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Address correspondence to: Alexander Ploss or Charles M. Rice, Center for the Study of Hepatitis C, The Rockefeller University, 1230 York Avenue, Box 64, New York, NY 10065. Phone: 212.327.7066; Fax: 212.327.7048; E-mail: aploss@rockefeller.edu (A. Ploss). Phone: 212.327.7046; Fax: 212.327.7048; E-mail: ricec@rockefeller.edu (C.M. Rice).

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Bidirectional homing of Tregs between the skin and lymph nodes

Hironori Matsushima and Akira Takashima

Department of Medical Microbiology and Immunology, University of Toledo College of Medicine, Ohio.

Although several homing receptors are known to be differentially expressed by Tregs in lymphoid tissues compared with those found in peripheral tissues, it remains unclear whether these cells traffic between the two locations. In this issue of the JCI, Tomura et al. report steady-state Treg migration from the skin to draining LNs in mice. Furthermore, they report that not only does skin inflammation exacerbate LN-directed Treg homing, it also triggers reverse circulation of Tregs from LNs to skin, whereby these cells contribute to regulation of the immune response. These results now form a new framework for our understanding of Treg homing.

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Memory T cells are disseminated to lymphoid and nonlymphoid tissues throughout the body, and their migration to respective tissues is tightly regulated by adhesion mol-

ecules and chemokine receptors (Table 1). For example, memory T cells that infiltrate the skin express a unique adhesion molecule, known as cutaneous lymphocyte-associated antigen (CLA), which is produced from P-selectin glycoprotein ligand-1 through posttranscriptional carbohydrate modification by fucosyltransferase VII. Skin-homing memory T cells also express specific chemokine receptors: CCR4, CCR6, and CCR10. In contrast, memory T cells that preferentially circulate through lymphoid tissues express CD62 ligand (CD62L; also known as L-selectin) and CCR7 (1).



Table 1
Differential expression of homing receptors by skin-homing versus LN-homing memory T cells

Target tissue	Homing receptor	Corresponding ligand(s)
Skin	CLA	E-selectin
Skin	CCR4	CCL17, CCL22
Skin	CCR6	CCL20
Skin	CCR10	CCL27, CCL28
LN	CD62L	PNA ^d
LN	CCR7	CCL19, CCL21

PNA^d, peripheral lymph node addressin.

Skin-resident Treg populations

The entire skin surface of an adult human is populated with approximately 2×10^{10} T cells, a majority of which express CLA, CCR4, and CCR6. Since approximately 10^{10} T cells circulate in peripheral blood, it has been estimated that normal skin contains twice as many T cells as are present in the blood circulation (2). Importantly, significant fractions (5%–20%) of those human skin-resident T cells appear to represent Tregs based on the phenotype of CD4⁺CD25^{hi}CD69^{lo} and the expression of Foxp3, a master transcriptional regulator of Treg development and function (2, 3). Conversely, large fractions of blood-circulating human Tregs express CLA (80%) and CCR6 (73%) (4). Thus, the skin, an outermost organ constantly exposed to external insults, appears to serve as a major site for the immunosuppressive action of Tregs. In fact, Foxp3-deficient *scurfy* mice develop autoimmune inflammatory lesions primarily in the skin and lung (5).

Analogous to homing mechanisms for memory T cells, adhesion molecules and chemokine receptors regulate tissue-specific trafficking of Tregs as well. A majority of Tregs found in secondary lymphoid tissues express CD62L and CCR7. In adoptive transfer experiments, Tregs isolated from CD62L-deficient mice were far less efficient than WT Tregs in their homing to peripheral LNs and spleen as well as in suppressing the onset of acute graft-versus-host disease (6). Likewise, adoptively transferred CCR7^{-/-} Tregs failed to achieve LN-directed homing and, thus, to suppress antigen-specific proliferation of CD4⁺ naive T cells (7, 8). Similar observations have been made in NOD type 1 diabetes and cardiac allograft models (9, 10). Because CD62L^{-/-} Tregs and CCR7^{-/-} Tregs are both comparable to their WT counterparts with respect to their in vitro suppressive potentials, these observations

imply that CD62L/CCR7-dependent homing of Tregs to secondary lymphoid tissues is required for their in vivo protective function.

