change in the aged lung that have not been investigated in the context of T_{RM} accumulation. DAVID analysis of the aged lung transcriptome indicates decreased cell cycle with increased extracellular matrix and cell adhesion gene programs (100). Human Lung Cell Atlas (HLCA) data indicates these changes are accompanied by increases in fibroblasts and neuroendocrine populations and a drop in Type II pneumocytes (100, 101). Additionally, the stroma may be more apt to prompt inflammation in lungs of aged individuals (102). Nevertheless, the data indicate that the aged environment enhances T_{RM} cell accumulation after a single *de novo* response, suggesting that the aged lung is fertile ground for T_{RM} cell differentiation. In contrast, there is a reduced generation of lung T_{RM} cells following influenza infection in infant mice, largely due to T cell-intrinsic defects (103).

Our data suggest that memory T cells can robustly accumulate in mucosal tissue during aging following a single round of viral challenge. Yet, aged individuals still have impaired protective responses following vaccines or respiratory viral infections which has been attributed to memory CD8 T cell function (104). To resolve the discrepancy, we performed single cell (sc) RNA-seq on young or aged T_{RM} cells against the major influenza protective epitope D^b-$NP_{366-374}$. Our results found that T_{RM} cells isolated from aged lungs lack a subpopulation characterized by high expression of molecules involved in TCR signaling and effector function (6). Consequently, we found that aged mice exhibit impaired T_{RM} cell-mediated protective immunity against heterologous viral rechallenge compared to those of young mice. Thus, aging facilitates the accumulation of dysfunctional T_{RM} cells in the respiratory tract, which explains the phenomena that aged individuals have increased susceptibility of influenza-associated severe diseases despite the robust presence of influenza-specific T_{RM} cells in the respiratory tract. Given the current spread of SARS-CoV2 infection among the elderly population, it would be important to determine whether SARS-CoV2-specific T_{RM} cells exhibit similar functional impairment during aging as the T_{RM} cell-mediated protection would be a key determinant of respiratory immunity during secondary exposure to the virus.

If these newly formed T_{RM} cells are not providing protection, what is their role in the tissue during aging? To address the question, we depleted either circulating, or circulating plus resident CD8 T cells and examined the long-term effects on organ-level transcription and histopathology (6). Depletion of the resident CD8 T cells that were not providing protection against subsequent influenza infection, led to resolution of pulmonary inflammation in aged hosts while concomitantly decreasing the inflammatory environment at the transcriptional level, particularly, chemokines involved in recruiting monocytes and neutrophils (**Figure 2**) (6). Further, long-term age-related infection-induced exacerbation of collagen deposition was mitigated in the absence of parenchymal CD8 T cells (**Figure 2**). Establishment of pulmonary T_{RM} in IAV infection models depends on local presentation of antigen, likely *via* monocyte-derived macrophages and/or dendritic cells, which we find sustained in the aged lung parenchyma (40, 46, 105). Infiltrating monocyte-derived macrophages have been shown to exacerbate collagen-deposition following influenza infection (106). Collectively, this could indicate the aged environment provokes accumulation of pulmonary T_{RM} cells that support ongoing inflammation of the organ contributing to its poor repair following respiratory viral pneumonia.

As discussed above, SARS-CoV2 infection disproportionately affects aged individuals. Of particular relevance is the observation of severe COVID-19 patients presenting both with CD8 T cell lymphopenia in the blood, but large number of T_{RM}-like CD8 T cells in the airways (107). Notably, emerging evidence

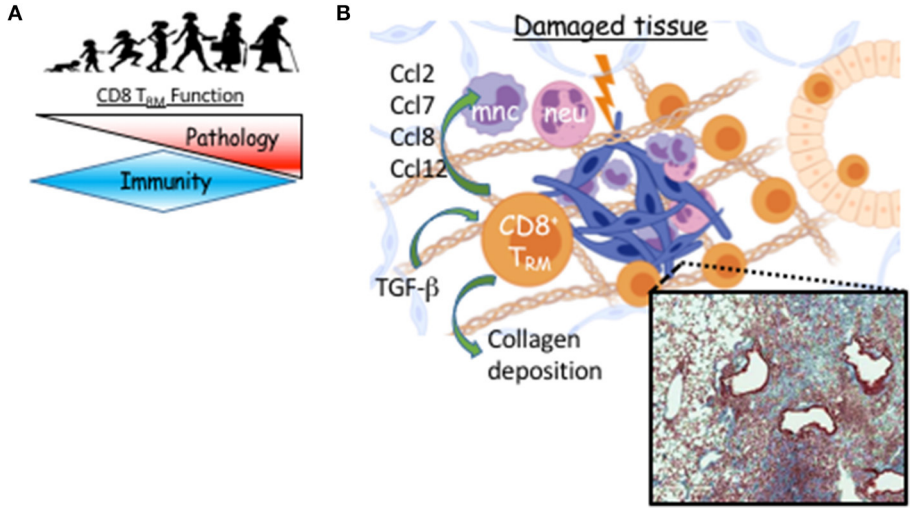

FIGURE 2 | T_{RM} cell-mediated long-term sequelae post viral pneumonia during aging. **(A)** T_{RM} function switches from immune protection to pathology as we age. **(B)** Following viral pneumonia, CD8 T_{RM} cells accumulate in aged lungs where their differentiation is TGF-b–dependent. Instead of providing increased immune protection, they provoke pathology, likely through direct or indirect recruitment of myeloid cells that contribute to unresolved inflammation and prevention of collagen degradation. Micrograph is Masson's trichrome stained lung from aged mouse 60 days post-H1N1 infection. Blue is digitally enhanced collagen deposition which is dependent on CD8 T_{RM} cells (6). Created with BioRender.

has suggested that a large proportion of COVID-19 patients exhibit pulmonary and extrapulmonary symptoms 6 months after recovery from the acute morbidity (108). Particularly, it is predicted that a large number of severe COVID-19 patients will develop persistent lung damage and fibrosis as observed in patients infected with SARS-CoV and MERS (109–113). Notably, TGF-β activating integrin is upregulated in fibrotic lung lesions in COVID-19 patients 2 months post-infection, which could support fibrosis and T_{RM} cell maintenance (114). It would be critically significant to examine whether malfunctional CD8 T_{RM} cells contribute to the long-term fibrotic sequelae of SARS-CoV2 infection.

While viral-specific pathogenic CD8 T cells have not been found in human tissue to-date, plausible candidates may now be on the radar. Age-associated granzyme K-expressing CD8 T cells are enriched in the T effector memory compartment in human blood (81). Age-associated CD8 T cell counterparts in mice were identified by expression of the effector molecule granzyme K, the checkpoint molecule PD-1, integrin CD49d, and the transcription factor TOX and are enriched in blood and across tissues (spleen, peritoneum, lungs, liver, and white adipose tissue) with age. The aged environment conferred this phenotype to young CD8 T cells in adoptive transfer models. While the TCR repertoires of age-associated CD8 T cells were clonally narrowed within each host across tissues, between hosts, their TCR sequences were diverse, suggesting either microbial-specific or stochastic differentiation. It is important to note that these age-associated CD8 T cells are transcriptionally distinct from senescent virtual memory CD8 T cells also enriched with age (88). It's unclear how granzyme K^+ age-associated CD8 T cells behave in an immune response. While their phenotype (PD-1^{Hi} TOX^+) is typically associated with CD8 T cell exhaustion, recombinant granzyme K augmented cytokine and chemokine production from senescent fibroblasts *in vitro* (81). Activation of local age-associated CD8 T cells may thus provoke inflammation and potentially influence tissue remodeling and senescence associated secretion phenotypes.

Age-Related Pulmonary Fibrosis

Examples of age-related increases in lung tissue disrepair abound and are found commonly in idiopathic pulmonary fibrosis (IPF) (115, 116). IPF is an interstitial pneumonic disease that results in alveoli involved in gas exchange being progressively replaced by scar tissue with a 20% 5 year survivability (117). No treatment can reverse the process once started. As its namesake would suggest, IPF has no known single cause and it is unclear how the tissue becomes damaged and fails to repair. It is notable that IPF shares some features of viral pneumonia sequelae including COVID-19, most prominent of which is collagen accumulation which can lead to fibrosis (118). We described an increased number of CD8 T cells in the parenchyma surrounding lesions in IPF patients (5). It is plausible that these patients lost the battle for homeostatic control of local memory T cells that can mediate bystander inflammation. Of note, respiratory T cells have a role in dysfunctional wound repair resulting in fibrosis in acute lung injury models (119). Further, one of the frontline treatments (Nintedanib) that slows development of IPF by presumably targeting the kinase activities of PDGF, FGF, and VEGF receptors, inhibits src family tyrosine kinases, including the crucial T cell activating kinase Lck, with similar IC_{50} values (120, 121). This could implicate dampened T cell activity as a partial mechanism slowing fibrotic progression in the lung. Thus, while lung damage and repair models can happen in lymphocyte-scarce environments,

certain T cell subsets exacerbate fibrosis and the jury may need to be recalled as to whether local T cells play a role in IPF pathogenesis and potentially viral pneumonic sequelae in humans.

CONCLUSIONS

Although pulmonary resident memory CD8 T cells have shown outstanding immune-protective capacity, this does not seem to be the case in aged hosts following respiratory viral infections. In contrast, resident CD8 T cells mediate pathology during the disease course leading to non-resolution of lung inflammation in aged hosts. Unexpectedly, aged hosts accumulate local T_{RM} cells despite a poor response in the circulation (6). This suggests efforts should be retooled to restore their protective immunity (122) and mitigate their pathogenic capacity rather than recruit more to the mucosa. These opposing features of T_{RM} cells in young and aged hosts may identify a balance between immune protection and pathology and shed light on their teleological existence in a vital organ. While recent work has highlighted the cellular and molecular networks that mediate pulmonary T_{RM} density in young healthy hosts, we are just beginning to understand the potential they have to mediate damage when homeostatic controls are lost, e.g. through the aging process. Understanding the mechanisms modulating the balance of T_{RM} cell-mediated immunity vs. pathogenicity will be important to selectively harness the beneficial function of T_{RM} cells and simultaneously mitigate their pathogenic potential.

AUTHOR CONTRIBUTIONS

NG and JS wrote and IC was responsible for editing the manuscript. All authors contributed to the article and approved the submitted version.

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The Role of CD4$^+$ Resident Memory T Cells in Local Immunity in the Mucosal Tissue – Protection Versus Pathology –

Kiyoshi Hirahara[1,2*], *Kota Kokubo*[1], *Ami Aoki*[1], *Masahiro Kiuchi*[1] *and Toshinori Nakayama*[1,3*]

[1] Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan, [2] AMED-PRIME, Japan Agency for Medical Research and Development, Chiba, Japan, [3] AMED-CREST, Japan Agency for Medical Research and Development, Chiba, Japan

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***Correspondence:**
Kiyoshi Hirahara
hiraharak@chiba-u.jp
Toshinori Nakayama
tnakayama@faculty.chiba-u.jp

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Memory T cells are crucial for both local and systemic protection against pathogens over a long period of time. Three major subsets of memory T cells; effector memory T (T_{EM}) cells, central memory T (T_{CM}) cells, and tissue-resident memory T (T_{RM}) cells have been identified. The most recently identified subset, T_{RM} cells, is characterized by the expression of the C-type lectin CD69 and/or the integrin CD103. T_{RM} cells persist locally at sites of mucosal tissue, such as the lung, where they provide frontline defense against various pathogens. Importantly, however, T_{RM} cells are also involved in shaping the pathology of inflammatory diseases. A number of pioneering studies revealed important roles of CD8$^+$ T_{RM} cells, particularly those in the local control of viral infection. However, the protective function and pathogenic role of CD4$^+$ T_{RM} cells that reside within the mucosal tissue remain largely unknown. In this review, we discuss the ambivalent feature of CD4$^+$ T_{RM} cells in the protective and pathological immune responses. We also review the transcriptional and epigenetic characteristics of CD4$^+$ T_{RM} cells in the lung that have been elucidated by recent technical approaches. A better understanding of the function of CD4$^+$ T_{RM} cells is crucial for the development of both effective vaccination against pathogens and new therapeutic strategies for intractable inflammatory diseases, such as inflammatory bowel diseases and chronic allergic diseases.

