Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice

Michio Tomura,[1] Tetsuya Honda,[2] Hideaki Tanizaki,[2] Atsushi Otsuka,[2] Gyohei Egawa,[2,3] Yoshiki Tokura,[4] Herman Waldmann,[5] Shohei Hori,[6] Jason G. Cyster,[7] Takeshi Watanabe,[3] Yoshiki Miyachi,[2] Osami Kanagawa,[1] and Kenji Kabashima[2,3]

[1]Laboratory for Autoimmune Regulation, Research Center for Allergy and Immunology, RIKEN, Yokohama City, Japan. [2]Department of Dermatology and [3]Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University Graduate School of Medicine, Japan. [4]Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu, Japan. [5]Sir William Dunn School of Pathology, Oxford, United Kingdom. [6]Research Unit for Immune Homeostasis, Research Center for Allergy and Immunology, RIKEN. [7]Howard Hughes Medical Institute and Department of Microbiology and Immunology, UCSF, San Francisco, California.

Tregs play an important role in protecting the skin from autoimmune attack. However, the extent of Treg trafficking between the skin and draining lymph nodes (DLNs) is unknown. We set out to investigate this using mice engineered to express the photoconvertible fluorescence protein Kaede, which changes from green to red when exposed to violet light. By exposing the skin of Kaede-transgenic mice to violet light, we were able to label T cells in the periphery under physiological conditions with Kaede-red and demonstrated that both memory phenotype $CD4^+Foxp3^-$ non-Tregs and $CD4^+Foxp3^+$ Tregs migrated from the skin to DLNs in the steady state. During cutaneous immune responses, Tregs constituted the major emigrants and inhibited immune responses more robustly than did LN-resident Tregs. We consistently observed that cutaneous immune responses were prolonged by depletion of endogenous Tregs in vivo. In addition, the circulating Tregs specifically included activated $CD25^{hi}$ Tregs that demonstrated a strong inhibitory function. Together, our results suggest that Tregs in circulation infiltrate the periphery, traffic to DLNs, and then recirculate back to the skin, contributing to the downregulation of cutaneous immune responses.

Introduction

Lymphocytes travel throughout the body to conduct immune surveillance. $CD4^+$ helper T cells are central organizers in immune responses. Upon stimulation, naive $CD4^+$ T cells differentiate into effector Th cells (1). $Foxp3^+$ Tregs represent a unique subpopulation of $CD4^+$ T cells that are important for maintenance of immunological homeostasis and self tolerance (2, 3). Naive T cells circulate between blood and secondary lymphoid tissues (4–7). However, it is debatable whether T cells travel through uninflamed peripheral tissues as part of their recirculation route. One type of peripheral tissue with the active afferent limb of the lymphatic system is, for example, the skin, and memory/effector T cells migrate to inflamed skin using CCR4 and CCR10 (8–10). Classic studies employing cannulation of afferent lymph vessels have shown that $CD4^+$ memory/effector cells make up nearly all cells in the afferent lymph of sheep (6, 11–13). On the other hand, Debes et al. have reported that $CD4^+$ cells, especially naive subsets, migrate from the skin in a CCR7-dependent manner using subcutaneous injection of fluorescent-labeled lymphocytes (14). However, the above experiments require traumatic or artificial procedures to follow or label T cells. Therefore, it is of interest to clarify whether T cells in the peripheral organs such as the skin migrate to draining LNs (DLNs) and to identify the T cell subsets of migration and their roles under physiological conditions.

To directly assess cells migrating from the peripheral tissue, we have devised a new experimental system that involves labeling resident cells using Tg mice expressing the Kaede protein. Kaede is a photoconvertible green fluorescence protein cloned from stony coral (15, 16) that changes its color from green to red when exposed to violet light (16). Therefore, the Kaede-Tg mouse system is an ideal tool for monitoring precise cellular movements in vivo at different stages of the immune response (17).

Here, we used the skin as a representative of the peripheral organs and observed the movement of cells from the skin using Kaede-Tg mice (17). A high proportion of the migrating cells into the DLNs were Tregs that had a stronger capacity to suppress acquired immune responses than LN-resident Tregs. Moreover, these migrating T cells recirculated into the skin upon elicitation to terminate immune responses.