A distinct Treg population expressing CD103 (also known as integrin α_E) has been recently identified. These CD103⁺ Tregs are characterized by elevated expression of conventional memory T cell markers (e.g., CD44, CD54, CD29, and CD69) and selective migratory responses to inflammatory chemokines (11). Importantly, CD103 is expressed by skin-resident Tregs (which account for greater than 20% of CD4⁺ T cells in normal mouse skin), and its functional contribution has been demonstrated in antibody-blocking and CD103^{-/-} Treg-transfer experiments (12, 13). Upon intradermal OVA injection, adoptively transferred, OVA-reactive CD103⁺CD62L⁺ Tregs acquired the expression of CD103, CCR4, and CLA, while losing CCR7 expression (12). Moreover, CCR4^{-/-} Tregs and CLA^{-/-} Tregs both exhibited impaired skin homing, signifying the equally important roles of this chemokine recep-

tor and adhesion molecule (12, 14). The skin inflammation observed in the *scurfy* mouse is preventable by the transfer of WT Tregs, but not CLA^{-/-} Tregs (15). Homing of Tregs to *Leishmania major*-infected skin lesions requires not only CCR4, but also CCR5 (16). Thus, Tregs expressing selected homing receptors preferentially migrate to the skin, where they presumably suppress the activation of effector T cells recognizing autoantigens or innocuous environmental antigens.

Direct tracking of Treg migration from the skin to draining LNs

As described above, Tregs employ distinct homing receptors for their preferential migration to lymphoid versus nonlymphoid epithelial tissues. A key question is whether Tregs are capable of trafficking between the two locations. In their current study in this issue of the *JCI*, Tomura et al. attempt to address this question by selectively “tagging” the skin-resident Tregs with the Kaede protein (17). Kaede (*Acer palmatum*, Japanese maple) decorates the autumn scenery by rapidly changing leaf color (Figure 1). The leaf is green during the summer because of the presence of chlorophyll, which also masks other pigments such as carotenoids. In the fall, the leaf turns bright red because of protease-dependent degradation of chlorophyll and de novo synthesis of anthocyanins. Kaede protein, named after the Japanese maple because of the parallel color change, is one of the recently developed photoconvertible fluorescent proteins that change their emission spectra in response to light exposure. Cloned from a stony coral, *Trachyphyllia geoffroyi*, Kaede undergoes

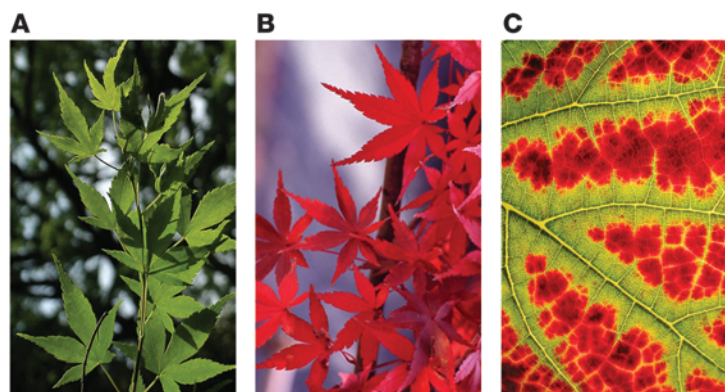
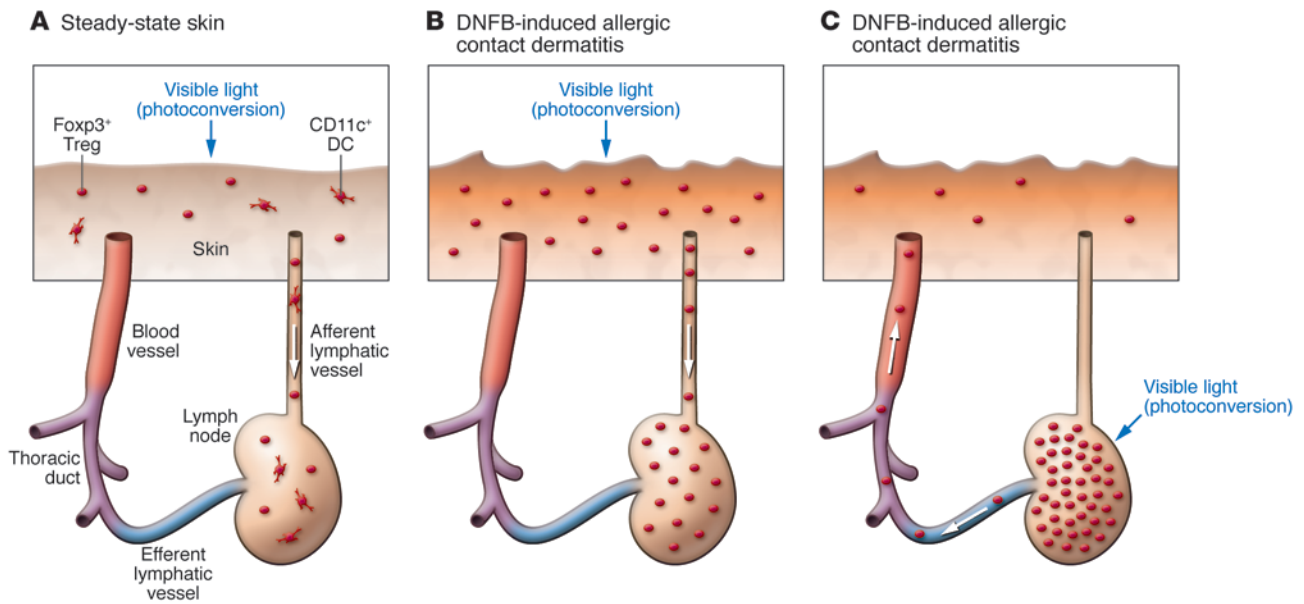


