Skin as a Peripheral Lymphoid Organ: Revisiting the Concept of Skin-Associated Lymphoid Tissues

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Antigen presentation to T cells is essential for the induction of adaptive immunity. This event takes place not solely in the lymph node (LN) but also in the skin. Recent *in vivo* trafficking studies using Kaede-transgenic mice reveal that skin-homing effector memory T cells alter their effector function and homing ability by transitioning to a central memory T cell-like phenotype through antigen recognition that occurs in the skin. In addition, these cells travel back and forth between the skin and draining LNs. These studies are evocative of the classic concept of skin-associated lymphoid tissues and underscore the critical role of skin as a peripheral lymphoid organ.

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INTRODUCTION

T cells localize within lymphoid and non-lymphoid organs throughout the body. Naive T cells are produced and educated in the bone marrow and thymus, which constitute the primary lymphoid organs. The secondary lymphoid organs such as lymph nodes (LNs) and spleen provide an area for antigen presentation and induce clonal expansion of antigen-specific memory T cells. Memory T cells then migrate to non-lymphoid peripheral organs to scan for antigens. The current understanding is that secondary lymphoid organs are the dominant sites of T cell activation, whereas non-lymphoid counterparts, such as skin, are viewed as sites for effector T cell function only.

In specialized submucosal areas such as the gastrointestinal tract and bronchial pathway, specific ''sentinel'' lymphoid tissues called mucosa-associated lymphoid tissue (MALT) are organized to be on the alert for any invading pathogens (Brandtzaeg *et al.*, 1999). In humans, for example, the oral and nasal pharynx areas are monitored by the tonsils and adenoids, and lymphoid follicles are present in the normal bronchi. Single lymphoid follicles are also distributed throughout the intestine and, in the distal ileum, lymphoid follicles are grouped in large clusters termed Peyer's patches. These tissues serve as antigen presentation sites in non-lymphoid peripheral organs.

Around 1980, cutaneous immunologists elucidated some key findings: (1) Langerhans cells are bone marrow derived and capable of antigen presentation, (2) a fraction of T cells display high affinity for the skin, and (3) epidermal cells profoundly affect T cell maturation by producing multiple cytokines and chemokines (Stingl *et al.*, 1978; Rubenfeld *et al.*, 1981). On the basis of these findings, researchers have proposed that lymphoid tissues analogous to MALT in submucosal areas may form within the skin under the inflammatory condition. They offered the term SALT (skin-associated lymphoid tissues) for these putative skin-associated tissues (Streilein, 1978, 1983, 1985). Although SALT are still conceptual tissues, this hypothesis proposes that the skin is not merely a physical barrier but also a component of the lymphatic system.

Although both MALT and SALT provide defined sites for antigen presentation within peripheral organs, there are distinct functional differences between these tissues. MALT contains significant numbers of B cells and forms lymphoid follicles, whereas virtually all lymphocytes within SALT are T cells. MALT lymphoid follicles are surrounded by T-cell-rich areas in which high endothelial venules are embedded and serve as entry points for naive T cells. Therefore, MALT is equipped to provide a field for antigen presentation to naive T cells as well as other secondary lymphoid organs. In contrast, high endothelial venules are not found in the skin, and the T cells in SALT are antigen-experienced memory T cells. Thus, SALT may function as a peripheral lymphoid tissue to provide a function distinct from other secondary lymphoid organs and tissues including MALT.

To understand the function and mechanism of antigen presentation in SALT, we must expand our knowledge of the biology of skin-resident antigen-presenting cells (APCs) and skin-homing T cells. Until recently, Langerhans cells were thought to have a major role in antigen presentation not only in the LNs but also in the skin

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Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; LN, lymph node; MALT, mucosa-associated lymphoid tissue; SALT, skin-associated lymphoid tissue; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; T_{PM}, peripheral memory T cell

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(Toews *et al.*, 1980; Grabbe and Schwarz, 1998). However, the role of Langerhans cells has recently been challenged by studies using transgenic (Tg) mice in which Langerhans cells can be selectively depleted. Intriguingly, contact hypersensitivity was amplified rather than abrogated in the absence of Langerhans cells (Kaplan *et al.*, 2005). As for skin-homing T cells, although the homing mechanism of T cells in general is an active area of research, the kinetics and trafficking of skin-directed T cells remain largely unknown partly because of the lack of tools for analysis. Recently, time-lapse imaging of skin immune responses has been obtained using two-photon microscopy, which allows the motility of skin-resident APCs and skin-homing T cells to be directly visualized *in vivo*. In addition, unique Tg mice that express a photoconvertible fluorescent protein Kaede have been developed (Tomura *et al.*, 2008). In these Kaede Tg mice, living cells in a particular area at a specific time point can be labeled and tracked thereafter.