Keywords: CD4+ resident memory T cells, Aspergillus fumigatus, lung fibrosis, ATAC-seq, inducible bronchus-associated lymphoid tissue (iBALT), pathogenic T cell

WHAT ARE TISSUE-RESIDENT MEMORY T CELLS?

"Immune memory" is a central and characteristic phenomenon of the acquired immune system. The long-term survival of the antigen-specific memory T cell population in response to invading harmful microorganisms is essential for the establishment of immune memory *in vivo*. Memory T cells can respond directly and rapidly to re-invading harmful microorganisms and efficiently eliminate them to protect the host.

Memory T cells were originally classified into two subpopulations, effector memory T (T_{EM}) cells and central memory T (T_{CM}) cells, based on (1) the expression pattern of cell surface molecules, (2) the orientation to specific tissues and (3) responsiveness to re-stimulation with a certain antigen (1). T_{EM} cells show the low expression of CCR7, a chemokine receptor that is crucial for homing to the secondary lymphoid organ and the low expression of the cell surface molecule CD62L. T_{EM} cells are mainly found in the non-lymphoid tissues and are responsible for peripheral immune surveillance and the immediate protective function in the host. T_{EM} cells respond quickly to re-stimulation of antigens and produce large amounts of proinflammatory cytokines, including IFN-γ, IL-5 and IL-4, but they showed shortened telomeres (2). In contrast, T_{CM} cells highly express both CCR7 and CD62L and migrate to sites with secondary lymphoid tissues, such as lymph nodes; T_{CM} cells primarily produce IL-2 upon antigen restimulation. After proliferation, T_{CM} cells efficiently produce large amounts of proinflammatory cytokines, such as IFN-γ and IL-4 (3, 4). Memory T cells are subdivided by various cell-surface markers, including CD27, CD127, CD43, CXCR3 and CX3CR1 (5–8). A study using CX3CR1-reporter mice reveals that CX3CR1^{hi} CD8$^+$ T_{EM} cells were largely excluded from peripheral tissues after viral infection, providing novel insight concerning CD8$^+$ T_{EM} cells (9).

Recently, non-circulating memory T cells have been identified, which are now referred to as tissue resident memory T (T_{RM}) cells (10). T_{RM} cells show the high expression of C-type lectin-like molecule CD69 and integrin E subunit molecule CD103. T_{RM} cells produce various kind of cytokines, including IL-2, IFN-γ, TNF-α, and IL-17 (11–16). Unlike T_{CM} cells and T_{EM} cells, which circulate throughout the body *via* blood vessels and lymphatic vessels, T_{RM} cells do not circulate throughout the body, but they reside in non-lymphoid tissues such as the lung, skin, and gut. However, a series of recent studies clearly show that re-activated CD8$^+$ T_{RM} cells rejoin the circulating pool and proliferate in draining lymph nodes (**Figure 1**) (17, 18). Regarding CD4$^+$ T_{RM} cells, CD4$^+$ T_{RM} cells account for 30% of the lymph node-CD4$^+$ T cell population, which is a larger proportion than that of CD8$^+$ T cells (19). However, the plasticity of subpopulations of memory CD4$^+$ T cell has remained unclear. Regardless, the functions of memory T cells are closely linked to their mobility in the body of the host.

In mucosal tissues, such as the skin and female reproductive tract, antigen-recognized CD8$^+$ T_{RM} cells produce IFN-γ and TNF-α to recruit other immune cells and activate dendritic cells and NK cells (12–14). In non-mucosal tissues, such as the brain and liver, CD8$^+$ T_{RM} cells reside in each organ and play crucial roles in the host defense against pathogens (20, 21). In the brain, IFN-γ and Perforin-producing CD8$^+$ T_{RM} cells act as an autonomous cytotoxic barrier to viral infection (21). In the lymphocytic choriomeningitis virus (LCMV)-infected brain, almost all CD8$^+$ T_{RM} cells express CD69, but these cells show heterogeneous expression patterns of CD103 (21). In the liver, CXCR3$^+$CD8$^+$ T_{RM} cells are essential for protection against liver-stage malaria (20). Human CD69$^+$CD103$^+$CD8$^+$ T_{RM} cells in the liver produce large amounts of IL-2 compared to CD69$^-$CD103$^-$ CD8$^+$ T cells (15).

Regarding CD4$^+$ T cells, recent studies have highlighted prominent populations of CD4$^+$ T_{RM} cells in various mucosal tissues, such as the skin (22–25), female genital tract (19, 22, 26, 27), small intestine (19, 28–30) and lung (16, 19, 22, 30–33). In the skin, CD4$^+$ T_{RM} cells protect hosts against invading pathogens, including *Leishmania major* (23, 24). *Candida albicans* infection also induces IL-17-producing CD4$^+$ T_{RM} cells in the skin (34). In the female genital tract, CD4$^+$ T_{RM} cells are crucial for antiviral defense against genital herpes simplex virus 2 (HSV-2) infection (26, 27). Helminth infection and *Listeria monocytogenes* infection cause the induction of functional CD4$^+$ T_{RM} cells in the intestine (28, 29). In the upper tract, pneumococcus infection induces CD4$^+$ T_{RM} cells that prevent pneumococcal colonization (33). Furthermore, lung CD4$^+$ T_{RM} cells are essential for protection against bacterial infection (16). Thus, similar to CD8$^+$ T_{RM} cells, CD4$^+$ T_{RM} cells may facilitate a rapid immune response to protect the host against re-exposure to pathogens in various mucosal organs.

In human, CCR7^{hi} CD4$^+$ T_{RM} cells are detected in the female genital tract (35). In infants, mucosal memory CD4$^+$ and CD8$^+$ T cells already show characteristics of tissue residency, such as the enhanced expression of CD69 and CD103, which suggests that local *in situ* priming to antigens causes the induction of T_{RM} cells (36). Investigations of human samples from the lung after lung transplantation have revealed that lung-infiltrating recipient CD4$^+$ and CD8$^+$ T cells gradually acquire T_{RM} phenotypes, such as the enhanced expression of CD69 and CD103, over several months *in vivo* (37). In non-mucosal sites, human brain CD4$^+$ T cells show the high expression of CD69 but a low expression of CD103 (38). More detailed information about human T_{RM} cells has been reviewed in other articles (39, 40). The roles of CD4$^+$ T_{RM} cells in the non-mucosal tissue have not been well elucidated.

In addition to the essential role of T_{RM} cells in the biological defense of mucosal and non-mucosal organs, T_{RM} cells and other tissue resident immune cells, including innate lymphoid cells (ILCs), play a critical role in tissue homeostasis (41).

THE MOLECULAR MECHANISMS UNDERLYING THE INDUCTION AND MAINTENANCE OF THE TISSUE RESIDENCY OF T_{RM} CELLS

The mobility of T cells among various organs throughout the body is tightly regulated by various cytokines, chemokines and cell surface molecules (42). Transforming growth factor β (TGF-β) is an essential cytokine for the development of CD8$^+$ T_{RM} cells in the mucosal tissues (43). TGF-β induces the expression of CD103 on CD8$^+$ T cells (44). In the skin, CD8$^+$ T_{RM} cells require transactivated autocrine TGF-β for epidermal persistence (45). An important cytokine for the survival of CD8$^+$ T_{RM} cells in the skin is IL-15 (46). In the skin, hair follicle-derived IL-15 and IL-7

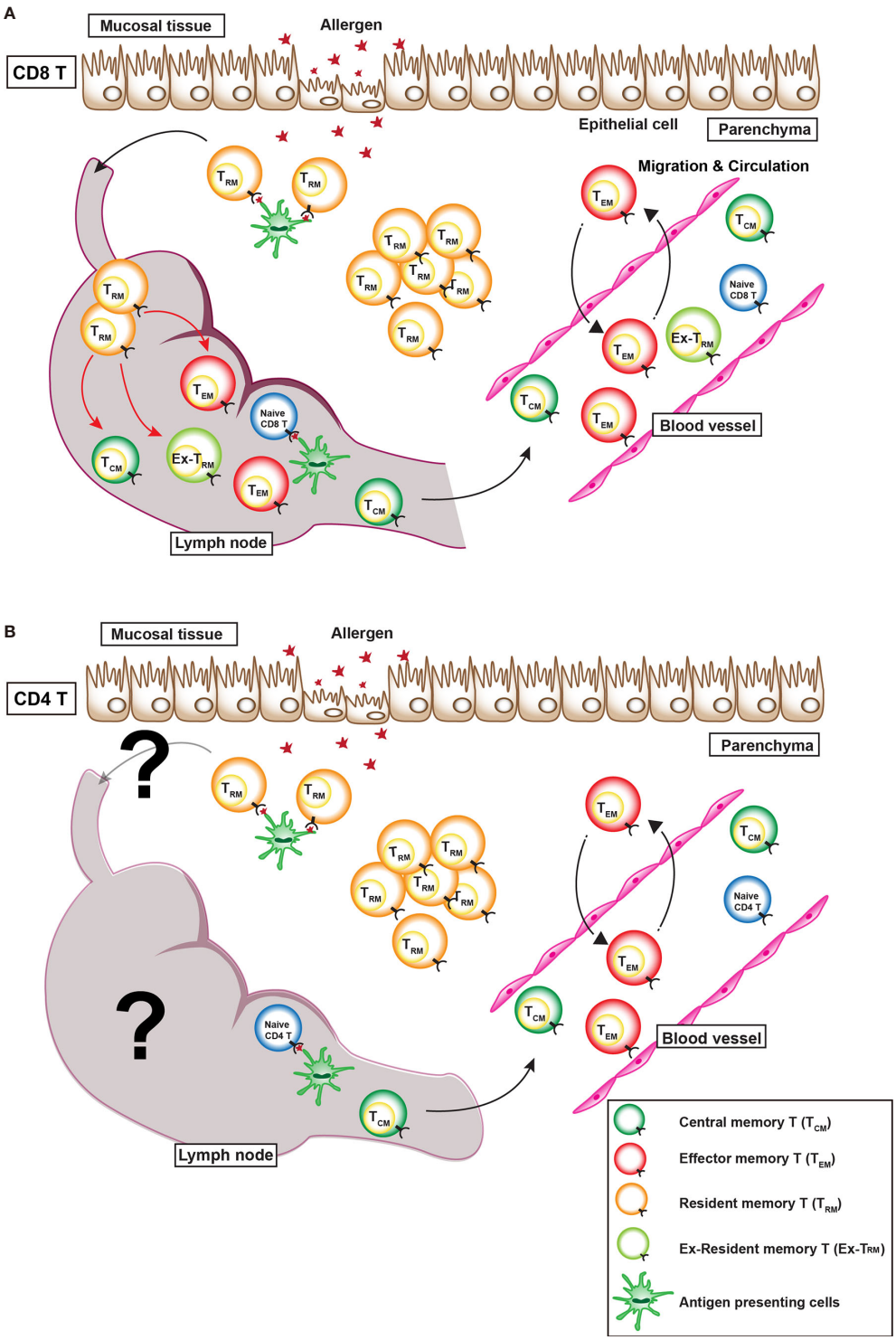

FIGURE 1 | Distribution of various memory T cells *in vivo*. There are three types of memory T cells *in vivo*: (1) central memory T (T_{CM}) cells, which mainly reside in secondary lymphoid tissues, (2) effector memory T (T_{EM}) cells, which circulate in the blood, non-lymphatic tissues, and secondary lymphoid tissues, and (3) resident memory T (T_{RM}) cells, which reside within non-lymphoid tissues. **(A)** A recent study revealed that CX3CR1^{hi} CD8$^+$ T_{EM} cells are largely excluded from peripheral tissues after viral infection (9). In case of CD8$^+$ T_{RM} cells, a series of recent studies clearly showed that re-activated CD8$^+$ T_{RM} cells rejoined the circulating pool and proliferated in draining lymph nodes (red arrows). Some T_{EM} cells move back and forth between the blood vessel and parenchyma. **(B)** However, whether or not CD4$^+$ T_{RM} cells rejoin the circulating pool and a re-activated in the draining lymph nodes is unclear.

are required for the maintenance of $CD8^+$ T_{RM} cells (47). During influenza viral infection, IFN-γ produced by $CD4^+$ T cells induces $CD8^+$ T_{RM} cells, which are crucial for protection against pathogenic viruses (44).