Results

Detection of cell migration from the skin in the steady state using Kaede-Tg mice. To monitor cell migration from the skin in vivo, the abdominal skin of Kaede-Tg mice was photoconverted by exposure to violet light for 10 minutes (see Methods). Before photoconversion, all the cells in the skin of Kaede-Tg mice expressed only Kaede-green fluorescence (Kaede-green) (Figure 1, A and B). Immediately after violet light exposure to the skin, the whole skin tissue (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI40926DS1) and the skin cells of the photoconverted area showed red signal (Kaede-red), whereas virtually no draining axillary LN cells (Figure 1, A and B, and Supplemental Figure 2) or blood cells (Supplemental Figure 2)

Authorship note: Michio Tomura and Tetsuya Honda contributed equally to this work.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J Clin Invest* doi:10.1172/JCI40926.

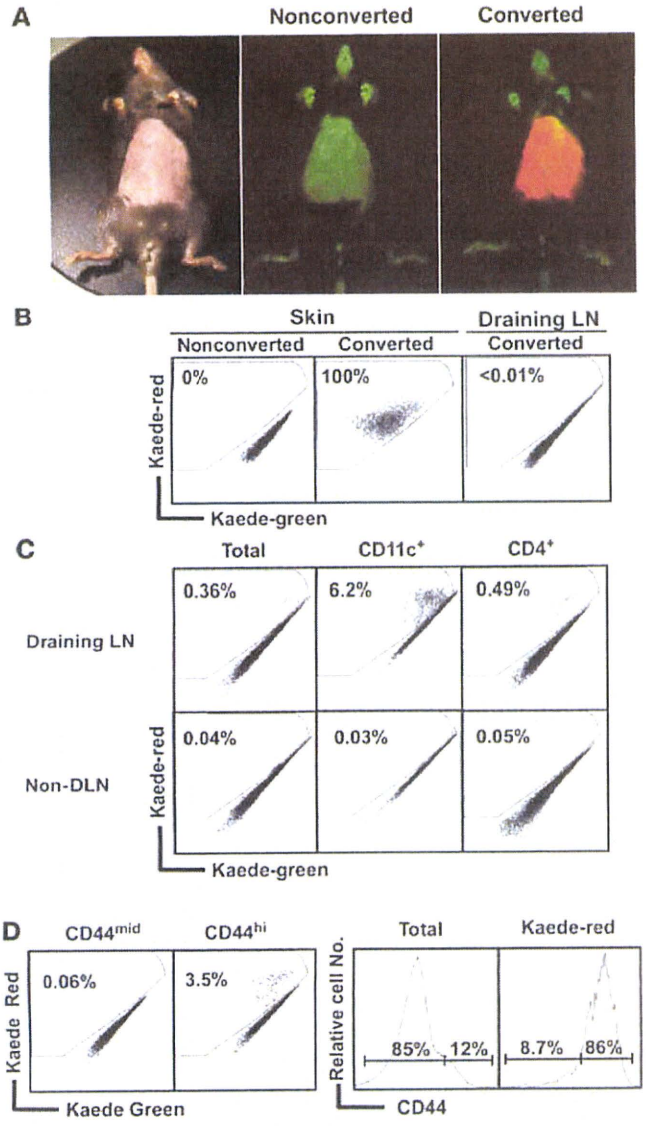

Figure 1

Cell migration from the skin to the DLN in the steady state. (**A**) Kaede-Tg mice were photoconverted on the clipped abdominal skin as described in Methods and observed with a fluorescence stereoscopic microscope. Nonphotoconverted clipped skin is shown as a control (middle). Note: nonclipped area remains black since light cannot reach. (**B**) Skin and draining axillary LN cells resected immediately after violet light exposure of the abdominal skin and resected skin cells not exposed to violet light were subjected to flow cytometric analysis to evaluate the photoconversion. (**C** and **D**) Twenty-four hours after photoconversion of the abdominal skin, cells from the draining axillary and other nondraining cervical and popliteal peripheral LNs were stained with CD11c and CD4 mAbs (**C**) and CD4 and CD44 mAbs (**D**) and subjected to flow cytometry. These data are representative of at least 5 experiments. Numbers within plots or histograms (**B–D**) indicate percentage of cells in the respective areas.

were photoconverted. Although we found that Kaede-red proteins could be detected in the extracellular fluids when incubated for 24 hours after photoconversion of the LN cells (Supplemental Figure 3), we confirmed that the extracellular photoconverted Kaede proteins could not be transferred into T cells in vitro (Supplemental Figure 4).