In this perspective, we first describe the properties of skin-resident APCs in terms of their heterogeneity, functions, and kinetics. We then address the effector function of T cells that migrate back and forth between the skin and LNs. Finally, we propose a serial development model for the generation of skin-associated memory T cells.

ANTIGEN-PRESENTING CELLS IN THE ANTIGEN-PRIMING PHASE

When the skin immune system encounters an antigen for the first time, Langerhans cells and dermal dendritic cells (DCs) capture the antigen and then migrate to the draining LNs and present antigen to naive T cells (Grabbe and Schwarz, 1998). Importantly, antigen presentation to naive T cells takes place only in skin-draining LNs but not within the skin, in contrast to mucosal epithelia that contain MALT. On the other hand, antigen presentation to memory T cells takes place within the skin (Clark, 2010; Figure 1a). Because no antigen-specific memory T cells exist in either the skin or blood at this time, subsequent skin inflammation is not induced. Such a scenario brings forward the concept of SALT whereby antigen presentation in the skin modulates T-cell functions. To establish the existence of SALT, it is important to elucidate whether memory T cells egress skin after antigen recognition. This issue will be discussed later.

Langerhans cell-depletion models have identified a subset of cells essential for antigen presentation in the LNs. Langerin is a C-type lectin that is selectively expressed in Langerhans cells and a minor population of dermal DCs. Dermal DCs are divided into several subsets by the differential expression of Langerin and other surface molecules (Bursch *et al.*, 2007; Ginhoux *et al.*, 2007; Poulin *et al.*, 2007). Transgenic mice engineered such that the diphtheria toxin receptor

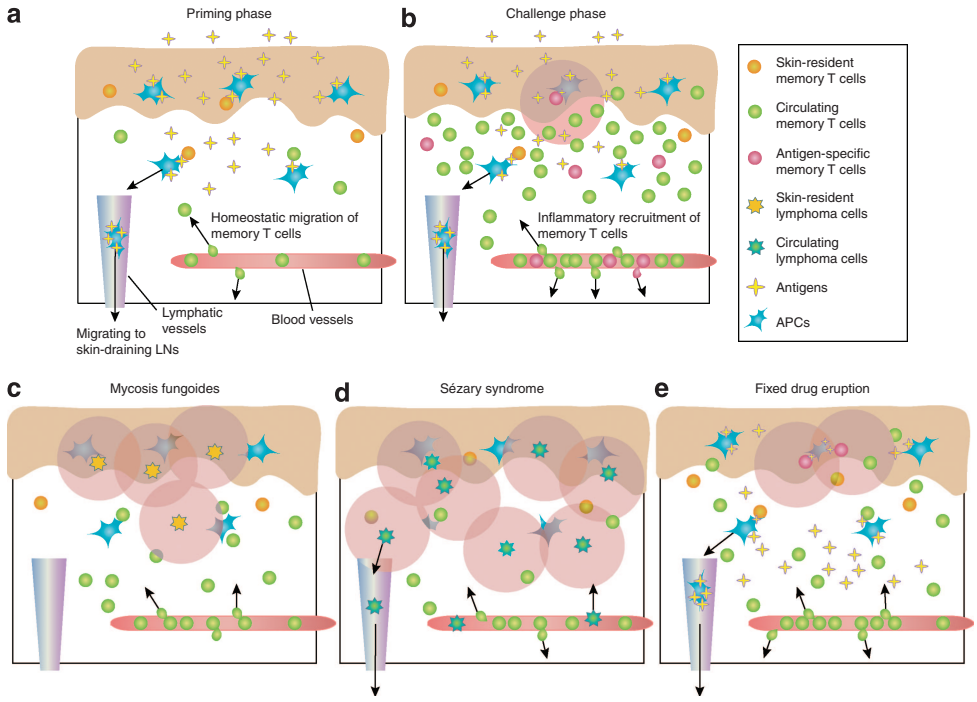

Figure 1. Antigen presentation to skin-resident and circulating memory T cells. (**a**) First encounter with antigens (priming phase). Antigens are captured by skin-resident APCs and presented to skin-resident and circulating memory T cells. The antigen-specific memory T cells are absent at this time point. At the same time, antigen-loaded APCs migrate to skin-draining lymph nodes to prime T cells. (**b**) Second encounter with antigens (challenge phase). The antigen-specific memory T cells induce subsequent inflammation. Antigen-specific and even nonspecific memory T cells, as well as other inflammatory cells, are recruited to the skin and activated by products of inflammation independently of antigen recognition. (**c**) Malignant cells in mycosis fungoides are resident in the skin; therefore, they are categorized as skin-resident lymphoma cells/T_{PM}. (**d**) The malignant cells in Sézary syndrome disseminate throughout the skin via blood and lymphatic circulation as circulating lymphoma cells/T_{CM}. (**e**) In the lesional skin of fixed drug eruption, the antigen-specific memory T cells exist only in the population of skin-resident T cells (T_{PM}), but not in the circulating memory T cells. APCs, antigen-presenting cells.