For the long-term survival of $CD4^+$ T_{RM} cells, IL-7 is needed in the skin (47). In the lung, IL-15 is required for the generation of $CD4^+$ T_{RM} cells (48).

Regarding chemokines and cell surface molecules, CD62L and CCR7 must be expressed on T cells to enter the peripheral lymph nodes (1), while Sphingosin-1-Phosphate Receptor 1 (S1P1), which binds the ligand Sphingosin-1-Phosphate (S1P), allows T cells to leave the lymph nodes and enter the lymphatic vessels (49). In humans, both $CD8^+$ and $CD4^+$ T_{RM} cells upregulate the adhesion molecules ITGAE (CD103) and ITGA1 (CD49a) as well as inhibitory molecules, including PD-1 and the dual specificity phosphatase DUSP6 (30). Both $CD8^+$ and $CD4^+$ T_{RM} cells show the down-regulated expression of S1PR1 (30). CD69 is a type 2 glycoprotein with a C-type lectin-like domain that acts as a homodimer (50). CD69 binds to S1P1 to promote the internalization and degradation of S1P1 in the cytoplasm. As a result, CD69-expressing T cells remain within lymphoid tissues, such as the thymus and lymph nodes (49). $CD8^+$ T_{RM} cells in the lungs of mice with influenza viral infection show the high expression of CD69, and a CD69-deficient environment was shown to be associated with a reduced number of $CD8^+$ T_{RM} cells in the lung (51, 52). In the skin and kidneys, CD69-deficiency in $CD8^+$ T cells also result in a markedly reduced number of $CD8^+$ T_{RM} cells (53, 54). $CD8^+$ T_{RM} cells show lower S1P1 expression levels (43). In addition, $CD8^+$ T_{RM} cells reveal the low expression of Krupple-like factor 2 (KLF2), a transcription factor that regulates the expression of S1PR1 (55). These findings suggest that CD69 plays a crucial role in $CD8^+$ T_{RM} cells, as more than a mere cell surface marker. Interestingly, though, $CD8^+$ T_{RM} cells are able to be maintained in the lung independently of the CD69 expression (52). Furthermore, experiments using pet mice with differing microbial experiences revealed that the CD69 expression on $CD8^+$ T cells was insufficient to interpret tissue residence (56). Indeed, the functional requirement for CD69 is evidently dependent on the tissue where $CD8^+$ T_{RM} cells exist (54). Thus, although CD69 is not a perfect cell surface marker for tissue residency, more detailed studies regarding the functional roles of CD69 in T_{RM} cells, especially $CD8^+$ T_{RM} cells, are needed to draw firm conclusions. In contrast, the role of CD69 in $CD4^+$ T_{RM} cells remains unclear.

The unique transcriptional features of T_{RM} cells have been well established in $CD8^+$ T_{RM} cells. The transcription factor homolog of Blimp1 in T cells (Hobit) is specifically expressed in $CD8^+$ T_{RM} cells (57). Hobit and Blimp1 cooperatively downregulate the expression of S1pr1 and Ccr7, which are required for tissue egress (57). Hobit and Blimp1 also repress the transcription factors Tcf7 and Klf2, which regulate survival and trafficking of circulating memory T cells (57). The transcription factor Runx3 plays a crucial role in establishing $CD8^+$ T_{RM} cells (57, 58). $CD8^+$ T_{RM} cells in the liver show an enhanced expression of *Hobit* (20). Without appropriate $CD4^+$ T cell help, lung $CD8^+$ T_{RM} cells show an enhanced expression of T-bet that suppresses the formation of $CD8^+$ T_{RM} cells by direct binding to the *Itgae* locus (44).

Regarding $CD4^+$ T cells, Hobit and Blimp1 are reported to attenuate $CD4^+$ T_{RM} cell-dependent colitis (59). Viral infection induced-CD4 T_{RM} cells show the enhanced expression of Hobit and Eomes (19). However, another group reports that T helper type 2 (Th2) CD4 T_{RM} cells do not preferentially express Hobit, Blimp1 or Runx3 in their RNA sequencing (RNA-Seq) data sets (60). In humans, the transcription factor c-MAF induces the tissue residency transcriptional program in Th17 cells (61). Although many of the phenotypic characteristics of $CD4^+$ T_{RM} cells are shared with $CD8^+$ T_{RM} cells, precise assessments regarding the transcriptional features of $CD4^+$ T_{RM} cells are required to identify the nature of $CD4^+$ T_{RM} cells (62).

Recent studies using human tissue resident memory T cells have revealed that both $CD4^+$ and $CD8^+$ T_{RM} cells are transcriptionally distinct from other memory T cell subsets (30, 63). A core gene signature including ITGA1, ITGAE, IL-2, CXCR6, and PD-1 shows differential regulation between T_{RM} cells and circulating T cells, suggesting the unique feature of human T_{RM} cells *in vivo* (30).

THE EXPERIMENTAL TECHNIQUES USED TO IDENTIFY T_{RM} CELLS *IN VIVO*

Proving the tissue residency of T cells is a major challenge. It is necessary to show at least that the cells are present in the same tissue for a certain period to prove tissue residency. Currently, experimental techniques, such as (1) parabiosis, (2) *in vivo* intravascular staining, and (3) tissue transplantation are used to prove the tissue residency of a certain population of cells (**Figure 2**).

Parabiosis is an experimental technique in which two mice are surgically linked and share a common circulatory system (**Figure 2**), which makes us possible to separate substances that are circulating in blood vessels and those that are not in the bloodstream. This method was established in France in the 19th century. In the second half of the 20th century, it has been widely used to investigate the endocrine system. In the field of immunology, parabiosis experiments are conducted to demonstrate the tissue residency of a certain cell population *in vivo*. In the tissue transplantation, the tissue—together with tissue-resident cells—is transplanted into congenic mice and then analyzed for the migration of donor-derived cells in the tissue to demonstrate tissue residency (10). Intravascular *in vivo* labeling is an experimental technique using the intravenous injection of cell-surface antibodies, such as anti-CD4 antibodies, to distinguish cells in tissue from those in blood vessels (**Figure 2**) (64). The advantage of this technique is its simplicity in comparison to parabiosis and tissue transplantation experiments. T cells in the vasculature were found to differ from those in the lung parenchyma, which were not stained with cell-surface antibodies (64). However, it is important to note that this experiment shows that unstained cells were not present in the

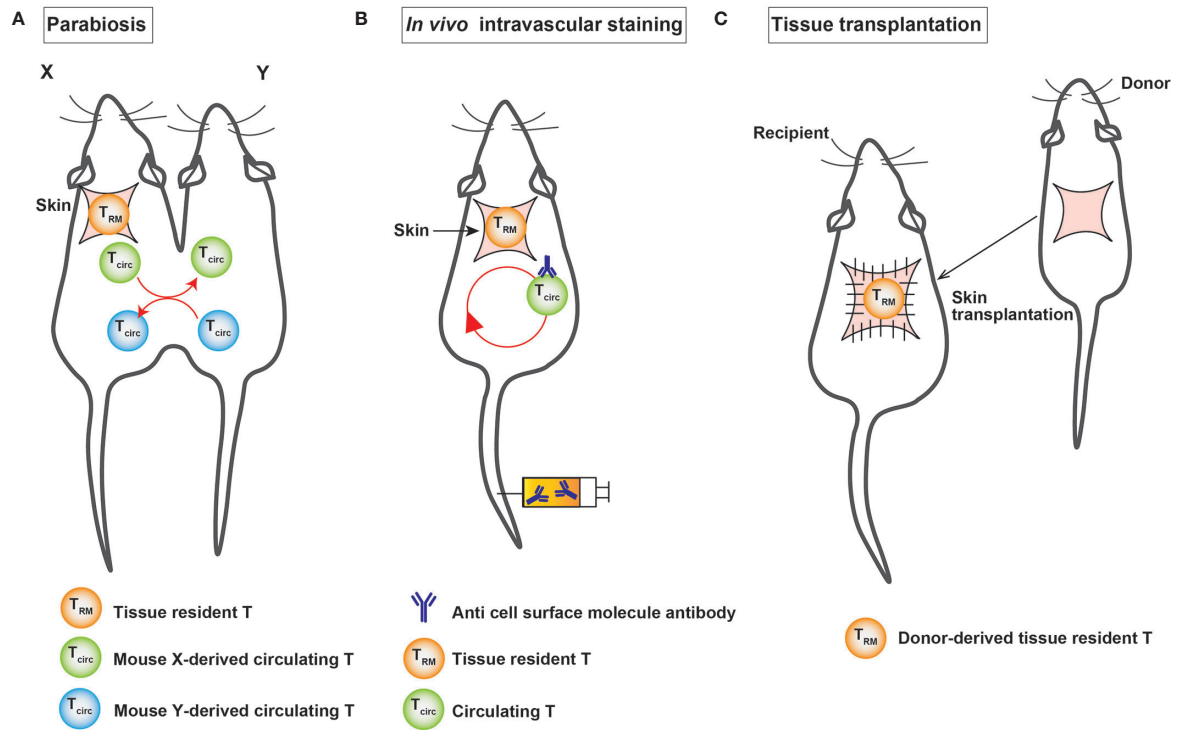

FIGURE 2 | A schematic illustration of the experimental techniques used to identify T_{RM} cells. **(A)** Surgical connection of two congenic mice allows them to share blood circulation. **(B)** *In vivo* intravascular staining marks circulating T cells through the intravascular injection of an anti-cell surface molecule antibody. **(C)** In tissue transplantation, donor-derived T cells are detected in the graft after transplantation.

vessels for a certain period of time after the intravenous injection of the antibody, because the cells were collected from each organ 3-5 minutes after the intravenous injection of the antibody under anesthesia.

As each of these techniques has certain limitations and addresses several specific criteria for residency, the definitive assessment of tissue residency of T cells should rely on supportive results obtained from multiple experimental techniques.

THE PROTECTIVE AND PATHOGENIC ROLES OF CD4$^+$ T_{RM} CELLS AT LOCAL INFLAMMATORY SITES

In addition to other memory T cell populations, such as T_{EM} and T_{CM} cells, T_{RM} cells play an important role in the body's defense against infection. In several experimental models in mice, CD8$^+$ T_{RM} cells have been revealed to be important in defending against viral, parasitic and other infections (20, 65–67). In humans, CD8$^+$ T_{RM} cells have been reported to be crucial in defending against herpes simplex type 1 virus infection in the skin (68).