Figure 1

The Kaede (Japanese maple) leaf changes color seasonally. (A) During summer, the leaf is green due to the presence of chlorophyll. (B and C) In the fall, protease-dependent degradation of chlorophyll and de novo synthesis of anthocyanins turn the leaf red.

**Figure 2**

Treg trafficking between the skin and draining LNs. In their study in this issue of the *JCI*, Tomura et al. used mice expressing the photoconvertible Kaede protein to directly track Treg migration between the skin and LNs in the steady state (**A**) and during DNFB-induced allergic contact dermatitis (**B** and **C**) (17). (**A**) Upon photoconversion from green to red of skin-resident cells, Kaede-red $CD4^+Foxp3^+$ Tregs and Kaede-red $CD11c^+$ DCs became detectable in draining LNs, representing steady-state homing of Tregs and DCs from the skin to LNs. They presumably migrate through afferent lymphatic vessels. (**B**) DNFB-induced allergic contact dermatitis lesions contained increased numbers of $Foxp3^+$ Tregs. An even more striking increase was observed in the numbers of Kaede-red $Foxp3^+$ Tregs in the draining LNs, demonstrating accelerated Treg homing from inflamed skin. (**C**) Upon photoconversion of LN-resident cells, Kaede-red $Foxp3^+$ Tregs became detectable in the DNFB-induced allergic contact dermatitis lesions. They presumably achieve this reverse migration by moving through efferent LVs, the thoracic duct, and blood vessels.

a green-to-red conversion within a few minutes after exposure to ultraviolet or violet light (18). Kaede protein contains an internal tripeptide, $His^{62}-Tyr^{63}-Gly^{64}$, serving as a green-emitting chromophore like other GFP species, but undergoes unusual photocleavage at His^{62} , thereby creating a new red-emitting chromophore (19). In Kaede-Tg mice, all the cell types constitutively exhibit Kaede-green fluorescence signals. Upon skin exposure to violet light, however, only those cells in the exposed skin begin to emit Kaede-red fluorescence signals.

Tomura et al. report that 24 hours after exposure of the abdominal skin of Kaede-Tg mice to violet light, small numbers of Kaede-red cells became detectable in the draining axillary LNs, but not in non-draining cervical or popliteal LNs (17). This cell population was composed of roughly equal numbers of $CD4^+$ T cells and $CD11c^+$ DCs. Importantly, a significant fraction (approximately 20%) of Kaede-red $CD4^+$ T cells expressed $Foxp3$, demonstrating steady-state trafficking of Tregs from the skin to draining LNs (Figure 2A) (17). To study skin-directed Treg hom-