or diphtheria toxin A is inserted into the Langerin locus have been generated (Kaplan *et al.*, 2008), allowing specific depletion of Langerin-positive cells. Surprisingly, when Langerin-expressing cells were depleted, sensitization to hapten was established normally (Bennett *et al.*, 2005). Moreover, the contact hypersensitivity response was significantly enhanced in Langerin-diphtheria toxin A mice (Kaplan *et al.*, 2005). These findings indicate that Langerhans cells are dispensable for hapten presentation in the draining LNs.

Recently, the morphology and kinetics of skin APCs during the antigen-priming phase have been visualized using confocal or two-photon microscopy. Even under steady-state conditions, Langerhans cells exhibit a unique motion, called the *dendrite surveillance extension and retraction cycling habitude* (dSEARCH; Nishibu *et al.*, 2006). On activation via antigen invasion, Langerhans cells enhance dSEARCH motion to facilitate more efficient antigen sampling. Moreover, they elongate dendrites upward beyond the tight junctions to capture foreign antigens (Kubo *et al.*, 2009). Time-lapse imaging studies have revealed that the cell body of Langerhans cells is almost static even under inflammatory conditions (Ng *et al.*, 2008; Sen *et al.*, 2010). In contrast, dermal DCs display less dendritic processes and actively migrate at a velocity of up to 4 μm minutes^{-1} under steady-state conditions. Upon antigen penetration into the dermis, dermal DCs decrease their migration speed and adopt a morphology characterized by multiple dendritic processes. Thus, although both Langerhans cells and dermal DCs have the capacity for antigen presentation, they exhibit different kinetics. As most of the above studies used hapten application models, the question of whether Langerhans cells are dispensable for the presentation of other types of antigen such as protein/peptide or metals remains open.

ANTIGEN-PRESENTING CELLS IN THE ANTIGEN-CHALLENGE PHASE

Upon antigen re-exposure to the skin, the antigen is captured and presented by skin-resident APCs, such as Langerhans cells, dermal DCs, and/or possibly macrophages (Figure 1b). These APCs stimulate the antigen-specific memory T cells that reside in the skin, or have entered from blood circulation, and induce the production of inflammatory cytokines such as IL-4 and IFN-γ to initiate antigen-specific skin inflammation. This local inflammation then induces upregulation of adhesion molecules on the endothelium of local skin vessels resulting in the recruitment of nonspecific memory T cells and other immune cells from the blood (Mori *et al.*, 2008). The recruited memory T cells are activated to produce inflammatory cytokines independently of antigen recognition.

Which subsets of APCs have an essential role in antigen presentation within the skin during the challenge phase is poorly understood. While Langerhans cells are thought to have a major role, a recent report demonstrated that Langerhans cell-depleted mice develop an antigen-specific response comparable to non-depleted mice upon transfer of hapten-sensitized T cells (Kaplan *et al.*, 2005; Bennett *et al.*, 2007). Moreover, the monocyte-derived DCs, so-called inflammatory DCs, are recruited to the skin inflammatory site from blood circulation (Le Borgne *et al.*, 2006; Ginhoux *et al.*, 2007; Leon *et al.*, 2007). In addition to professional APCs, skin static cells such as keratinocytes and macrophages can contribute to antigen presentation in the skin. It is possible that the essential APCs in antigen presentation within the skin may vary depending on the type and size of antigen.

The immune response during the challenge phase has been also visualized by two-photon microscopy *in vivo*. Time-lapse imaging studies using phorbol-12-myristate-13-acetate application to the skin reveal that skin-migrating T cells display an active and random pattern of movement in the dermis with a mean velocity of 6 μm minutes^{-1} (Egawa *et al.*, 2011). In contrast, in the contact hypersensitivity model with 2,4-dinitro-1-fluoro-benzene, 2,4-dinitro-1-fluoro-benzene-sensitized T cells halted migration for more than 1 hour in the vicinity of APCs. A similar observation was reported with a peptide-induced skin delayed-type hypersensitivity model (Matheu *et al.*, 2008). Imaging tools also aided identification of a subset of APCs essential for the challenge phase. The system consisted of labeled Langerhans cells and dermal DCs obtained from CD11c-YFP mice, in which a yellow fluorescent protein (YFP) complementary DNA is inserted into the CD11c locus along with dermal phagocytes such as DCs and macrophages labeled by intradermal injection of fluorescent-labeled antigens. Using this system, the interactions of APCs and T cells in the skin were examined and long-term interactions between skin-migrating T cells and DCs and phagocytes could be demonstrated (Matheu *et al.*, 2008; Egawa *et al.*, 2011). These studies verified the existence of T cell–APC interactions in the skin and demonstrated that antigen presentation in the skin is facilitated not only by DCs but also by other phagocytic cells as well.