Regarding CD4$^+$ T cells, CD4$^+$ T_{RM} cells are important for optimal protection against respiratory virus infection *via* the enhanced production of IFN-γ (11). CD4$^+$ T_{RM} cells play key roles in the elimination of HSV-2 and chlamydia in the vagina (26, 69). HSV-2-specific CD4$^+$ T_{RM} cells are enriched in local inflammatory sites, and the chemokine CCL5 is important for the retention of CD4$^+$ T_{RM} cells in vaginal tissues (26). These CD4$^+$ T_{RM} cells also produce large amounts of IFN-γ (26). In an LCMV infection model, CD4$^+$ T_{RM} cells play a key role in local immunosurveillance along with CD8$^+$ T_{RM} cells (19). CD4$^+$ T_{RM} cells also play a protective role against pneumococcal infection in the lung (70). In this model, IL-17-producing CD4$^+$ T_{RM} cells recruit neutrophils to the lung, which is crucial for protecting the host against bacterial infection (70). In humans, an increased frequency of donor T_{RM} cells in the lung of patients with lung transplantation is associated with a reduced rate of adverse clinical events, such as primary graft dysfunction (37). This finding suggests the protective roles of donor T_{RM} cells in the rejection of transplanted tissue.

However, T_{RM} cells are also involved in the pathogenesis of various human immune-related diseases. In psoriasis, an autoimmune disease of the skin, CD8$^+$CD49a$^-$ T_{RM} cells produce IL-17 at the local inflammatory site and are involved in the pathogenesis of the disease. In vitiligo, CD8$^+$CD49a$^+$ T_{RM} cells produce IFN-γ in the inflammatory tissue and are involved in the pathogenesis of the disease (71). In addition, using experimental autoimmune encephalomyelitis, a mouse model of multiple sclerosis, CD8$^+$ T_{RM} cells have been shown to be involved in the onset and relapse of disease (72).

Mucosal tissues that include a large number of T_{RM} cells are susceptible to environmental stresses, such as cell damage, cell death, and changes in partial oxygen pressure. T_{RM} cells play important roles in maintaining local tissue homeostasis, including tissue repair and regeneration as well as defense against infection and the pathogenesis of immune-related diseases. Indeed, $CD8^+$ T_{RM} cells localize within local inflammatory sites during tissue regeneration after influenza virus infection (52). This suggests that $CD8^+$ T_{RM} cells are involved in the processes of tissue repair and regeneration. However, overactivation of the tissue repair process causes tissue fibrosis (73). Various stimuli, including HDM and fungal infection, cause fibrosis in the lung (73–75). In fact, house dust mite (HDM)-induced allergic airway inflammation has been demonstrated to be dependent on HDM antigen-specific $CD4^+$ T_{RM} cells in the lungs in experimental mouse models (74, 76). IL-2 signaling is required for the residency of HDM antigen-specific $CD4^+$ T_{RM} cells, which are sufficient to induce airway hyper-responsiveness (76). Interestingly, chronic exposure of HDM induces the infiltration of both $CD4^+$ and $CD8^+$ T cells into the lung tissue; however, only $CD4^+$ T_{RM} cells persist in the lung for a long time (77). Another group reported that allergen-specific $CD4^+$ T cells were able to survive for over 70 days in the lung (74). A dominant type 2 immune response is induced by repetitive HDM exposure, and Th2 T_{RM} cells are functionally and transcriptionally distinct from circulating memory Th2 cells in the lungs of mice with HDM-induced allergic inflammation (60). Th2 T_{RM} cells express increased levels of *Il5* and *Il13* (60). Thus, $CD4^+$ T_{RM} cells play a critical role in shaping various pathologies, such as airway hyper-responsiveness and eosinophilic inflammation during chronic type 2 inflammation.

Furthermore, Th2 T_{RM} cells show the enhanced expression of metalloproteases, extracellular matrix (ECM) components and regulators for ECM (60). These unique transcriptomic feature of Th2 T_{RM} cells suggests the pathogenic role of Th2 T_{RM} cells in the induction of fibrotic responses. Regarding fungal infection, patients with allergic bronchopulmonary aspergillosis/mycosis (ABPA/ABPM) have recurrent bronchial asthma attacks accompanied by bronchial dilatation and fibrotic changes in the lung (75). In the lungs of mice with repeated exposure to the *Aspergillus fumigatus* antigen, $CD4^+$ T_{RM} cells, which produce various type of inflammatory cytokines accompanied by the low expression of CD103 and the enhanced expression of fibrosis-related genes, induce fibrotic responses (78). In addition, $CD103^-$ $CD4^+$ T_{RM} cells also express the metalloprotease *Adam8* (78). An assay for transposase-accessible chromatin using a sequencing (ATAC-Seq) analysis revealed that the characteristic features of these $CD4^+$ T_{RM} cells populations were regulated at the chromatin level. For example, the regulatory elements of inflammatory cytokines, such as *Il4*, *Il5*, and *Il13*, were specifically accessible in CD103-negative $CD4^+$ T_{RM} cells (**Figure 3**). At the same time, CD103-positive $CD4^+$ regulatory T (Treg) cells are induced in the inflammatory lung. These CD103-positive Treg cells regulate the fibrotic responses induced by CD103-negative $CD4^+$ T_{RM} cells in chronic allergic inflammation caused by repeated exposure to the *A. fumigatus* antigen *in vivo* (78) (**Figure 3**). Thus, $CD103^-$ $CD4^+$ T_{RM} cells are involved in the fibrotic response processes in the lung. Taken together, these findings suggest that $CD4^+$ T_{RM} cells play pathogenic roles in the fibrosis induced by various stimuli, such as HDM and fungi.

The protective roles of $CD4^+$ T_{RM} cells have been elucidated in various infectious diseases. However, the pathogenic roles of $CD4^+$ T_{RM} cells in chronic inflammation other than type 2-related diseases, such as allergic inflammation, have been unclear. Thus, we await the further investigation of the pathogenic roles of $CD4^+$ T_{RM} cells in various immune-related diseases, including multiple sclerosis and psoriasis, the induction of which reportedly involves type 17 inflammation.

PLASTICITY AND EPIGENETICS OF T_{RM} CELLS

It is now clear that memory T cells comprise several subsets, including T_{CM} cells, T_{EM} cells and T_{RM} cells. Researchers have shown that $CD8^+$ T_{CM} cells become $CD8^+$ T_{RM} cells *via* an adoptive transfer experimental system (79). In fact, adoptively transferred $CD8^+$ T_{CM} cells reside in the skin of donor mice accompanied by the enhanced expression of CD69 and CD103 after viral infection (79).

But what about the opposite direction of re-differentiation? In other words, do $CD8^+$ T_{RM} cells have the ability to re-differentiate to $CD8^+$ T_{CM} cells? T_{RM} cells are localized within specific organs for a long time, indicating their involvement in first-line protective responses against local reinfection. If $CD8^+$ T_{RM} cells can re-differentiate to $CD8^+$ T_{CM} cells, T_{RM} cells may be involved in systemic memory immune responses. Experiments using $CD8^+$ T_{RM} cells accompanied by an analysis of the methylation state of the CpG region have shown that the function of T_{RM} cells is not fixed, and T_{RM} cells have the ability to change their function *in vivo* (17). A machine learning-based analysis using the methylation state of the CpG region in $CD8^+$ T_{RM} cells showed that $CD8^+$ T_{RM} cells were able to re-differentiate (17). Furthermore, using an experimental system of virus-infected mice, researchers showed that some reactivated $CD8^+$ T_{RM} cells returned to the systemic circulatory system and re-differentiated into $CD8^+$ T_{CM} cells. Using a $CD8^+$ T_{RM} cell-restricted transcription factor Hobit-reporter system, another group showed that $Hobit^+$ $CD8^+$ T_{RM} cells proliferate in draining lymph nodes after viral re-infection (18). Importantly, $Hobit^+$ $CD8^+$ T_{RM} cells re-differentiated into $CD8^+$ T_{EM} cells together with the downregulation of the *Hobit* expression and contributed to the generation of the systemic immune responses (18). These results suggest that immune memory maintained in the local inflammatory sites may also be involved in systemic memory immune responses, at least in the case of $CD8^+$ T_{RM} cells.

An IL-17A tracking-fate mouse experimental system showed that $CD4^+$ T_{RM} cells were derived from effector Th17 cells (16). In humans, $CD4^+$ T_{RM} cells in the bone marrow show unique DNA methylation profiles among memory T cell subsets, indicating their specialized function (80). However, in contrast

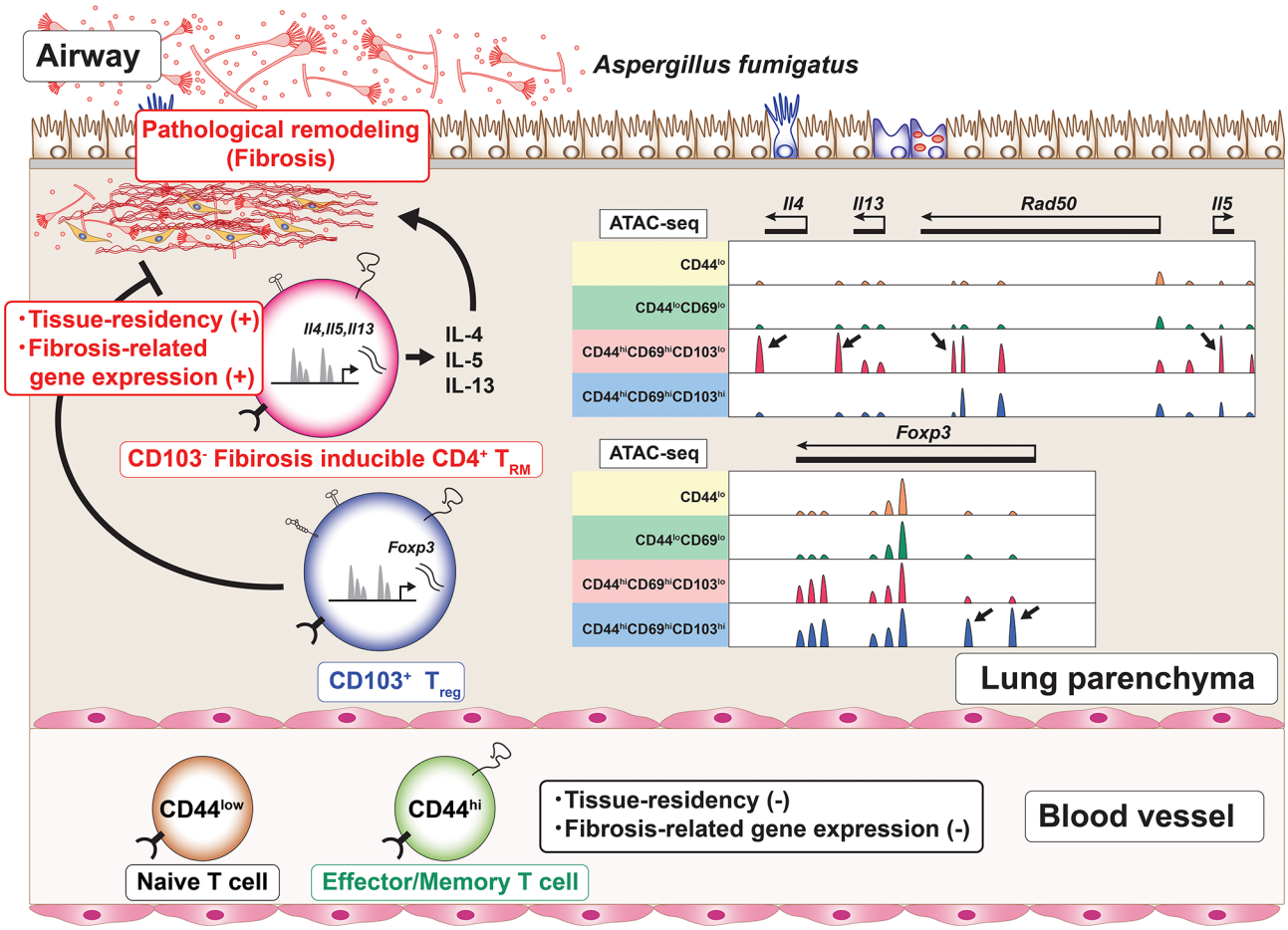

FIGURE 3 | The induction of CD4+ T_{RM} cells with a unique regulome signature. Chronic allergic inflammation with fibrosis of the lung induced by repeated exposure to *Aspergillus fumigatus* antigen causes the induction of two cell populations, CD103-negative CD4+ tissue-resident memory T (T_{RM}) cells and CD103-positive regulatory T (Treg) cells, which are involved in the pathogenesis of fibrotic responses. Each of these cell populations has its own characteristic regulome. For example, CD103-negative CD4+ T_{RM} cells produce proinflammatory cytokines and show specific peaks of ATAC-Seq in the Th2 cytokine loci (arrows). In contrast, CD103-positive Treg cells show specific peaks of ATC-Seq in the *Foxp3* locus (arrows).

to findings concerning CD8+ T cells, the plasticity of the CD4+ memory T cell population has remained unclear.

