Multilayered T-cell memory in human skin

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The skin represents a highly complex immunological microenvironment capable of protecting the organism from infectious pathogens. Accordingly, the human skin harbors large quantities of immune cells, including billions of memory T cells (1,2). While some of these T cells are constantly recirculating through the skin and thus are in equilibrium with their counterparts in the blood, others remain permanently resident in the skin and never return to the circulation (2). A recent study published in *Science Translational Medicine* by Watanabe and colleagues (3) has shed new light on the composition of these recirculating and resident T-cell subsets in human skin, and has linked the malignant transformation of individual subsets to the variable clinical presentation of cutaneous T-cell lymphoma (CTCL).

Memory T cells provide long-lived pathogen-specific immunity in lymphoid and peripheral tissues, including barrier locations such as skin and mucosa. These tissues contain a combination of recirculating and permanently resident memory T (T_{RM}) cells and studies in mice have recently identified the latter (T_{RM} cells) as the central mediators of localized protective immunity (4-9). However, T_{RM} cells may also drive aberrant immune responses that are associated with autoimmunity, transplant rejection and malignancies (10,11). In skin for instance, such pathogenic T-cell responses are observed in psoriasis, alopecia, contact hypersensitivity and CTCL. Consistent with a T_{RM}-cell involvement, such diseases often present with chronic or recurrent lesions in fixed anatomical locations (10-14). While detailed information on the mode of tissue residency, *in situ* differentiation and protective function of T_{RM} cells

in skin has emerged from mouse studies (11,15,16), obvious technical and ethical constraints have thus far limited our knowledge about the relative proportions and effector activities of resident and recirculating T-cell subsets in human skin.

Watanabe and colleagues employed an elegant skin xenograft model to study T-cell dynamics in human skin (3). They transplanted human neonatal foreskin onto immune-deficient mice and, at the same time, transferred allogenic human peripheral blood cells from unrelated donors. The transferred T cells were activated in the skin grafts via allorecognition, which resulted in the induction of a dermatitis and subsequently, in the generation of various populations of graft-resident memory T-cells. A subset of these T cells up-regulated CD69 shortly after migration into the grafts and some of these cells co-expressed the integrin subunit CD103, thereby resembling the T_{RM} cells described in mouse skin (5,6). There were also T cells that displayed a central memory T-cell (T_{CM}) phenotype, as indicated by expression of the lymph node (LN)-targeting migration receptors CCR7 and L-selectin. Importantly, all subsets identified in human-engrafted mice were also detected in healthy adult skin not subjected to tissue transplantation, therefore further emphasizing the validity of the xenograft model.

A previous study by the same group has shown that circulating T cells in CTCL patients can be depleted with low doses of alemtuzumab, a humanized anti-CD52 antibody, while skin T_{RM} cells remain unaffected by this treatment (17). In the present study, Watanabe and colleagues found that the same was true for human-

engrafted mice. Alemtuzumab treatment lead to depletion of T cells from the blood but spared some of the T-cell subsets in the graft, making it possible to discriminate between recirculating and skin-resident memory T cells. Analysis of remaining and thus non-recirculating T_{RM} cells in alemtuzumab treated human-engrafted mice and CTCL patients revealed that virtually all of them expressed CD69. This is consistent with studies in mice, where T_{RM} cells commonly express CD69 (15,16). This molecule has been shown to promote prolonged effector T-cell retention in skin by interfering with the tissue exit receptor, sphingosine-1-phosphate receptor 1 (18). A fraction of both CD4+ helper and CD8+ killer T cells remaining in the grafts for at least 3 weeks of alemtuzumab treatment also expressed CD103, the α-subunit of the αEβ7 integrin thought to mediate tethering of T_{RM} cells to their microenvironment by binding to epithelial cells expressing the CD103 ligand, E-cadherin (15). Interestingly, CD103+ T_{RM} cells were enriched in the epidermal layer, although considerable populations of helper and killer CD103+ cells were also detected in the dermis. Given that a similar epidermotropism of CD103+ T_{RM} cells has also been described in mice (5,19,20), it is tempting to speculate that the dermal CD103+ cells may preferentially associate with skin appendages of epithelial origin, such as glands or hair follicles, and/or represent specialized subsets such as regulatory T cells, of which some express CD103 in human skin (21). Functional assays on isolated T cells further revealed that both CD103+ and CD103- T_{RM} subsets exhibited a heightened capacity to produce effector cytokines, but had lower proliferative potential when compared to their recirculating CD69- counterparts. This is an important piece of data fitting well with the overall concept that T_{RM} cells provide superior local immune defense.