TISSUE-SPECIFIC HOMING ABILITY OF T CELLS

The T-cell homing system is tightly regulated by the expression of adhesion molecules and chemokine receptors called addressins. T cell subsets that have high affinity for the skin and gut, as well as for secondary lymphoid organs, have been identified (Table 1). Thus, it is clear that defending the outermost membranes, the skin and gut, is a high priority for the immune system.

Naive T cells are found primarily within the blood and secondary lymphoid organs. The expression of CD62L (also known as L-selectin) and CCR7 supports the migration of naive T cells into secondary lymphoid organs where they encounter antigen-bearing APCs from peripheral tissues. When naive T cells recognize their cognate antigen, they differentiate into effector memory T cells (T_{EM}). During this differentiation process, T cells are imprinted to express tissue-specific homing addressins, while losing CD62L and CCR7 expression, and to circulate in the blood and peripheral tissues. Most T_{EM} disappear within several weeks following the

Table 1. Receptors involved in tissue-specific homing

	Cell type	Receptor	Ligand
Peripheral LNs	Naive T, T_{CM}	CCR7	CCL19, CCL21
	Naive T, T_{CM}	CD62L	sLex
Gut	T_{EM}	CCR9	CCL25
	T_{EM}	α4β7-Integrin	MAdCAM-1
Skin	T_{EM}	CLA	E-selectin
	T_{EM} (Th1)	CXCR3	CXCL9, CXCL10
	T_{EM} (Th2)	CCR4	CCL17, CCL21
	T_{EM} (Th2)	CCR10	CCL27, CCL28
	T_{EM} (Th2)	CCR8	CCL8

Abbreviations: CLA, cutaneous lymphocyte-associated antigen; LNs, lymph nodes; MAdCAM-1, mucosal vascular addressin cell adhesion molecule-1; sLex, sialylated Lewis x; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; Th1, T helper 1; Th2, T helper 2; Th17, T helper 17.

peak of the immune response, and a second population, central memory T cells (T_{CM}), becomes predominant. Analogous to the homing mechanism used by naive T cells, T_{CM} express CD62L and CCR7, and preferentially circulate through secondary lymphoid tissues. This memory T cell subset contributes to long-lasting immunological memory. T_{CM} have lower levels of effector functions, but can proliferate vigorously upon antigen rechallenge. When compared with naive T cells, T_{CM} are less dependent on costimulation, thus providing more effective feedback to secondary antigen stimulation.

T_{EM} that infiltrate the skin express a unique adhesion molecule, cutaneous lymphocyte-associated antigen (CLA). The expression of CLA is defined by its reactivity with a unique mAb HECA-452, which recognizes glycosylated P-selectin glycoprotein ligand-1; Fuhlbrigge *et al.*, 1997). E-selectin, a ligand of CLA, is expressed by post-capillary venules in the skin and is upregulated in response to inflammation (Chong *et al.*, 2004). On interaction with E-selectin, CLA-bearing cells are able to transmigrate into the skin. Skin-homing memory T cells also express specific chemokine receptors: CXCR3, CCR4, CCR6, CCR10, and CCR8. CXCR3 and its CXC ligands have a major role in T helper 1 (Th1)-induced inflammatory processes. CCR4 and CCR10 are mainly expressed on Th2 cells, whereas CCR6 is expressed predominantly on Th17 cells (Campbell *et al.*, 1999; Homey *et al.*, 2002; Singh *et al.*, 2008). CCR8 is a newly identified skin-homing chemokine receptor expressed on an IL-5-enriched subset of Th2 cells (Islam *et al.*, 2011). Memory T cells that express α4β7-integrin and CCR9, on the other hand, have high affinity to intestinal mucosa.