THE MAINTENANCE OF T_{RM} CELLS IN THE NON-LYMPHOID TISSUE

Inducible bronchus-associated lymphoid tissue (iBALT), a type of ectopic lymphoid tissue, is often formed in response to various stimuli, including infection, smoking, and collagen disease, in the inflamed lung (81). iBALT includes MHC class II-positive cells, B220-positive cells, CD11c-positive cells, VCAM1-positive stromal cells, and CD21-positive follicular dendritic cells. CD11c-positive dendritic cells are crucial for the reactivation of CD8+ T_{RM} cells in the lung (82). Memory CD4+ T cells are maintained within iBALT in lungs with chronic allergic inflammation (83). Furthermore, Thy1-positive IL-7-producing lymphoid endothelial cells are essential for the survival of memory CD4+ T cells due to their production of IL-7 in the inflammatory tissue of the lung (83). Interestingly, the maintenance of allergen-specific CD4+ T cells is dependent on IL-7 signaling in the lung (74). Single-cell RNA sequencing of the lung from mice with bacterial infection has revealed the enhanced expression of *Il7* by lymphatic endothelial cells, which are colocalized with CD4+ T cells (16). Based on these findings, it is likely that CD4+ T_{RM} cells, which are induced by repeated exposure to *Aspergillus fumigatus* antigen, are also maintained within iBALT in the inflamed lung. In fact, repeated exposure to *Aspergillus fumigatus* antigen induces the enhanced formation of iBALTs in the inflamed lung. However, the molecular mechanisms underlying the differentiation, induction, and maintenance of CD4+ T_{RM} cells in the lung and the role of iBALT in these processes remain unclear and require further research. In another mucosal tissue, the skin, the formation of ectopic lymphoid tissue called inducible skin-associated lymphoid tissue (iSALT) was reported (84). CD4+ T_{RM} cells accumulate

within iSALT following skin inflammation (84, 85). IL-7 is a key cytokine supporting the long-term survival of $CD4^+$ T_{RM} cells in the skin (47).

More detailed information regarding the tissue-specific anatomical niches for the maintenance of $CD4^+$ T_{RM} cells has been reviewed in other articles (62, 86).

T_{RM} CELLS AND THE "PATHOGENIC TH CELL DISEASE INDUCTION MODEL"

We proposed a model for the pathogenesis of immune-related inflammatory diseases called the "pathogenic Th-cell disease induction model" (87). In our proposed "pathogenic Th-cell disease model", a certain population of memory $CD4^+$ T cells is highly pathogenic, and the generation of pathogenic T cells is important for the pathogenesis and regulation of various inflammatory diseases. In other words, various immune-related chronic inflammatory diseases are not induced by an imbalance between the subsets of $CD4^+$ T cells (e.g., Th1 cells, Th2 cells or Th17 cells), rather, they are induced by a specific population of pathogenic cells (pathogenic $CD4^+$ T cells) that arise in peripheral tissues under certain conditions. For example, we identified IL-5 high-producing-pathogenic Th2 cells that produce large amount of IL-5 and induce eosinophilic airway inflammation (88). We also identified fibrosis-inducing-pathogenic Th2 cells that produce Amphiregulin, a tissue repair factor, and induce tissue fibrosis *via* the activation of eosinophils (89, 90). These pathogenic Th2 cells have also been found in tissue, as they are maintained within the iBALT.

The CD103-negative $CD4^+$ T_{RM} cells that we identified recently are also pathogenic $CD4^+$ T cells, which coexist with pathogenic Th1/Th2/Th17 cells due to the nature of the pathological model of *Aspergillus fumigatus* antigen administration. Interestingly, both pathogenic $CD4^+$ T_{RM} cells and regulatory T cells are induced simultaneously in chronic inflammatory tissues. Thus, multiple functional $CD4^+$ T_{RM} cell populations are involved in the pathogenesis of refractory immune-related inflammatory diseases, such as bronchial asthma and atopic dermatitis. We need to investigate the diversity of $CD4^+$ T_{RM} cells in the lung using a single cell RNA-sequencing (scRNA-seq) analysis.

CLOSING REMARKS

Tissue-resident memory T cells represent a relatively new cell population that has only been attracting attention for approximately 10 years. Regarding $CD8^+$ T cells, the tissue-resident memory T cell population is being actively studied worldwide, and novel findings about $CD8^+$ T_{RM} cells have emerged one after another, including the identification of transcription factors such as *Hobit*, *Blimp1*, and *Runx3*, which are important for the induction of $CD8^+$ T_{RM} cells (57, 58). As described previously, the plasticity of $CD8^+$ T_{RM} cells has also been analyzed at the epigenomic level.

On the other hand, the mechanisms underlying the differentiation, maintenance, and plasticity of $CD4^+$ T_{RM} cells remain unclear. $CD4^+$ T_{RM} cells play a protective role in the lungs against infections such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis* (70, 91). $CD4^+$ T_{RM} cells also play an important role in the elimination of HSV-2 and chlamydia in the vagina (26). The intranasal administration of pneumococci induces IL-17-producing $CD4^+$ T_{RM} cells that protect the host against pneumococcal colonization (33). Intranasal vaccination of influenza virus induced the accumulation of both $CD4^+$ and $CD8^+$ T_{RM} cells in the lung of mice (92). Moreover, intranasal vaccination with Venezuelan equine encephalitis replicons (VRP) encoding a severe acute respiratory syndrome coronavirus (SARS-CoV) $CD4^+$ T cell epitope resulted in airway memory $CD4^+$ T cell-dependent protection against SARS-CoV (93). In humans, increased frequencies of $CD4^+$ T_{RM} cells in the airway are associated with surviving severe disease of SARS-CoV-2 infection (94). Furthermore, $CD4^+$ T_{RM} cells may promote the generation of antibodies by B cells against pathogenic microorganisms in mucosal tissues, including the lung. In fact, a subpopulation of $CD4^+$ T_{RM} cells promotes humoral responses in the lung after viral infection (95, 96). This subpopulation shows the follicular helper T (Tfh)-like phenotype, including a high expression of PD-1 and CXCR5 (95). The differentiation of this subpopulation depends on B cells and the intrinsic expression of Bcl6 (95). Importantly, $Bcl6^{hi}$ $CD4^+$ T_{RM} cells, which are colocalized with B cells in iBALT, promote local antibody production and help $CD8^+$ T_{RM} cells *via* the enhanced production of IL-21 (95, 96). Thus, $CD4^+$ T_{RM} cells are a promising target cell population in terms of the development of next-generation vaccine therapies (97). In the future, more intensive research on $CD4^+$ T_{RM} cells is expected to reveal new cellular mechanisms and molecular mechanisms for $CD4^+$ T_{RM} cells.

AUTHOR CONTRIBUTIONS

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REVIEW
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Interplay of Inflammatory, Antigen and Tissue-Derived Signals in the Development of Resident CD8 Memory T Cells

Curtis J. Pritzl, Mark A. Daniels and Emma Teixeiro *

Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO, United States

CD8 positive, tissue resident memory T cells (T_{RM}) are a specialized subset of CD8 memory T cells that surveil tissues and provide critical first-line protection against tumors and pathogen re-infection. Recently, much effort has been dedicated to understanding the function, phenotype and development of T_{RM}. A myriad of signals is involved in the development and maintenance of resident memory T cells in tissue. Much of the initial research focused on the roles tissue-derived signals play in the development of T_{RM}, including TGFß and IL-33 which are critical for the upregulation of CD69 and CD103. However, more recent data suggest further roles for antigenic and pro-inflammatory cytokines. This review will focus on the interplay of pro-inflammatory, tissue and antigenic signals in the establishment of resident memory T cells.

Keywords: resident memory, inflammation, antigenic stimulation, tissue-derived signals, memory differentiation

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***Correspondence:**
Emma Teixeiro
teixeiropernase@missouri.edu

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INTRODUCTION

Over the course of an infection, naïve CD8 T cells become activated in the lymphoid tissues and differentiate into CD8 effector T cells. As effector T cells abandon the secondary lymphoid organs and migrate to tissue, they need to integrate a multitude of signals coming from cytokines, chemokines and antigen in order to gain access to infected cells, clear the pathogen and differentiate into memory T cells. Among the T cell responders with effector function, the vast majority die and only a few persist as memory T cells. We do not yet fully understand what endows T cells with the potential to become memory T cells, although we do know that the level of exposure to antigenic and pro-inflammatory signals play an important role (1–6). We also know that a balance in the level of a set of transcription factors is crucial (i.e. Eomes/T-bet, Bcl-6/Blimp-1, Id-2/Id-3, ZEB1/ZEB2, BACH/AP-1, NR4A1/IRF4) (7, 8); that specific costimulatory and homeostatic cytokines signals impart maturing memory cells with longevity properties (9, 10); and that dramatic metabolic and epigenetic changes are essential (11, 12). Precursors of memory T cells (or MPECs) have been well defined as $KLRG1^{lo}$ and $IL\text{-}7R^{hi}$ (2) and are readily present early in the immune response albeit at small frequencies. Yet, as most of antigen specific-T cell responders progress through the immune response and die off (Short lived effectors/SLECS $KLRG1^{hi}$ $IL\text{-}7R^{lo}$ expressors), MPECs continue their process of maturation towards memory. Consequently, T cell memory is the result of a combination of early signals which configure the transcriptional/epigenetic memory program, and late signals that during the same immune response help to fully execute this program (13, 14). T cell

memory differentiation becomes even more complex when considering that memory T cells come in different "flavors" (T cell memory subsets) and with different benefits (T cell memory functions and locations). Thus, a T cell transitioning to memory, may become a central memory (T_{CM}), an effector memory, (T_{EM}), a stem-cell memory (T_{SCM}), or a resident memory (T_{RM}). Each population has evolved to fill a specific niche required to protect the host. T_{CM} ($CCR7^+$ $CD62L^+$ expressors) circulate between the blood and secondary lymphoid tissues and retain an extraordinary proliferative potential. T_{EM} ($CCR7^-CD62L^-$), in turn, circulate between the blood and peripheral tissues and are very efficient at exerting immediate effector functions upon antigen restimulation [reviewed recently in (15)]. T_{SCM} have been described in humans ($CD122^+$, $CD95^+$, $CCR7^+$, $CD62L^+$, $CD45RA^+$, $CXCR3^+$) and share the proliferative, self-renewal and pluripotency potential of T_{CM} cells (16).