To evaluate cell migration from the skin in the steady state, the clipped abdominal skin of Kaede-Tg mice was exposed to violet light as in Figure 1A, and 24 hours later, the draining axillary and nondraining cervical and popliteal LN cells were subjected to flow cytometry. We found that 0.36% of the DLN cells showed the Kaede-red phenotype (Figure 1C), suggesting a fraction of cells in the skin migrate to the DLNs. It is generally thought that dendritic cells are the major migrants from the skin in the steady state, and in fact 6.2% of CD11c$^+$ dendritic cells were of the Kaede-red phenotype in the DLNs (Figure 1C). In contrast, almost no Kaede-red CD11c$^+$ dendritic cells were detected in the non-DLNs (Figure 1C). We next evaluated CD4$^+$ T cell migration from the skin and found that 0.49% of CD3$^+$CD4$^+$ T cells in the DLNs had the Kaede-red phenotype (Figure 1C). Although the frequencies of the Kaede-red positivity among dendritic cells and CD3$^+$CD4$^+$ T cells differed, the absolute numbers of Kaede-red dendritic cells and CD4$^+$ T cells were comparable (CD4$^+$ T cells vs. CD11c$^+$ dendritic cells: 11621 ± 2716 cells per LN vs. 9063 ± 2333 cells per LN, n = 5 each, average ± SD). Moreover, the ratio of Kaede-red cells was higher in CD44^{hi} memory T cells than in CD44^{mid} naive T cells (Figure 1D). Consistently, the majority of Kaede-red migratory cells were of the CD44^{hi} memory phenotype (Figure 1D). These results suggest that predominantly T cells with the memory surface phenotype migrate from the skin into DLNs, even in the steady state.

Migration of Tregs from the skin to the DLNs. Immune responses and homeostasis are regulated by the functions of memory/effector T cells and Tregs. To determine the behaviors of these populations, we intercrossed Kaede-Tg mice with Foxp3 reporter mice expressing human CD2 and human CD52 chimeric protein, which are designated as Kaede/Foxp3$^{hCD2/hCD52}$ mice. Since Foxp3$^+$ cells coexpress hCD2 on the cell surface, live Foxp3$^+$ Tregs could be labeled and sorted with anti-hCD2 monoclonal Ab. The DLN cells from Kaede/Foxp3$^{hCD2/hCD52}$ mice in the steady state were analyzed by flow cytometry. A majority of CD25$^+$ cells were hCD2 positive, but a substantial number of hCD2$^+$ cells were detected even in CD25$^-$ cells (18) (Figure 2A), which is consistent with the previous findings by the other group (19). Therefore, the following studies were performed using Kaede/Foxp3$^{hCD2/hCD52}$ mice, and hCD2$^+$ cells were considered to be Tregs.

To evaluate T cell migration from the skin in the steady state, the clipped abdominal skin of Kaede/Foxp3$^{hCD2/hCD52}$ mice was exposed to violet light as in Figure 1A, and 24 hours later, the draining axillary LN cells were subjected to flow cytometry. Consistent with the previous results (Figure 1D), a substantial percentage (0.83%) of photoconverted CD4$^+$ T cells were observed in the DLNs (Figure 2B). Among hCD2$^-$ non-Tregs and hCD2$^+$ Tregs, the frequency of Kaede-red cells was comparable (0.79% vs. 0.98%) (Figure 2C), and the frequency of Kaede-red cells was higher in the CD44^{hi} memory subset than in the CD44^{mid} naive subset (Figure 2C). In addition, Kaede-red CD4$^+$ cells included a higher percentage of Tregs (22.7%) than total CD4$^+$ cells (14.1%) (Figure 2D). In total CD4$^+$ populations, the number of CD44^{hi} memory cells was lower than that of CD44^{mid} naive cells in both non-Tregs and Tregs (Figure 2E). In contrast, consistent with Figures 2C and 2D, CD44^{hi} memory cells were the major Kaede-red migrants from the skin among non-Tregs and Tregs (Figure 2E).