These tissue-specific homing abilities of memory T cells are also functional in lymphoma cells. In cutaneous T cell lymphomas (CTCL), including mycosis fungoides and Sézary syndrome, most lymphoma cells are $CLA^+CD4^+CD45RO^+CCR4^+$ (i.e., a subset of Th2-like memory T cells) and extensively infiltrate the skin, but rarely invade mucosal epithelia. Thus, it is plausible that an anti-CCR4 mAb may serve as a therapeutic modality for the treatment of cutaneous T cell lymphomas.

TRACKING T CELL MOBILITY: DIRECT EVIDENCE OF T CELL MIGRATION FROM SKIN TO DRAINING LNs

The fate of skin-directed memory T cells is, at this point in time, largely unknown, and a majority of these cells progress to apoptosis after termination of skin inflammation. Recently, the trafficking of memory T cells between the skin and draining LNs has been examined *in vivo* using Kaede protein. Kaede protein is a newly developed photoconvertible fluorescent protein that can change emission spectra in response to light exposure (Figure 2a). Kaede means maple in Japanese, and the Japanese maple leaves turn from green to red in the autumn. This protein was cloned from stony coral, *Trachyphyllia geoffroyi*, and contains an internal tripeptide, His^{62}-Tyr^{63}-Gly^{64}, which serves as a green-emitting chromophore, but undergoes photocleavage at His^{62} upon irradiation with violet light (436 nm), thereby creating a new red-emitting chromophore (Mizuno *et al.*, 2003). In Kaede-Tg mice, all cell types constitutively exhibit Kaede-green fluorescent signals. Immediately after violet light exposure to the skin, cells in the exposed area began to emit Kaede-red fluorescent signals, thus labeling *in vivo* skin T cells under physiological conditions.

By using Kaede-Tg mice, we have demonstrated that approximately 5% of $CD11c^+$ cells and 0.5–1% of $CD4^+$ T cells in the skin-draining LNs are skin-derived cells, suggesting that memory T cells as well as cutaneous DCs can constantly migrate from the skin to draining LNs, even under steady-state conditions (Tomura *et al.*, 2010). Importantly, all skin-derived Kaede-red T cells express not only CD44, a marker of memory T cells in mice, but also CCR7 and CD62L, suggesting that they exhibit a unique homing receptor expression profile that resembles that of T_{CM}. The trafficking of skin-associated memory T cells was also evaluated in the inflammatory state (Figure 2b). Kaede-Tg mice were sensitized with hapten on a dorsal area of skin and challenged with the same hapten on abdominal skin. The antigen-challenged site was then exposed to violet light. After the photoconversion, the number of Kaede-red cells in the draining LNs was increased by approximately 10 times over steady state, reflecting the accumulation of memory T cells into the abdominal skin. Surprisingly, when another site, the ear skin, was rechallenged, Kaede-red $CD4^+$ T cells were detected both in the blood and in the ear skin (Figure 2c). These findings indicate that a portion of skin-directed T_{EM} recover LN homing ability, CCR7 and CD62L