ing further, the authors intercrossed the Kaede-Tg mice with $Foxp3$ reporter mice and then induced allergic contact dermatitis by sensitization with 2,4-dinitro-1-fluorobenzene (DNFB) on the dorsal skin, followed by DNFB challenge on the abdominal skin. The resulting inflammatory skin lesions were found to contain roughly 5-fold more $Foxp3^+$ Tregs compared with control skin sites challenged with vehicle alone. An even more striking (~20-fold) increase was observed in the numbers of Kaede-red $Foxp3^+$ Tregs in the axillary LNs, unveiling exacerbated Treg migration from inflamed skin to LNs (Figure 2B). As expected, those skin-derived Kaede-red Tregs expressed higher levels of $CD103$, $CD44$, $CCR4$, and $CCR5$ and lower levels of $CD62L$ and $CCR7$, compared with the Kaede-green Treg counterparts in the same LNs. However, the difference observed between the two Treg populations was rather modest, perhaps reflecting a major limitation of the experimental system; that is, Kaede-green Treg populations contain not only LN-resident Tregs, but also skin-derived Tregs that have migrated

from skin before violet light exposure. Nevertheless, the observations reported by Tomura et al. provide the first experimental evidence for the concept that $CD103^+CCR4^+CCR5^+$ Tregs frequently observed in the skin are indeed capable of migrating to draining LNs.

New questions

Normal human skin and mouse skin are both populated with large numbers of Tregs, and their numbers further increase under inflammatory conditions. What are the mechanisms for maintaining such a large pool of Tregs? Do they divide in situ? Although Tregs were classically regarded as anergic, based on their extremely low in vitro mitotic activities, recent in vivo experiments have revealed their striking proliferative potentials. $CD44^{hi}CD62L^{lo}$ Treg populations, but not their $CD44^{lo}CD62L^{hi}$ counterparts, constantly incorporate BrdU, a synthetic analog of thymidine, into newly synthesized DNA in lymphoid tissues (20). Even more profound BrdU incorporation has been observed in the Tregs infiltrating *Leishmania major*-infected skin lesions (21).



Do skin-derived Tregs found in the LNs migrate back to the skin? To test this, Tomura et al. (17) photoconverted the LN cells and tracked their subsequent trafficking. A majority of the Kaede-red Tregs disappeared from the light-exposed LN within 48 hours, indicating their rapid efflux. Strikingly, a small, but significant, number of Kaede-red Tregs became detectable in DNFB-induced contact hypersensitivity skin lesions, unveiling their previously unrecognized ability to reverse-traffic from LNs to inflammatory skin (Figure 2C). Do the adhesion molecules and chemokine receptors known to mediate Treg migration from the skin to LNs also facilitate reverse Treg trafficking? Alternatively, Tregs may switch their homing receptor expression profiles depending upon the direction of their trafficking. Indeed, Tregs have been reported to employ largely overlapping, but slightly distinct, sets of homing receptors for their trafficking from the blood circulation to peripheral tissues and for their subsequent migration to draining LNs (22).

In the absence of Tregs, DCs and CD4⁺ helper T cells form stable cell-cell contacts in an antigen-specific manner. Interestingly, Tregs interrupt this cell-cell contact formation by primarily interacting with DCs (23). Because not only Tregs, but also DCs, were reported by Tomura et al. to migrate spontaneously from the skin to draining LNs (Figure 2A) (17), it is tempting to speculate that those Tregs migrating from the skin may dampen the antigen-presenting capacity of the comigrating skin-derived DCs, thereby preventing the activation of autoreactive CD4⁺ helper T cells in the LNs. In this regard, dermal DCs, but not epidermal Langerhans cells, have been reported to simultaneously trigger the production of the proinflammatory cytokine IFN- γ by helper T cells and of the antiinflammatory cytokine IL-10 by Tregs (24).

In summary, Tomura et al. have uncovered a unique ability of Tregs to migrate back and forth between the skin and LNs (17). Their experimental system should serve as an extremely useful model for answering the above and other important questions regarding Treg homeostasis and function.

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Address correspondence to: Akira Takashima, Department of Medical Microbiology and Immunology, University of Toledo College of Medicine, 3000 Arlington Ave., Toledo, OH 43614-5806. Phone: 419.383.5423; Fax: 419.383.3002; E-mail: akira.takashima@utoledo.edu.

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