# Skin Tissue-Resident Memory T Cells

Department of Dermatology, Cutaneous Biology Research Institute,
Yonsei University College of Medicine, Seoul, Korea

**Chang Ook Park**

Memory T cells provide rapid and highly effective protective immunity to previously encountered antigens derived from pathogen, tumor, or environmental proteins. It was previously thought that T cells consisted of two major subsets: central memory T cells ($T_{CM}$) and effector memory T cells ($T_{EM}$)[1]. $T_{CM}$ express the chemokine receptor CCR7 and the vascular addressin L selectin (CD62L), permitting them to access and enter lymph nodes from blood. $T_{EM}$ express low levels of CCR7 and CD62L but have receptors that allow them to access peripheral tissues (e.g., the E selectin ligand Cutaneous Lymphocyte Antigen, or CLA) which grants them access to the skin, and $\alpha 4\beta 7$ which is an integrin that allows them access to the gut[2,3].

Over the past decade, it has become clear that there is another important subset of memory T cells–tissue resident memory T cells, or $T_{RM}$[4]. $T_{RM}$ reside in epithelial barrier tissues at the interface between the host and the environment, such as the gastrointestinal tract, respiratory tract, reproductive tract, and skin. $T_{RM}$ can respond rapidly to pathogen challenge at these sites without recruitment of T cells from the blood[4,5]. They thus mediate the rapid protective immunity that is the hallmark of adaptive immune memory[5]. $T_{RM}$ in a tissue are enriched for T cells specific for pathogens and other antigens that have been encountered previously through that barrier epithelium. Thus, the TCR repertoire of skin $T_{RM}$ is different from lung $T_{RM}$, and both are different from gut $T_{RM}$[6]. However, $T_{RM}$ are not simply memory T cells in an unexpected location; rather, they have a transcriptional program that distinguishes them from peripheral blood $T_{EM}$ and $T_{CM}$[7].

The cell signaling interactions that maintain $T_{RM}$ in their resident tissues is the subject of much investigation. The role of $T_{RM}$ in human tissue specific immune and inflammatory diseases is just beginning to be appreciated[6]. In addition while there is good logic for $T_{RM}$ to be stationed at our interfaces with the environment, $T_{RM}$ have also been found in brain, kidney, joint, and other non-barrier tissues. $T_{RM}$ that appear in non-barrier tissues have similar transcriptional programs[8], and their biology and behavior make it likely that they play a role in chronic relapsing and remitting diseases of non-barrier tissues.

## Common features of $T_{rm}$ in barrier tissues

$T_{RM}$ are characterized by their inability to re-circulate between tissue, lymph node, and blood[5,9-13], although understanding the factors that help them achieve this is an active area of research. The glycoprotein CD69 is a marker of $T_{RM}$, and is expressed on $T_{RM}$ in skin, lung, GI tract, and everywhere $T_{RM}$ have been identified[5-7,14-17]. CD69 was originally thought to be a marker of recent T cell activation in the lymph node;[18] however most $T_{RM}$ in tissues are at rest. CD69 appears to be involved in peripheral tissue retention of $T_{RM}$ which appears to involve the downregulation of the G protein coupled receptor for sphingosine 1 phosphate (S1P1)[19]. There is a gradient of levels of sphingosine 1 phosphate in the body in humans and mouse, with the lowest levels in peripheral tissue, intermediate levels in lymph node, and the highest levels in blood[17,20,21]. These S1P gradients normally function to guide T cells out of tissues to lymph node, and out of lymph nodes into blood. Expression of CD69 by $T_{RM}$ interferes with cell surface expression and function of S1P1, thus blocking the capacity of these T cells to sense S1P gradients and supporting their stationary nature[17]. The transcription factor Kruppel-like Factor 2, which normally enhances S1P1 expression, is downregulated in $T_{RM}$, thus indirectly enhancing CD69 expression[20]. The mechanism by which CD69 and S1P1 compete with each other for cell surface expression is not completely understood[21].