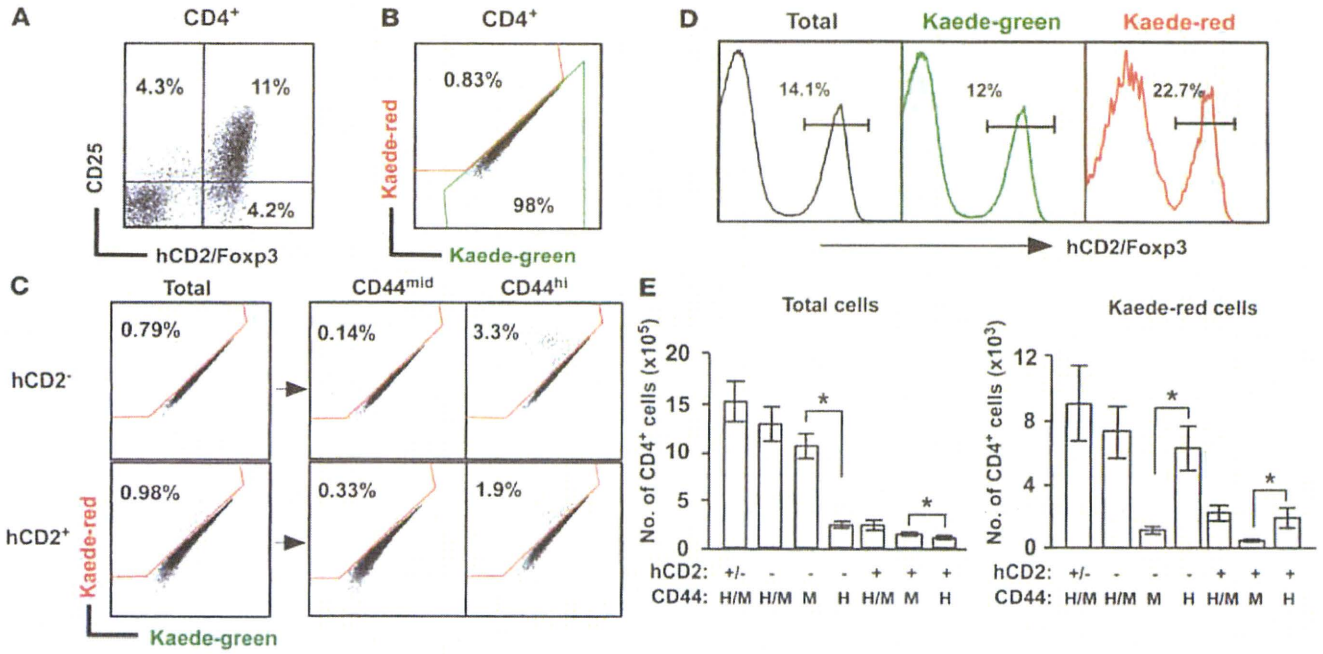

Figure 2

Migration of Tregs from the skin to DLNs. (**A–E**) The DLN cells of Kaede/Foxp3$^{hCD2/hCD52}$ mice photoconverted on the abdomen 24 hours prior were stained with CD4, CD25, and hCD2 mAbs. Shown here are the flow cytometric plots for hCD2/Foxp3 and CD25 staining among CD4$^+$ cells (**A**) and Kaede-red and Kaede-green expression on hCD2$^+$CD4$^+$ cells among skin DLN cells (**B**). (**C**) The DLNs and non-DLNs from the mice 24 hours after photoconversion were stained with CD4, hCD2, and CD44 mAbs and subjected to flow cytometry. (**D**) hCD2/Foxp3 expression in total (Kaede-red plus Kaede-green), Kaede-red, and Kaede-green CD4 cells was compared by flow cytometry. (**E**) The numbers of CD44^{mid} naive (M), CD44^{hi} memory (H), and naive plus memory (H/M) phenotypes of hCD2$^-$CD4$^+$ non-Tregs (–), hCD2$^+$CD4$^+$ Tregs (+), and total (hCD2$^-$ and hCD2$^+$; +/–) CD4$^+$ T cells in total CD4$^+$ (Kaede-red plus Kaede-green) cells and Kaede-red cells in the DLNs were counted. Data are presented as means ± SD and are representative of 3 independent experiments. Student's *t* test was performed between the indicated groups. $^*P < 0.05$. Numbers within plots or histograms indicate percentage of cells in the respective areas (**A–D**).