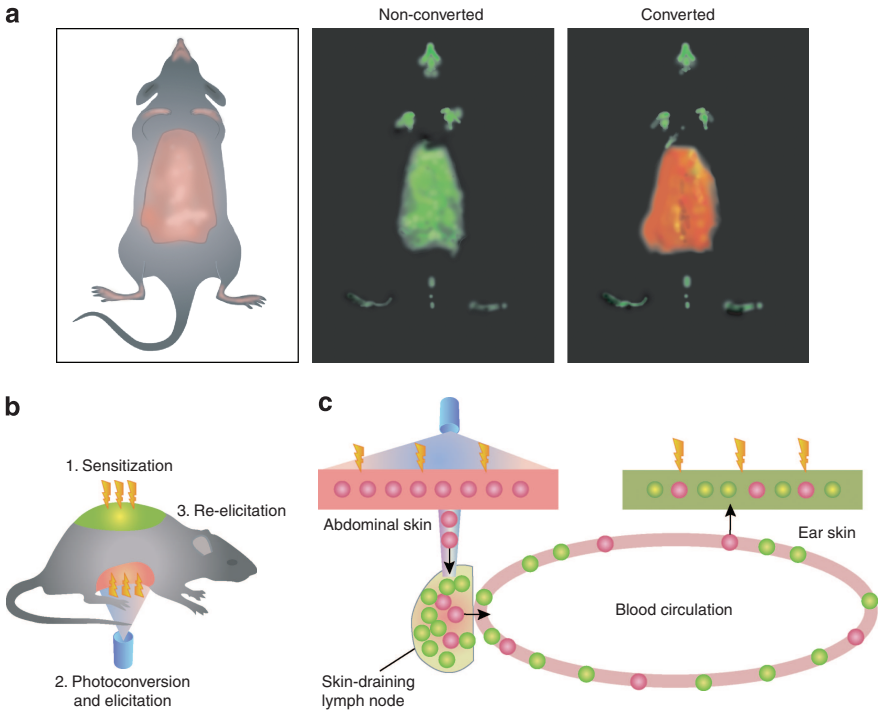

Figure 2. Photoconversion of Kaede-transgenic mice. (**a**) Kaede-transgenic (Tg) mice are photoconverted with violet light exposure. (**b**) The dorsal skin of Kaede-Tg mice are sensitized with hapten and are challenged at another site (abdomen) on the skin with the same hapten and exposed to violet light. The fluorescent color in all cells in the exposed skin changes to red (Kaede-red). (**c**) Kaede-red cells enter the blood circulation and remigrate into the ear skin in response to hapten rechallenge.

expression, and return to skin-draining LNs especially in the inflammatory state. Moreover, these cells re-enter the blood circulation system, and even more surprisingly, recover skin-homing adressins, or produce skin-homing T_{EM} upon antigen rechallenge.

MODULATION OF MEMORY T CELL ACTIVITY WITHIN THE SKIN

The above findings raise an additional question regarding the role of these Kaede-red memory T cells migrating from the skin into the LNs. This issue is addressed from the perspective of Foxp3$^+$ T regulatory cells (Tregs). Under steady-state conditions, a significant fraction (over 20%) of skin-derived CD4+ T cells in the draining LNs were Tregs. When skin inflammation was induced by hapten application, a striking (approximately 20-fold) increase was observed in the number of skin-derived Tregs in the LNs. In addition, skin-derived Tregs have a stronger capacity for inhibition of hapten-specific T cell proliferation than do LN-resident Tregs. Importantly, skin-derived Tregs contain a CD25^{high} fraction that expresses much higher levels of IL-10, transforming growth factor-β1, and cytotoxic T-lymphocyte-associated antigen-4 compared with LN-resident Tregs, suggesting that skin-derived Tregs have strong immunosuppressive activity. In fact, in the absence of Tregs, DCs, and CD4$^+$ naive T cells form stable cell–cell contacts in an antigen-specific manner in the LNs. Tregs interrupt this cell–cell contact formation primarily by interacting with DCs (Tadokoro *et al.*, 2006). Moreover, when Tregs were depleted in the elicitation phase of a contact hypersensitivity response, the skin inflammation was significantly prolonged and exacerbated (Tomura *et al.*, 2010). These findings indicate that skin inflammation exacerbates Treg migration to the skin, and that some of the skin Tregs enhance their immunosuppressive activity within the skin, then return to draining LNs, and affect termination of immune responses. This finding raises the possibility that the skin is a lymphoid organ wherein memory T cell activities are modulated.

PERIPHERAL MEMORY T CELLS, A NEW CONCEPT FOR SKIN-RESIDENT MEMORY T CELLS

It is believed that memory T cells migrate into the peripheral tissues only under conditions of active inflammation (Kupper and Fuhlbrigge, 2004). Recent studies, however, have revealed that, even in the absence of inflammatory stimuli, many memory T cells enter the peripheral tissues to scan for antigens (Clark, 2010; Figure 1a). In fact, an adult human skin contains ~20 billion T cells, which corresponds to nearly twice the number of blood-circulating T cells. Most of the T cells (>95%) in normal human skin are CLA$^+$CD45RO$^+$ memory T cells, and a subset express skin-homing addressins such as CCR4, CCR8, and CCR10. The majority of T cells (>80%) in the skin lack CCR7 and CD62L expression, suggesting that their identity is T_{EM}. Importantly, significant fractions (5–20%) of T cells residing in human skin are Foxp3$^+$ Tregs. Tregs produce IL-10 on antigen recognition in the skin to limit the function of other subsets of effector T cells (McLachlan

et al., 2009). In fact, Foxp3-deficient scurfy mice develop autoimmune inflammatory lesions mainly on the skin and lungs (Chen *et al.*, 2005). Moreover, the skin inflammation of scurfy mice was not rescued by the transfer of Tregs, the skin-homing ability of which was impaired because of α-1, 3-fucosyltransferase VII deficiency (Dudda *et al.*, 2008). Thus, skin-resident T cells are essential for maintaining normal immune homeostasis of the skin.

A series of studies examining herpes simplex virus (HSV) infection in mouse skin have provided information about the characteristics of skin-resident memory T cells (Gebhardt *et al.*, 2009). Such cells are generated during the primary infection of HSV, and remain in the same location for over 100 days. Most of these T cells lack CD62L expression and fail to re-enter the blood circulation. They are present in highest numbers at the primary site of infection, but are also detectable at other sites in the skin. Re-infection with HSV leads to local proliferation of these resident T cells and also recruitment of T_{EM} from the blood. In humans, a similar HSV-specific skin-resident memory T cell population was detected more than 2 months after the clearance of a primary HSV infection (Zhu *et al.*, 2007).