Tissue resident memory T cells persist in the peripheral tissues following infection and act as front-line sentries against pathogen re-infection. The response of CD8 T_{RM} triggers fast innate (17–19) and adaptive immune responses in the site of re-infection (20). Furthermore, CD8 T_{RM} have also been linked to defense against tumors, with its presence correlating with good prognosis (19, 21, 22). CD8 T_{RM} are present in almost every tissue, including secondary lymphoid organs (23). However, there is also phenotypic diversity of the T_{RM} subset depending on the tissue. This suggests that local tissue signals may play a critical role in positioning T_{RM} in specific locations to perform specialized functions (24). In spite of how much we have learned in recent years about T_{RM}, there is still little known about how cytokines, antigens and other tissue signals "crosstalk" intracellularly to program the generation and maintenance of CD8 T_{RM} (**Figure 1**). In this review article we will discuss how much the field has advanced in this aspect and point out to the gaps that still remain uncovered.

TISSUE RESIDENT MEMORY CD8 T CELLS

As mentioned before, tissue resident memory CD8 T cells have been found in peripheral healthy tissues such as lung, brain, gut, liver, skin, oral, nasal and female reproductive tract mucosal tissue, and also in tumors, transplants and organs subjected to autoimmune reactions (23). Most interestingly, tissue resident memory T cells also re-populate tissue draining lymph nodes upon antigen recall. Even at the memory stage, tissue T_{RM} can occupy local draining lymph nodes, most likely, to warrant extended protection (25, 26). All together this puts T_{RM} as the most abundant memory T cell in our bodies and especially so as we age. In mice, it is difficult to evaluate the lifespan of T_{RM} beyond one year. However, in humans, it has been shown that T_{RM} are stably maintained from childhood well into old age, at levels that are tissue specific (27, 28). Surprisingly and in contrast to mice (where naïve T cells largely reside in lymphoid organs), in humans naïve T cells are also long-term resident of tissues, although they are quickly outnumbered by memory T cells in mucosal sites (29). Resident memory T cells are extremely efficient at mounting protective innate and adaptive secondary responses upon re-infection (17, 30) and can control pathogen spread without the need of other T cell memory subsets (31). Yet whether this helps to spare the naïve and central memory population in lymph nodes from activation, and further maintain diversity in the T cell repertoire remains to be shown.

T_{RM} ontogeny is also still poorly understood as well as the relationship of the T_{RM} subset with the other T memory subsets. Initially MacKay, Carbone and Gebhardt described $KLRG1^{lo}$ epithelium expressors that encounter IL-15 and TGFβ signals as precursors of skin T_{RM}. This led to the idea that T_{RM} cells deviate from the T effector differentiation path once in tissue (32, 33). More recently, other studies have confirmed that even before tissue entrance circulating T cells can commit to the T_{RM} fate. This is readily concluded when considered that: (1) T_{CM} and T_{RM} share a common clonal origin (34); (2) even at the naïve stage, T cells can be pre-condition to "walk" the T_{RM} differentiation journey (35) and (3) that circulating effectors with a skewed T_{RM} transcriptional profile that preferably become T_{RM} exist (36). Whether this also applies to the ontogeny of T_{RM} in other tissues is still uncertain. Indeed, in contrast to the skin T_{RM} studies, scRNA sequencing studies in the gut have identified T_{RM} precursors in tissue very early upon infection (37). From all these data, one thing is still clear, regardless of the potential for becoming T_{RM}, circulating effectors will not be able to fulfill this potential unless exposed to tissue signals.

At the point T cells commit to the T_{RM} fate, are they deadlock in this identity? or on the contrary, do they retain pluripotency to generate other T cell memory subsets upon recall? Fonseca et al. answered this question recently and provided evidence supporting the idea that T_{RM} cells are not completely locked into the resident lineage. Upon rechallenge, ex-T_{RM} cells epigenetically retained the potential to become T_{CM} and T_{EM} (38), however, they preferentially re-differentiate into T_{EM} and T_{RM} that homed back to their original tissue (38, 39).

Another important issue in the field is T_{RM} diversity of heterogeneity. T_{RM} diversity is defined by changes in transcription profile, phenotype, location and function (37). However, despite the heterogeneity within the T_{RM} compartment, all T_{RM} share a specific transcriptional profile characterized by expression of Runx3, Blimp-1, and Hobit and reduction of Eomes, T-bet, and KLF-2 levels (40–43) (**Figure 1**). This transcriptional profile enables the expression of molecules that permit recruitment and lodging to tissue in addition to special adaptation to unique tissue signals for T_{RM} survival. What is less known is how the different signals a T cell encounters in its journey to T_{RM} regulate this transcriptional program.

A more precise view of T_{RM} development is arising. Cumulative evidence supports a multistep differentiation process where T cells have the potential to enter in the T_{RM} path at different stages (naïve, in circulation, in tissue). Yet how much the quality or amount of signals a T_{RM} precursor receives conditions its resident potential is unclear. Additionally, it is still ill-defined whether the same signals regulate T_{RM} development, maintenance, function, retrograde migration to draining lymph nodes and/or pluripotency upon recall. Initial findings pointed to

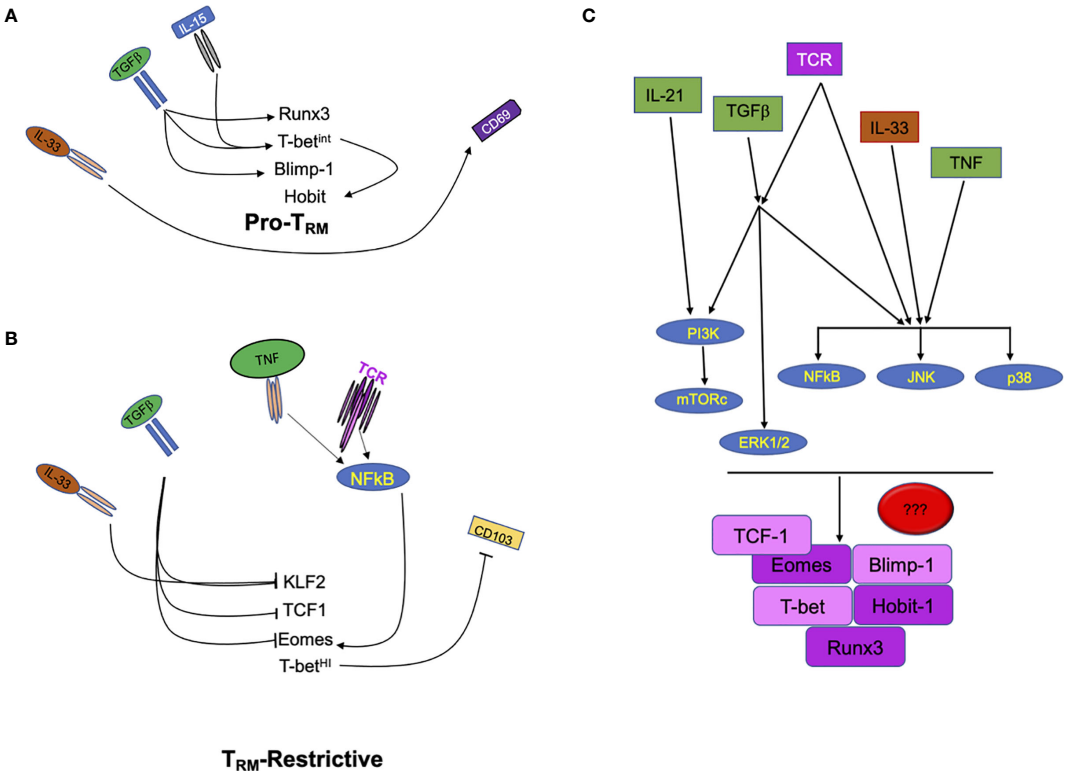

FIGURE 1 | Extracellular factors regulate multiple signals in CD8 T cells to drive or repress T_{RM} development. **(A)** Schematic of signals including IL-33, TGFβ, and IL-15 which promote the development of tissue resident T cell memory through the increase of transcription factors Runx3, Hobit, Blimp1, and the tuning of T-bet expression. **(B)** Tissue cytokines such as IL-33 and TGFβ also inhibit transcription factors (KLF2, TCF1, and Eomes) that can restrict the development of CD8 T_{RM}. In contrast, pro-inflammatory cytokine and antigenic/T cell receptor signals can modulate the expression of Eomes which can, then, interfere with CD8 T_{RM} development. **(C)** Signaling crosstalk between pro-inflammatory, tissue and antigenic signals. PI3K, MAPKs (ERK, JNK and p38 MAPK) and NFκB are potential nodes where extracellular cues converge to tune CD8 T_{RM} programming, differentiation and maintenance.

various cytokine signals and antigen within local tissues as main triggers to support CD8 effectors to CD8 T_{RM} differentiation. TGFβ has been shown to be a major contributor to this pathway along with IL-33 and IL-15. Roles for both antigenic stimulations along with inflammatory signals such as IL-12, IL-21, and TNF have been linked to the regulation of CD8 T_{RM} development as well (**Figure 1**).

TISSUE SIGNALS INVOLVED IN CD8 T_{RM} DEVELOPMENT

Tissue cytokines have been shown to act synergistically in establishing the resident memory phenotype in tissues such as the gut, skin, brain, and the lungs (40, 44–49). Hereafter, we will discuss what it is known of how each one of these signals contribute to T_{RM} development and maintenance and discuss the synergism of the signaling pathways they trigger.

TGFβ Signaling

TGFβ is a crucial cytokine for T cell development and differentiation. TGFβ is involved in thymic development, in the maintenance of naïve T cells, and also in CD8 T cell effector activation (50, 51). Seemingly, TGFβ has also been linked to the formation of CD8 T_{RM} in different organs such as skin, the gut and lung (32, 44, 45, 52, 53).

Although TGFβ and its receptor are ubiquitous in many cells, TGFβ activity is tightly controlled at multiple levels. At the extracellular level, TGFβ activity depends on induced cleavage of latent TGFβ that is associated to the extracellular matrix or presentation by cells (such as T regs, epithelial cells, fibroblasts, keratinocytes or DCs). Large latent TGFβ can be cleaved by ECM proteases. Alternatively, it can bind to integrin receptors in the membrane of cells, which *via* the actin cytoskeleton promote a conformational change in TGFβ that enables the mature TGFβ release process (54). TGFβ modulates T_{RM} in a manner that is contingent on the presence of immune cells expressing a specific set of integrin receptors. Thus, in the draining lymph nodes of the skin, specialized migratory DCs that express α_v integrins present active TGFβ to naïve T cells and pre-condition them to become epithelial CD8 T_{RM} (35). More recently, Hirai et al. provided data showing that keratinocytes activation and presentation of TGFβ to fully matured skin CD8 T_{RM} is crucial for their maintenance. Especially, if these T_{RM} had been generated in a bystander manner. Even more striking is that

skin CD8 T_{RM} produce their own TGFβ, thereby, contributing to their own maintenance (55). These new compelling roles of TGFβ in skin CD8 T_{RM} add to the already known role of TGFβ in CD8 T_{RM} differentiation (32, 40). However, they also open up new exciting questions. For instance, do these new roles of TGFβ apply to T_{RM} in other tissues? Or what is the relative contribution of autocrine CD8 T_{RM} TGFβ to T_{RM} lineage identity versus T_{RM} survival?