Treg migration from the skin during a cutaneous immune reaction. We tracked the extent of CD4$^+$ T cell migration from the skin during an immune response and sought to evaluate the role of CD4$^+$ T cells migrating from the skin. The dorsal skin of Kaede/Foxp3$^{hCD2/hCD52}$ mice was sensitized with 2,4-dinitro-1-fluorobenzene (DNFB), and 5 days later, the abdominal skin was challenged with DNFB. Two days after challenge, the abdominal skin was exposed to violet light for photoconversion, and another 24 hours later, the draining axillary LN cells were analyzed by flow cytometry (Figure 3A). The frequency of Kaede-red cells among CD4$^+$ T cells in the DLNs was increased up to 3% (Figure 3B) from that in the steady state (0.83%; Figure 2B). In addition, although 21% of total CD4$^+$ cells were Tregs, the number of hCD2$^+$ Tregs became comparable to that of non-Tregs in Kaede-red phenotype (49%; Figure 3, C and D). Again, the CD44^{hi} memory cells were major migrants from the challenged skin similarly to the steady state (Figure 3D and Figure 2E). The number of total CD4$^+$ T cells in DLN increased by 3-fold during contact hypersensitivity (CHS) compared with that in the steady state. However, the number of Kaede-red migratory non-Tregs and Tregs during CHS increased more drastically, by about 10- and 20-fold, respectively (Figure 2E and Figure 3D).

Consistent with increase of CD4$^+$ T cells migrating from the challenged skin into DLN, the numbers of both CD4$^+$ Tregs and CD4$^+$ non-Tregs were elevated when mice were sensitized and challenged compared with the steady state, and the ratio of Tregs to CD4$^+$ T cells during the immune response became higher than that in the steady state (Figure 3E). These results suggest that more Tregs than non-Tregs accumulate in the skin during the cutaneous immune response.

It is known that cutaneous dendritic cells migrate into the DLNs in a CCR7-dependent manner (20) and that in humans, most circulating Tregs express skin-homing receptors and CCR7 (21). To address whether skin T cells have the potential to migrate into the regional LNs, skin cell suspensions were obtained from the ears of mice sensitized on the abdomen and challenged on the ear with DNFB and applied to a transwell assay. The Tregs showed good chemotactic responses to CCL21 comparable to that of MHC class II$^+$ cutaneous dendritic cells (Figure 3F). Similar chemotactic activity to CCL21 was seen in CD4$^+$ non-Tregs (data not shown). Since the ratio of Tregs and non-Tregs in Kaede-red CD4$^+$ T cells in LNs was comparable to that in the skin at the time of photoconversion, Tregs and non-Tregs in the skin seem to have equivalent propensity to migrate to the DLN. In addition, we evaluated the CCR7 expression of Tregs in the skin before and after challenge and found that Tregs in the skin expressed CCR7 both before and after challenge and that the expression level of CCR7 of Tregs after challenge was slightly lower than that before challenge (Supplemental Figure 5).

Role of Tregs in the elicitation phase of CHS. As shown above, Tregs accumulate in the skin and they have the capacity to migrate to DLNs during the CHS response. These results prompted us to