The chemokine receptor CCR7 is another G protein coupled receptor that senses molecular gradients of its ligands CCL19 and CCL21, and directs T cells and dendritic cells from skin to lymph node via afferent lymphatics[22]. Expression of CCR7 allows T cells to migrate in response to gradients of its chemokine ligands, which are normally not abundant in tissue but are at their highest levels in lymph node and afferent lymphatics. It was recently shown in a mouse model that CD4+ T cells in skin require CCR7 to migrate to afferent lymphatics, and that blocking CCR7 expression prevented T cells from leaving skin[23]. In human skin, expression of CCR7 was seen on a population of T cells that migrated out of skin (so called T migratory memory or $T_{MM}$ cells), while CCR7- T cells remained in skin as $T_{RM}$[24]. The relative contributions of S1P1 and CCR7 expression on T cells to migration out of tissues have not been determined.

The integrin CD103 (also known as $\alpha$E, and which pairs with $\beta$7) is another marker of $T_{RM}$; however, its expression is more predominant on CD8 than CD4 $T_{RM}$. It is a known ligand of E-cadherin, a homotypic adhesion molecule expressed by epithelial cells in barrier tissues[25]. In mouse models, CD8 T cells specific for HSV-1 enter the skin lacking CD103 expression, and then in response to epidermal TGF$\beta$ upregulate CD103[7]. CD103 is also found expressed by $T_{RM}$ in the lung and GI tract, and even in $T_{RM}$ in the brain upon CNS viral infection[8,15,26,27]. It is tempting to assume that $\alpha$E$\beta$7 on these cells is binding to epithelial cells via interactions with E cadherin. However, binding to E cadherin is not required for tissue residence, as CD103+ CD4 and CD8 $T_{RM}$ can be found in the dermis, and CD103+ dendritic cells are plentiful in the dermis without ever entering the epidermis[28]. While

E cadherin is expressed during brain development, it is absent in adult CNS tissue[29], despite abundant CD103 on brain CD8 $T_{RM}$. Thus while its role is incompletely understood, it does appear that CD103 expression is a marker of differentiation of $T_{RM}$[7] rather than a functional requirement for tissue residence. It is notable that CD103 $T_{RM}$ have less proliferative potential and more significant effector cytokine production capacity than CD103− T cells in several human and mouse models[8,15,26,27,30–32]. CD103 expression is also not a strict requirement for human skin cells being $T_{RM}$[24]. A recent report suggested that CD103− $T_{RM}$ may play a different role in gut in a mouse model, being generated in inflammatory microenvironments in the lamina propria and playing a unique role in controlling infection[33].

Less is known about CD4 $T_{RM}$ than CD8 $T_{RM}$ in part because these cells are less efficiently generated by viral infection in mouse models in which $T_{RM}$ have been most completely characterized. Studies of HSV infection of the female mouse reproductive tract suggest that local chemokine gradients from tissue mononuclear cells maintain CD4 $T_{RM}$ in place[34]. In skin, evidence suggests that CD4 $T_{RM}$ do not preferentially localize to the epidermis, and express lower levels of CD103 than CD8 T cells[5,32]. HSV specific CD4 T cells in mouse skin may be more mobile than CD8 $T_{RM}$, and limited to the dermis[35]. CD4 T cells in skin may express CCR7 and/or CD69[6,23]. In a recent study the authors treated highly immunocompromised NOD/Scid/IL-2R$\gamma$-deficient (NSG) mice bearing human skin xenografts with alemtuzumab (an antibody that binds human CD52, a molecule present on all T cells). This humanized antibody has been shown to deplete human T cells in blood but not tissue[24,36]. Two populations of CD4 T cells could be isolated from skin of these mice: those that expressed both CCR7 and L selectin (markers of $T_{CM}$), and those that expressed CCR7 but not CD69 (dubbed T migratory memory, or $T_{MM}$ by this group). The two populations of CD4 T cells that remained within the skin both expressed CD69 and lacked CCR7 (and were thus unresponsive to S1P and CCL19/21 gradients), and contained CD103+ and CD103− populations. Thus, four distinct populations of CD4 T cells could be identified in human skin, two of which were short term residents and could exit skin into blood, and two that were true $T_{RM}$[24].

## Skin $T_{RM}$

In 2006, it was discovered that normal resting human skin contained twice as many T cells as blood[6,37,38], and it is now appreciated that the majority of these cells are $T_{RM}$[24]. Thus, memory T cells previously generated in response to pathogens in the cutaneous environment are present in abundance in the skin, allowing for immediate response to pathogenic invasion[6]. These cells have a diverse T cell receptor repertoire and can be activated by pathogens at a much lower threshold than circulating T cells via the T cell receptor[37]. Moreover, they are heterogeneous; they include $CD4^+$ and $CD8^+$ T cells that produce IL-17, IFN$\gamma$, TNF$\alpha$, IL-9, IL-13, and other cytokines, alone or in

combination[6,37–42].