CD103 is one of the most thoroughly described targets of TGFβ in T_{RM} cells (32, 44, 45, 52, 56, 57). CD103 is an integrin (alpha E) that associates with integrin beta 7. The αEβ7 integrin complex binds to E cadherin and facilitates migration and retention of CD8 T cells (32, 58, 59). While not exclusively required for development of all T_{RM} cells, CD103 has an important role in the establishment of tissue residency within certain tissues, such as gut and skin. Sheridan et al., showed that upon oral *Listeria monocytogenes* infection, the majority of the intestinal effector cells rapidly upregulated CD103, but this population was lost when TGFβ signals were blocked (52). In the lung, it has been reported that CD1c+DCs control CD103 expression on CD8 T cells, enabling their accumulation in lung epithelia through a membrane-bound TGFβ dependent process (60). Lack of access to active TGFβ from fully matured skin CD8 T_{RM} also lead to a loss of CD103 expression, although this loss appears to correlate better with the amount of active TGFβ than with a defect in CD8 T_{RM} differentiation (55). This raises the question as to whether CD103 only provides signals for localization or whether it also activates signal transduction pathways that promote T_{RM} lineage stability. The former is supported by the fact that in several tissues (female reproductive tract, liver, lung, and lamina propria) CD103 is not expressed by all resident memory cells (23, 61). It is also important to mention that CD103 is an integrin able to trigger bidirectional signaling and that it can cooperate with TCR signals to enable T cell migration and effector function (62). This suggests that synergism between antigenic and integrin signaling at the epithelium may be relevant for T_{RM} maturation.

Despite the important role of CD103 in CD8 T_{RM} adhesion, migration and retention in TGFβ rich environments, TGFβ receptor deficient cells are more compromised than CD103 deficient T cells for tissue long-term retention (44). Thus, the TGFβ role in CD8 T_{RM} development must be broader than CD103 regulation. Indeed, several studies have pointed to other roles. TGFβ has been found to induce apoptosis of short-lived effector cells (SLECs) by antagonizing the survival effects of IL-15 (63). Since CD8 T_{RM} maintenance in some tissues depends on both cytokines, it is possible that TGFβ contributes to the removal of SLECS, thereby favoring MPEC survival and retention in tissue (**Figure 1**). Comparative *in vitro* analysis also demonstrates a great overlapping between T_{RM} and TGFβ transcriptional signatures (64). More precisely, TGFβ signaling regulates the expression of transcription factors involved in T_{RM} development, such as Runx3 (65) and Blimp1 (66) and repress transcription factors (Eomes, TCF1, and T-bet) (40, 46), which are classically associated with CD8 terminal effector and central memory differentiation (5, 67–70). Achieving the right balance in the levels of all of these transcription factors appears to be crucial for the development of CD8 T_{RM}. Thus, while some T-bet expression is necessary for the expression of IL-15Rβ to receive sufficient IL-15 signals to lodge and survive in tissue (40, 47), over activation of T-bet can also result in the loss of CD103 expression (40, 71). Similarly, high levels of Eomes have been shown to repress T_{RM} development (40). It is still unclear how these transcriptions factors cooperate to establish the T_{RM} program. Yet, they seem to operate under different transcriptional rules than those regulating effector CTL differentiation (where all transcription factors work together in a synergistic way) (68).

Another role of TGFβ is to control tissue lodging by suppressing the expression of Krupple-Like Factor 2 (KLF2), which in turn regulates the expression of S1PR1 (42). Skon et al. reported that TGFβ can control the lodging of CD8 T_{RM} by downregulating KLF2 in a PI3K/Akt dependent manner (42). Curiously, canonical TGFβ signaling classically occurs through the induction of the SMAD pathway and involves formation of activated Smad2/3/4 complexes (54). However, Smad4 appears to be dispensable for CD8 T_{RM} development (72, 73). This implies that non canonical TGFβ signaling may be more important than anticipated for CD8 T_{RM}. TGFβR engagement can activate MAPKs p38, JNK, and ERK, NFκB, PI3K, and mTOR signaling pathways independently of Smad proteins (72–74), although the role of these pathways in CD8 T_{RM} remains elusive. MAPKs (**Figure 1**), in particular, might be especially relevant as recent transcriptional studies have found an association between JunB and FosL and T_{RM} differentiation (37).

Lastly, it is important not to underestimate the crosstalk of TGFβ with other tissue signals which may further tune TGFβ signaling and pay attention of how these signals interaction may account for further diversity or differences in CD8 T_{RM} longevity and/or function (54, 74).

IL-33 Signaling

Along with TGFβ, IL-33 has also been involved in the establishment of CD8 resident memory. IL-33 is a part of the IL-1 family of cytokines. It is expressed by non-hematopoietic cells, constitutively in epithelial cells and inducible in activated DCs, necrotic cells, and tumor cells. It works as an alarmin in response to infection or injury [reviewed in (75, 76)]. CD8 T cells express low levels of the IL-13R or ST2 but IL-33 signaling is still important for effector function (77) and antiviral protective responses (78). Following the initial characterization of CD8 T_{RM}, Casey et al. showed in *in vitro* experiments, that IL-33 could act synergistically with TGFβ to induce CD69 among CD8 T cells in the gut (45). The role of IL-33 was further defined to include the down regulation of KLF2, again in synergism with TGFβ (42). More recently, Harty's group explored the role of IL-33 in the formation and maintenance of lung CD8 T_{RM} *in vivo*. They found that when ST2 was blocked with a neutralizing antibody, the accumulation of influenza specific CD8 T_{RM} was significantly reduced. Yet no effect on conversion to a T_{RM} phenotype was observed (79). In another study, McLaren et al. also showed a loss of CD8 and CD4 T_{RM} ($CD69^{+}CD103^{-}$ or

$CD69^+CD103^+$) in the lungs and salivary glands of IL-33-deficient mice upon MCMV infection (49). Collectively, these data strongly support a critical role of IL-33 in the establishment of the T_{RM} pool in the lung, although whether this role impinges on CD8 T_{RM} differentiation, maintenance and/or recruitment is unclear. Similarly, it is still unknown whether IL-33 impacts CD8 T_{RM} in a CD8 T cell intrinsic manner or through an indirect mechanism. The *in vitro* experiments mentioned above (45), however, point out to a direct role in synergism with TGFβ.

IL-33 signals through MyD88/NFκB can inhibit TGFβ signals through Smad6/7 (74). Furthermore, IL-33 can synergize with IL-12 to promote the expression of T-bet and Blimp-1 while repressing Eomes and TCF-1 (77) (all transcription factors linked to CD8 T_{RM} differentiation) (**Figure 1**). Taking all together (**Figure 1**), it is tempting to speculate that CD8 T_{RM} differentiation and maintenance will be likely dependent on the relative levels of these cytokines in tissue and how their signaling networks crosstalk.

INFLAMMATORY SIGNALS AND RESIDENT MEMORY

Tumor Necrosis Factor

TNF is a cytokine that has pro- and anti- inflammatory functions. TNF is first expressed as a biological active transmembrane homotrimer, which can either be released after cleavage and bind to TNFR1 or TNFR2 or remain bound to the membrane and signal upon binding to TNFR2. TNFR1 is expressed universally on almost all cell types, whereas TNFR2 is mainly restricted to immune cells and some tumor cells. TNF, by contrast, can be produced by T and B cells and innate immune cells (dendritic cells, monocytes, neutrophils, mast cells). TNF is an inflammatory mediator that is heavily induced upon infections such as influenza or tuberculosis but their long -term effects are frequently associated with pulmonary diseases such as asthma, COP, ALI, and ARDS (80). In T cells, TNF can promote the activation and proliferation of naïve and effector T cells, but it also promotes cell death of highly activated effector T cells, further determining the size of the memory T cell pool (81). *In vitro* studies have shown that TNF can synergize with TGFβ and IL-33 to regulate the expression of molecules associated with a T_{RM} signature (CD103, CD69 and Ly6C) in the gut, as well as regulate the expression of the transcription factor KLF-2 (facilitating the retention of T_{RM} in tissue) (42, 45, 82). Additionally, in experiments aiming to test the role for cytokines in the conversion of circulating memory T cells to lung T_{RM}, the authors found that neutralizing TNF levels resulted in a significant reduction in the frequency of CD8 T_{RM} in the parenchyma (79). Altogether, these studies strongly support a role for TNFα in the establishment of T_{RM}, however, whether TNF effects act directly on CD8 T_{RM} precursors *via* their TNFR1 or TNFR2 or indirectly *via* other cells it is still unclear. A study showed that mice lacking TNFR1 expression were inefficient at controlling vaccinia virus in the skin, rather due to defects in resident innate cells and not to the generation of skin memory T cells (82). On the other side, other studies have implicated both TNFR1 and TNFR2 in survival of airway CD8 effectors during influenza infection (83) and also in the generation of memory T cells (81, 84). Thus, when considering the multifaceted roles of TNF signals in the progressive differentiation of CD8 T cells, more studies are needed to assess when and how TNF impacts CD8 T_{RM} and if this happens for all tissues.

Members of the TNF superfamily OX-40 (85), 4-1BB (86, 87) and LIGHT (88) have also been linked to the establishment of CD8 T_{RM}. 4-1BB and LIGHT appear to be crucial for the survival of effector CD8 T cells as they differentiate to T_{RM} (86–88), whereas OX40 signals rather seem to impact the generation of effector and, therefore, accumulation of memory T cells in tissue. One feature in common among all members of the TNF superfamily (TNF included) is the activation of NFκB PI3K, Akt, MAPK and JNK pathways (89), which most likely allow for enhanced survival. However, all TNF superfamily members are also notorious for their dependence on TCR (for costimulatory functions or expression) or cytokine signals (i.e. TNF synergism with TGFβ signals). This points to a more complex picture regarding how all these factors play together in tissue as T cells differentiate and are maintained as CD8 T_{RM} (**Figure 1**). Given the therapeutic value of neutralizing antibodies and fusion proteins targeting TNF family members to decrease inflammation, addressing these gaps of knowledge will aid to improve current strategies directed to boost CD8 T cell immunity in organs or tumors. Similarly, and because anti-TNF treatments are often administered to diminish inflammation in diseases such as Crohn's and rheumatoid arthritis (90–92), knowing the impact of these treatments in the generation and maintenance of the T_{RM} pool in patients is also important.

Interleukin 12, Type I IFN, IL-18, IL-21, and IL-6

Both IL-12 and Type I IFN are the prototypic pro-inflammatory cytokines that provide signal 3, which with signal 2 (costimulation) and signal 1 (antigen/TCR) enable full effector and memory differentiation (93–96). It has also been shown that high levels of these pro-inflammatory cytokines skew effector T cells away from memory (2, 97, 98). Intestinal proinflammatory microenvironments have elevated IFN-β and IL-12 and several studies have shown that both cytokines are critical drivers of CD8 T_{RM} in the gut. Bergsbaken et al. identified intestinal $CCR2^+$ macrophages as the main source of both pro-inflammatory cytokines in the gut and showed that either deletion of these innate population or deletion of the receptors for IL-12 or Type I IFN on CD8 T cells could severely reduce the differentiation and persistence of gut $CD103^-CD69^+$ CD8 T_{RM} cells. Importantly, this was not a consequence of defects in expansion or survival of effector CD8 T cells early in the infection, but rather it was connected to the integration of pro-inflammatory cytokine signals (IL-12, IFNβ, or IL-18) and TGFβ signals in tissue (99). Another report has also shown that IL-12 acting together with IL-15 and CD24 signals is essential for the development of potent

CD8 resident memory responses in the skin. In this case, a migratory BATF3$^+$ dendritic cell population was the main source of IL-12. When tissue IL-12 signaling was inhibited using antibody blockade, sub-optimal CD8 T_{RM} generation was observed in the skin of vaccinia virus-infected mice (100).

IL-12 can also contribute to the establishment of skin CD8 T_{RM} through the expression of the adhesion receptor CD49a, which is specifically critical for CD8 T_{RM} persistence and IFNγ production upon recall (101). At the transcriptional level, IL-12 is a known regulator of master regulators of CD8 T_{RM} Eomes, T-bet and Blimp-1 (102, 103). T-bet is required for the expression of CD122 and input of IL-15 signals necessary for CD8 T_{RM} survival (40, 47), suggesting that IL-12 indirectly facilitates CD8 T_{RM} survival. At the same time, high levels of T-bet may be detrimental for CD8 T_{RM} (40). Since all the studies so far have evaluated the blockade of IL-12 signals to test the role of this cytokine in CD8 T_{RM}, it would be interesting to test whether high levels of IL-12 (which can naturally occur in cytokine storms) could be detrimental, perhaps by exceeding the T-bet threshold that transcriptionally supports T_{RM} (40, 104).