Although not readily replicated in the xenotransplant model, the authors further identified a forth T-cell subset in the skin of healthy individuals and CTCL patients. This additional subset, termed migratory memory T cells (T_{MM}), expressed CCR7 but not L-selectin and therefore, differed from the CCR7+ L-selectin+ T_{CM} cells. Remarkably, these T_{MM} cells were the most abundant population amongst skin-tropic T cells in the blood of healthy individuals, as identified by their expression of the skin homing molecule, cutaneous leukocyte antigen. Importantly, T_{MM} cells were depleted in both the circulation and the skin of CTCL patients treated with alemtuzumab, although depletion from skin was much slower compared to that of TCM

cells. Thus, T_{MM} cells represented a recirculating T-cell subset with distinct migration kinetics. Furthermore, given that these cells lacked expression of L-selectin and therefore should be excluded from entering LNs from the blood, the authors speculated that T_{MM} cells might also have a specialized recirculation pattern. In support of this hypothesis, Watanabe and colleagues reported an intriguing link between the T_{CM}/T_{MM} phenotypes of malignant skin-tropic T-cell clones and the varying clinical pictures in CTCL patients.

CTCL can present as skin-limited forms, such as mycosis fungoides, or leukemic forms, including the Sézary syndrome (22). Patients with early stage mycosis fungoides have fixed skin lesions and transformed T-cell clones are usually absent from LNs and blood. By contrast, leukemic forms of CTCL present with more disseminated lesions with ill-defined borders that in their most extreme form can result in generalized erythroderma. The malignant T-cell clones in these patients are usually found in the blood and lymphoid tissues, including skin-draining as well as systemic LNs. Elegant earlier work by Clark and Kupper and colleagues has demonstrated that the leukemic CTCL forms are caused by recirculating skin-homing memory T cells, which can be successfully depleted by alemtuzumab treatment (17,23). Mycosis fungoides on the other hand originates from malignant T_{RM} cells that are unresponsive towards the same treatment (17,23). In the current study, Watanabe and colleagues analyzed malignant T-cell clones in leukemic CTCL patients with different clinical manifestations and found a pattern consistent with the proposed differential migration of T_{CM} and T_{MM} cells. Patients with malignant CCR7+ L-selectin+ T_{CM} cells showed diffuse skin lesions with local as well as systemic LN involvement. By contrast, patients harboring malignant clones with a predominantly CCR7+ L-selectin- T_{MM} phenotype displayed more discrete skin lesions, albeit with ill-defined borders, and had involvement of skin-draining, but not systemic LN. Thus, these clinical data are consistent with a scenario where T_{MM} cells migrate between the skin and blood via the lymphatics and skin-draining LNs. Importantly, however, the T_{MM} cells are excluded from entering lymphoid tissues draining other organs due to their lack of L-selectin expression and their inability to enter LN from the blood. Conversely, T_{CM} cells similarly recirculate between skin and blood but also access remote LNs via high endothelial venules in a L-selectin and CCR7-depedendent manner (*Figure 1*). Future studies will have to further validate this concept of specialized migratory