There are therefore two distinct populations of memory T cell subsets, tissue-resident memory T cells and circulating blood T_{EM}, both capable of responding to antigen rechallenge (Figure 1a and b). In mice, populations of tissue-resident memory T cells have been described in the skin, gut, and lungs. $CD8^+$ T cells persist in the lung several months after recovery from viral infections (Hogan *et al.*, 2001). In the gut, resident T cells are scarce at birth but progressively increase in number throughout life. Here, to distinguish from circulating short-lived T_{EM}, we designate such tissue-resident memory T cells as peripheral memory T cells (T_{PM}). T_{PM} comprise a functionally distinct, non-migratory population and persist long term in peripheral tissues to provide effective protection against local antigen rechallenge. The precise molecular mechanism of the induction and maintenance of T_{PM} remains an unsolved and important issue.

T_{PM} AND CLINICAL RELEVANCE

Although the mechanism by which T_{PM} are produced and maintained in peripheral tissues is not clear, the different behaviors of T_{CM} and T_{PM} may be demonstrated by the clinical manifestations that we observe in cutaneous T cell lymphoma (Figure 1c and d). In patients with mycosis fungoides, lymphoma cells are confined to the skin and present stable patches and plaques. In Sézary syndrome, lymphoma cells migrate throughout the skin and also colonize the blood and LNs, as well as present erythroderma and lymphadenopathy. Recent studies revealed that lymphoma cells in mycosis fungoides are $CLA^+CCR4^+CCR7^-$, whereas lymphoma cells in Sézary syndrome are $CLA^+CCR4^+CCR7^+$ (Campbell *et al.*, 2010). Thus, lymphoma cells in mycosis fungoides are of the T_{PM} phenotype and sessile by nature, and rarely translocate to blood/lymphatic circulation. In contrast, lymphoma cells in Sézary syndrome are of the T_{CM} phenotype, and disseminate throughout the skin and LNs via blood and lymphatic circulation.

Fixed drug eruption is another distinctive model that demonstrates the nature of skin-resident T_{PM} (Figure 1e). It occurs following ingestion of a causative drug, and spontaneously resolves when the drug is discontinued. Intriguingly, it recurs in the same location years or even decades later when the drug is taken again. The fixed location of this eruption may reflect the sessile nature of skin-resident memory T cells. In fact, a large population of IFN-γ-producing $CD8^+$ memory T cells are detected even in resting fixed drug eruption lesions (Teraki and Shiohara, 2003). Consistent with this, positive patch test reactions are observed only at the site of previous lesions, which harbor significant numbers of intraepidermal $CD8^+$ T cells (Shiohara, 2009). These findings indicate that T_{PM} may retain within the epidermis for long periods of time without antigen stimulation. The involvement of T_{PM} in the pathogenesis of other skin diseases that present sessile lesion, including psoriasis and prurigo nodularis, should be addressed in the future study.

CONCLUSION AND PERSPECTIVE—A NEW MODEL FOR MEMORY T CELL DEVELOPMENT

The memory T cell pool functions as a repository of antigen-experienced T cells that accumulate throughout life. As described above, memory T cells contain distinct populations of T_{EM}, T_{CM}, and T_{PM} T cells that are characterized by distinct homing abilities and effector functions. The tissue-resident T_{PM} and circulating T_{EM} exhibit an immediate response to antigen invasion in peripheral tissues. On the other hand, T_{CM} that home the T cell areas of secondary lymphoid organs rapidly proliferate to become T_{EM} in response to antigen stimulation (Veiga-Fernandes *et al.*, 2000).

Although the existence of several subsets of memory T cells is now well documented, the precise mechanism of memory T cell differentiation is still under debate. Whether these subsets develop in parallel or in series is also unknown. The current idea is that T cell receptor (TCR) signal strength is a major factor in determining T cell differentiation (Figure 3a; Sallusto *et al.*, 2004). By using an *in vivo* priming system in which the strength of antigenic stimulation is tightly controlled, a strong or prolonged antigen signal was demonstrated to efficiently generate T_{EM}, whereas insufficient or short antigen presentation led to the development of T_{CM} (Iezzi *et al.*, 2001). According to this model, T_{EM} and T_{CM} develop in parallel because TCR stimulation is a stochastic event. This model is, however, insufficient because antigen presentation within peripheral tissues is not taken into account and also because it does not elucidate a mechanism for T_{PM} differentiation.