IL-21 is another pro-inflammatory cytokine that is primarily expressed by CD4 T cells, although macrophages, NKT, B, DC, and CD8 T cells can express it at low levels (105). Recently, it has been shown that IL-21R CD8 T cell intrinsic signaling is important for the development of lung and brain CD8 T_{RM} *via* oxidative metabolism (106, 107). IL-21 has been shown to synergize with other cytokines (IL-2, IL-15, IL-10) and TCR signals for regulating CD8 T cell differentiation (108). IL-21R, in turn, transduces signals *via* STAT-1/3/5, but it also shares the activation of PI3K and MAPK with other tissue signals (antigen, TGFβ, TNF), establishing in this way a potential system of check and balances that warranties CD8 T_{RM} [reviewed in (105)] (**Figure 1**).

IL-6 shares functional features with IL-21, and it is produced in certain tissues (bone, lung, liver, adipose tissue, muscle) to fulfill homeostatic functions as well as in response to infection, cancer and tissue injury (109–111). IL-6 signals through STAT3 and together with TGFβ is primordial for Th17 differentiation (112). Furthermore, IL-6 stimulates the production of IL-21 by CD4 T cells (113) and exerts a pro-survival role that can impact the effector/memory population in the context of infection (114, 115). In CD8 T cells, IL-6, together with IL-15 and IL-7, contributes to CD8 T cell proliferation and effector function (116) and to the generation of super IL-21 producer CD8 T cells that can then, help B cells in the lung (117). The connection between IL-6 and tissue resident T cell memory is still poorly understood, although a recent report has identified a distinct population of memory helper CD8 T cells in humans that singularly express IL-6R and exhibit a skin T_{RM} transcriptional signature (118). Interestingly, these IL-6R CD8 memory T cell population is altered in psoriasis (118) and asthma (119), although a role for these type of T cells during infection is still lacking.

Experimental evidence supports that an interaction between local tissue signals and pro-inflammatory cytokines is essential for the establishment of CD8 T_{RM} during infection. Yet, often in systemic infections, cancer therapies (CART) and autoimmunity (rheumatoid arthritis, psoriasis), levels of these pro-inflammatory cytokines or signaling can become dysregulated and cause disease. IL-6 is, indeed, together with TNF, IL-1, IL-18, IL-33, IFNγ a soluble mediator of cytokine storms (120) in mucosal tissues, although whether high levels of inflammatory cytokines are beneficial for CD8 T_{RM} establishment or maintenance still remains to be investigated.

HOMEOSTATIC SIGNALS IL-7, IL-15 AND IL-10

Dendritic cells are key to initiating immune responses and often for directing those responses to the appropriate tissues *via* delivery of antigen, co-stimulation and pro-inflammatory cytokines. What is less studied is how their contribution to homeostatic signals shape the immune response. Iborra et al. recently showed that DNGR-1+ dendritic cells cross present antigen and produce IL-12, IL-15 and CD24 signals which were required for CD8 T_{RM} formation in the skin and lungs (100). IL-15, together with IL-7, is a homeostatic cytokine whose role in T_{CM} and T_{EM} cell memory maintenance is well established (121–123).

In the context of resident memory, IL-7 is almost dispensable while IL-15 has been shown fundamental for survival of CD8 T_{RM} in some tissues (such as skin, kidney, lung and salivary glands but not in FRT, gut, pancreas) (32, 47, 124). In the skin, IL-15 contributes to lodging and maintenance of CD8 T_{RM} by keeping balanced levels of T-bet and the transcription factor Hobit (40, 43). Hobit, in turn, is expressed exclusively in the resident memory population and has the potential to bind to regulatory regions of TCF1, KLF2 and S1PR1, all crucial for CD8 T cell tissue migration (43). In the liver, skin, and small intestine, Hobit has been shown to act in conjunction with Blimp-1 to drive T_{RM} development as well (43). However, in the lung, Blimp-1, rather than Hobit drives T_{RM} formation (125). This is despite the fact that persistence of a subset of lung CD8 T_{RM} (CD103$^+$CD69$^+$) is completely dependent on IL-15 (40). Interestingly, the patterns of Hobit expression and function in mice and humans are different (126), but whether the results in the mouse models remain true in humans will require further investigation. Contrary to Hobit, Blimp-1 promotes CD8 T_{RM} development in the lung while reducing the generation of CD8 T_{CM}. This is particularly critical for CD103$^+$ CD25$^+$, but not CD103$^-$ CD25$^-$ lung T_{RM} (125). While this points out to a potential role of IL-2 and IL-15 in regulating the levels of Blimp-1 the evidence remains controversial. *In vitro* studies have attributed a role for IL-2, but not IL-15, in the induction of Blimp-1 (127). By contrast, *in vivo* studies delivering IL-15 complexes have clearly shown that acute exposure (but not prolonged) to IL-15 signals can promote Blimp-1 expression (128). As IL-12 is also an inducer of Blimp-1 (103), it is possible that specialized DCs able to produce IL-15 and IL-12 (100), together with IL-2, contribute to the induction of Blimp-1 and generation of lung CD8 T_{RM} in sites with residual inflammation.

Another cytokine that is often induced in response to infection is IL-10. CD4 regulatory T cells (Tregs) are producers of IL-10 (129). Both, Tregs and IL-10, play a critical role late in the immune response in the generation of memory CD8 T cells (130). Similarly, Type 1 Tregs (T-bet-) also promote the generation of CD8 T_{RM}. In this case a distinct role for IL-10 was not clearly identified. Instead, the authors found that CD4 Tregs express CXCR3 and by positioning themselves close to CD8 T cells make functional TGFβ available to promote their T_{RM} differentiation (131).These findings were consistent with previous studies indicating that TGFβ-dependent production of TGFβ resulted in increased expression of CD103 on brain CD8 T cells upon CNS infection (132).

T CELL RECEPTOR SIGNALS AND RESIDENT MEMORY CD8 T CELLS

T cells recognize pathogenic or self-antigens *via* their T Cell Receptors (TCRs). TCR signaling is critical for memory T cells (5). Strikingly though, while T cell proliferation and some effector functions are supported by strong antigenic signals, T cell memory ensues regardless, in response to both strong and weak antigens (1, 6). These studies mainly looked at central and effector memory differentiation and found that weak TCR signals specifically favor central memory development *via* expression of high levels of Eomes. Moreover, TCR signal strength inversely regulated the input of inflammation by controlling the expression of inflammatory cytokine receptors and enabling a higher frequency of CD8 T cells that have been stimulated by weak antigens to become central memory T cells (1, 133). In the case of resident memory differentiation, the role of TCR signaling has been largely overlooked until recently. Fiege et al. have shown that while both high and low affinity TCR stimulation support the formation of CD8 T_{RM}, low affinity TCR signals favored the resident memory population (134) mirroring what happens for central memory (1).

Among the signaling cascades the engaged TCR can trigger, the ones able to provide a digital type of signaling, such as Itk/Calcium and ERK (which regulate transcription factors, IRF4 and AP-1 family members) seem to be preferentially involved in promoting terminal effector differentiation (133, 135, 136). Their role in CD8 T_{RM} remains unknown. By contrast, signaling pathways/networks leading to transcription factors that do not strictly fit the rules of TCR signal strength, appear to favor T cell memory fate (BACH2, TCF-1, Eomes) by repressing transcription factors that favor terminal effector differentiation (BACH2 represses AP-1 binding while NR4A1 represses IRF4) (1, 137–146). One of these signals is the NFκB pathway, which appears to be especially critical to the regulation of T cell memory (5, 67, 147). Both, strong and weak TCR signals use this pathway, at least to regulate central memory differentiation (147). NFκB, however, does not seem to regulate the T cell effector versus central memory decision but rather, it controls the survival of CD8 T cells during the transition to memory *via* maintenance of high levels of Eomes and Bcl2, which are crucial for central memory (67, 69, 70). This is possible thanks to a feedback loop where NFκB-Pim1K- Eomes drive a continuum of NFκB signals that extend beyond the peak of the immune response. These proteins also ensure memory maintenance, as memory T cells devoid of either of these failed to survive and respond (67). Whether NFκB signaling has a distinct way to regulate resident memory is unknown. NFκB signaling is also an important driver of inflammation with broad effects. From the induction of pro-inflammatory cytokines (IL-6, etc) to the signaling by inflammatory cytokines (i.e. TNF etc), NFκB holds the potential to inhibit [TGFβ (74)] or potentiate [IL-33 (148)] tissue signals that are essential for CD8 T_{RM} [reviewed in (149)]. Although still unexplored, our previous findings and the fact that Eomes negatively modulates CD8 resident memory development (40), strongly suggest that NFκB may be an important regulator of CD8 T_{RM}.

It is also important to mention that TCR signals are not sufficient for CD8 T cell memory and are often tuned by other environmental signals (**Figure 1**). This is the case of inflammatory cytokines IL-12 (102), IL-10 (150) or IL-21 (108) and metabolic signals (151). The metabolic signaling pathway, mTOR, which can also be activated by TCR and IL-12 (152), has been linked to CD8 T_{RM} (153). Although, whether mTOR impacts on migration to tissue and/or T_{RM} survival is still unclear.

Another important question to answer is when antigenic signals are required for establishing resident T memory. Besides the obvious need for antigenic signals to activate naïve T cells, it is widely accepted now that effector T cells that migrate from the draining lymph node to the tissue need to receive a second antigenic hit in the tissue and then, further differentiate into T_{RM} (33, 154). Yet, depending on the tissue the continuous need to maintain antigenic signals to avoid the erosion of T_{RM} remains contentious. Thus, several studies support that antigenic signals are required in brain, lung, female reproductive tract and skin (155–159) to accumulate T_{RM} while in other tissues, re-exposure to antigen may be dispensable (42, 45, 157, 160). These studies only referred to cognate pathogenic antigen and did not address whether local antigenic signals were required once T_{RM} had already been established. Moreover, while it has been shown that CD8 T cell memory does not require self-peptide-MHC signals for its maintenance or establishment (9, 161, 162), the role of self-peptide-MHC in the context of resident memory has not been sufficiently explored yet.

CONCLUSION

CD8 T_{RM} are a critical first line of defense against pathogen infections and a promising tool in the fight against tumors. However, the development of CD8 resident memory requires a complex milieu of signals both from the tissues such as TGFβ, IL-33, and IL-15 and from inflammatory cytokines including IL-12 and TNF. Not only are multiple signals required, as this review discusses, specific quantities and timing of the signals are likely to be necessary. While these signals contribute to the development of CD8 resident memory, excessive amounts of some inflammatory

cytokines may also limit the differentiation of CD8 T_{RM}. Moreover, pharmaceutical treatments such as TNF blockade or other anti-inflammatory regimes may interfere with the development of the regulation of these signals and could possibly alter the development of CD8 T_{RM}. As the transcriptional and epigenetic mechanisms that regulate CD8 T_{RM} are becoming clearer, it is also critical that the field puts the effort to fully understand biochemically how tuning antigen, inflammatory and local tissue signals in time affect T_{RM}. This information can be extremely valuable to the treatment of diseases where T_{RM} are involved (infection, cancer, autoimmunity, allergies and transplantation).

AUTHOR CONTRIBUTIONS

CJP wrote and edited the manuscript as well as organized the review. MAD edited and contributed to the discussion of the manuscript. ET wrote, edited, and contributed to the discussion of the manuscript. All authors contributed to the article and approved the submitted version.

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