Human peripheral blood T cells enriched in skin (CLA), gut ($\alpha4\beta7$), or lung (CLA/$\alpha4\beta7$–) tropic memory T cells are specific to previously encountered pathogens of those tissues[42]. Mouse models have been instrumental in our understanding of skin $T_{RM}$. Early studies showed that mice transfused with transgenic T cells specific for HSV peptides, and then infected with HSV, showed that HSV specific CD8 T cells could be transferred from one mouse to another by a previously infected skin graft, and that these cells maintained their ability to clear virus upon challenge[32]. In another study it was shown that skin scarification by vaccinia virus (VACV) was far superior to other routes of immunization in generating skin resident CD8 T cells[43]. These investigators also showed that skin $T_{RM}$, in the absence of $T_{CM}$ and antibody, could clear virus on re–challenge. Furthermore, skin scarification generates lung $T_{RM}$ that, in the complete absence of circulating antibodies and $T_{CM}$ can partially protect naive mice from an otherwise lethal pulmonary challenge with VACV[43]. Thus, skin immunization can lead to widespread $T_{RM}$ throughout skin and also in distant barrier tissues. Another study showed that after HSV challenge in mice, CD8+ $T_{RM}$ migrate to the epidermis and acquire a sessile phenotype, while CD4+ $T_{RM}$ localize to the dermis and show greater mobility[35]. This is not only at the site of infection, but also at distant sites, and more CD8 $T_{RM}$ accumulate throughout the skin after multiple infections at distinct sites[5]. However, CD8 $T_{RM}$ do not re–circulate, and mice that contain $T_{CM}$ but lack $T_{RM}$ are cannot effectively clear VACV from skin, in contrast to mice that have immune skin $T_{RM}$[5].

Interestingly, $T_{RM}$ from skin, lung, and gut have transcriptomes that have common core features in mouse[7]. This same study showed that localization of $CD8^+$ $T_{RM}$ in the epidermis and CD103 expression of $T_{RM}$ was induced in the epidermis by TGF$\beta$, these $CD8^+$ $T_{RM}$ cells homed to epidermis by an uncharacterized chemokine mediated process[7]. Mouse CD8 $T_{RM}$ were also shown to occupy epidermal niches formerly filled by a population of T cells that seed the epidermis prior to birth––$\gamma\delta$ Dendritic EpidermalT Cells––, and when viewed by intravital microscopy moved laterally between keratinocytes, unlike sessile $\gamma\delta$ DETC. These CD8 $T_{RM}$ interacted transiently with Langerhans cells, suggesting that they were scanning the environment for antigen[44]. In humans, there are two isoforms of the dimeric CD8 molecule on T cells, composed of $\alpha\beta$ or $\alpha\alpha$ chains, respectively. After cutaneous HSV infection, CD8$\alpha\alpha$ $T_{RM}$ localize at the dermal epidermal junction. These cells, but not CD8$\alpha\beta$ T cells, protected against reactivation of HSV and lesion formation[45].

Such $T_{RM}$ cells have been studied most carefully in murine models of viral infection[46], which have focused on CD8+ T cells that produce IFNg on activation. Candida albicans is a dimorphic fungus to which humans are exposed early in life; by adulthood, it is part of the mycobiome of skin and other tissues. We adapted a highly reproducible model of skin infection with C. albicans. Prior to C. albicans infection, IL–17 producing cells in murine skin were composed entirely of dermal gd T cells. At 30 days after C. albicans infection, however, CD4 ab

T cells become the predominant producers of IL-17, replacing dermal gd T cells. By intravital microscopy, these cells resided in the papillary dermis in previously infected mice and were sessile. These $T_{RM}$ made frequent contacts with CD11c+ dermal dendritic cells (DCs). Next, we confirmed that normal human skin CD4 T cells also produced significant IL-17 when incubated with heat-killed C. albicans. These studies demonstrate that C. albicans infection of skin preferentially generates CD4+ IL-17 producing $T_{RM}$, which mediate durable protective immunity[47].

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