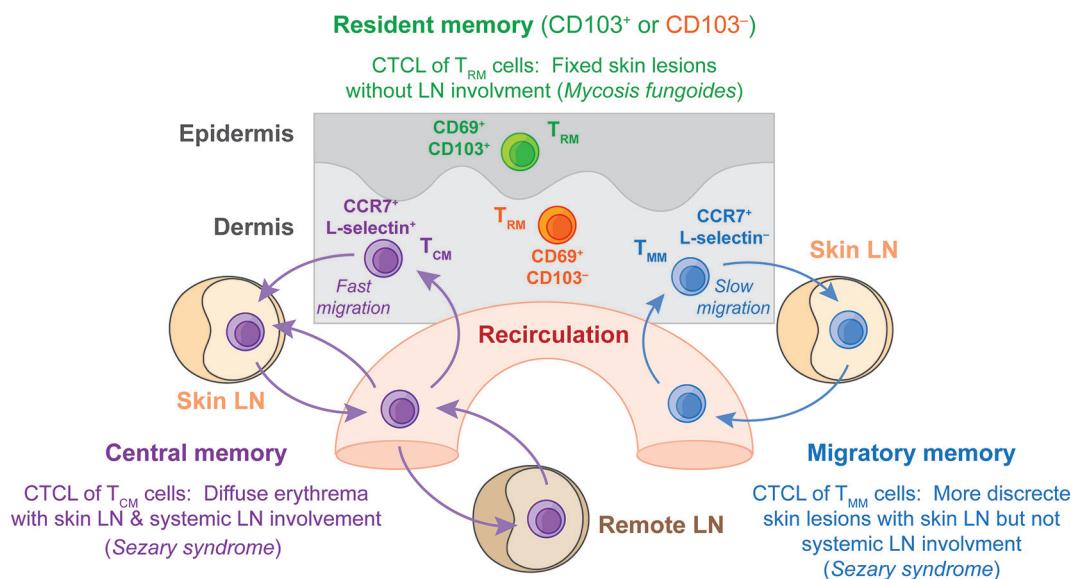
Figure 1 Four subsets of memory T cells with distinct recirculation patterns, effector functions and proliferative potential populate the human skin under normal conditions. Their malignant transformation can give rise to various forms of CTCL. Mycosis fungoides, a form of CTCL with fixed skin lesions and without LN involvement, is caused by transformed cells that resemble non-recirculating $CD69^+CD103^+$ (green) or $CD69^+CD103^-$ (orange) T_{RM} cells. By contrast, transformed T cells that recirculate through the body, albeit with subset-specific patterns, cause leukemic forms of CTCL. $CCR7^+L$-selectin$^+$ T_{CM} cells (purple) migrate rapidly through skin, draining LNs, blood and distal LNs, and can give rise to Sézary syndrome with diffuse erythema and generalized LN involvement. $CCR7^+L$-selectin$^-$ T_{MM} cells (blue) recirculate with slower kinetics and cannot enter distal LNs from the blood due to their lack of L-selectin expression. Accordingly, their malignant transformation may result in a Sézary syndrome with more discrete skin lesions with skin LN but not systemic LN involvement. CTCL, cutaneous T-cell lymphoma; T_{RM}, resident memory T; T_{CM}, central memory T-cell; LN, lymph node; T_{MM}, migratory memory T cells.

memory T cells for other organ systems and will have to investigate the contribution of skin-tropic T_{CM} and T_{MM} cells to other types of skin diseases.

Taken together, Watanabe and colleagues have made an important contribution to our understanding of T-cell responses in human skin. Careful interpretation of clinical data in combination with the use of sophisticated xenograft models allowed the authors to identify four distinct populations of skin T cells with vastly different migration patterns and effector potential (*Figure 1*). As well as extending our basic concepts of T-cell immune surveillance in skin, this work also highlights the predictive value that the identification of the various T-cell subsets has for therapy outcomes in patients with various forms of Sézary syndrome. Likewise, determining the contribution of these distinct memory subsets to the pathogenesis of other diseases may improve future treatments in T-cell mediated inflammatory skin conditions, such as psoriasis, vitiligo, alopecia or contact hypersensitivity. Finally, understanding the precise roles recirculating and resident

T cells play in immune defense against infectious diseases will be critical for the development of novel vaccination strategies that aim to protect the body's surfaces in skin and mucosa.

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Footnote

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