In this article, we have explored the importance of antigen presentation in peripheral tissues, especially within the skin. A trafficking study with Kaede-Tg mice revealed that skin-directed T_{EM} can transition to a T_{CM}-like phenotype upon antigen recognition within the skin and can then return to the skin-draining LNs. Moreover, these T_{CM}-like

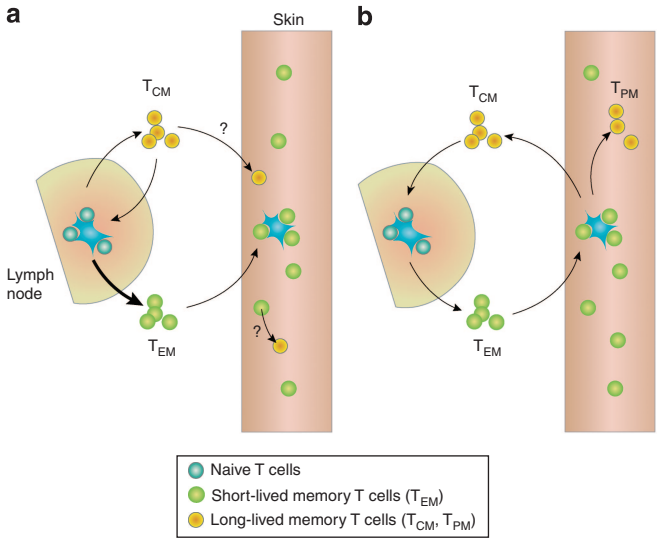

Figure 3. Two models for memory T cell generation. (**a**) Signal strength model. Differentiation to T_{EM} and T_{CM} takes place in parallel and depends on the strength of T cell receptor signaling. The strength of antigenic stimulation is indicated by the thickness of arrows. (**b**) Serial development model. T_{EM} are generated in secondary lymphoid organs, and then transition into T_{CM} and T_{PM} through antigen recognition in peripheral tissues. T_{CM}, central memory T cell; T_{EM}, effector memory T cell; T_{PM}, peripheral memory T cell.

cells circulate in the blood and may, upon antigen rechallenge, produce T_{EM} that again infiltrate inflamed skin. Consistent with this finding, interconversion from T_{EM} to T_{CM} was demonstrated in adoptive transfer experiments in mice (Wherry *et al.*, 2003). Considering these findings, it is tempting for us to envision a novel model of memory T cell development, which we call the serial development model (Figure 3b). Naive T cells that recognize cognate antigens within secondary lymphoid organs differentiate into T_{EM}. In this model, T_{EM} are defined as short-lived memory T cells that have experienced cognate antigen in secondary lymphoid organs, but not in peripheral tissues. T_{EM} upregulate the expression of tissue-homing addressins and lose CCR7 and CD62L expression. These cells circulate between the blood and peripheral tissues and disappear within several weeks unless they encounter antigen in peripheral tissues. Once T_{EM} recognize cognate antigen within peripheral tissues, they transition into long-lived memory T cell subsets, such as T_{CM} and T_{PM}. During this differentiation process, some T_{EM} reacquire CCR7 and CD62L expression and differentiate into T_{CM}, whereas some retain their homing receptor profile and persist in peripheral tissues as T_{PM}. Because most of the memory T cells recruited to inflamed skin are nonspecific bystander cells (Figure 1b), most skin-homing memory T cells disappear when inflammation is terminated. If these assumptions are valid, it follows that the context of TCR activation is important for T cell development. Initial TCR activation within the primary lymphoid organ induces maturation into naive T cells (positive selection). A second TCR activation within secondary lymphoid organs activates naive T cells to produce T_{EM}, and a third TCR activation within peripheral tissues may induce T_{CM} and T_{PM} development.

In summary, we now must determine the mechanism of antigen presentation within the skin, which can affect the function and differentiation of memory T cells. The classic concept of SALT is based on multiple studies that have revealed the existence of T cells and APCs in the skin. When Streilein developed the concept of SALT, he thought that the skin, at least under homeostatic conditions, is a non-lymphoid organ that is only functionally connected with the draining LNs. Recent studies have established that the interaction of T cells and skin-resident APCs are real events and have demonstrated that a subset of T cells that migrate to the skin can egress and return to skin-draining LNs, and even more, circulate in the blood and other tissues. These findings flesh out the concept of SALT with the consequence that, even under the homeostatic condition, skin is an active organ of immune system and immune reactions in the skin could influence systemic immunity. Thus, the study of T cell biology in the skin may be essential for our understanding of the pathogenic state of other tissues.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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