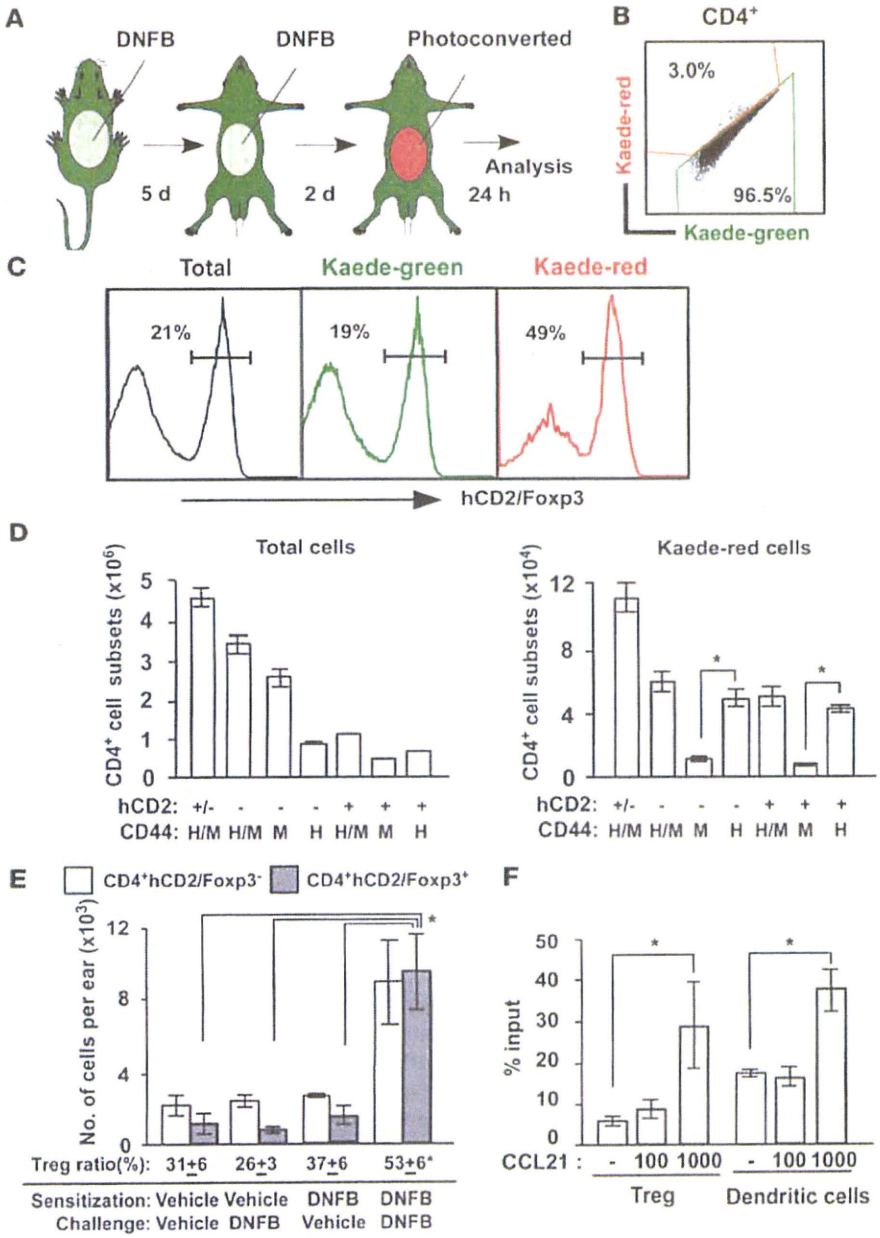

Figure 3
Cell migration from the skin to DLN during a cutaneous immune response. (**A**) Scheme of the experimental protocol is as follows: the dorsal skin of Kaede/Foxp3$^{hCD2/hCD52}$ was sensitized, and 5 days thereafter the abdominal skin was challenged. 2 days after challenge, the painted areas were photoconverted, and 24 hours after photoconversion, cells from the skin DLNs were analyzed by flow cytometry. (**B** and **C**) The frequency of Kaede-red and Kaede-green cells among CD4$^+$ cells, and the frequencies of hCD2/Foxp3+ cells in total, Kaede-green, and Kaede-red cells among CD4$^+$ cells were analyzed. Numbers within plots or histograms indicate percentage of cells in the respective areas. (**D**) The numbers of CD44^{mid} naive (M), CD44^{hi} memory (H), and naive plus memory (H/M) phenotypes of hCD2$^-$CD4$^+$ non-Tregs (–), hCD2$^+$CD4$^+$ Tregs (+), and total (hCD2$^-$ and hCD2$^+$; +/–) CD4$^+$ T cells among total CD4$^+$ cells and Kaede-red cells in the DLNs were counted. (**E**) Number of Tregs and non-Tregs in the skin. The mice were painted with DNFB or vehicle on the abdomen, followed by DNFB or vehicle application on the ears. The number of CD4$^+$ Tregs and CD4$^+$ non-Tregs and the percentage ratio of Tregs among CD4$^+$ T cells in the ears were measured. (**F**) Transwell assay. The number of hCD2$^+$CD4$^+$ cells and CD11c$^+$ cells of skin-cell suspensions from Foxp3$^{hCD2/hCD52}$ mice that migrated to the lower chamber was analyzed. Data are presented as means ± SD (**D**–**F**) and are representative of 3 independent experiments. Student's *t* test was performed between the indicated groups. $^*P < 0.05$ (**D**–**F**).

evaluate the role of Tregs in the cutaneous immune response. In a murine CHS model, we found that administration of Campath-1G Ab (a depleting Ab for the human CD52 antigen; ref. 22) resulted in a marked decrease in the number of Tregs in the DLNs and the skin, 1–3 days after injection (Figure 4A and data not shown). Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized with DNFB on the abdomen and treated in the presence or absence of Campath-1G Ab. The ear thickness changes after the challenge on the ears were significantly prolonged by the treatment with Campath-1G Ab at each time point compared with in control mice (Figure 4B). This enhancement of CHS response by Campath-1G Ab was not observed when C57BL/6 (B6) wild-type mice were used, which excluded the possibility of the nonspecific effect of Campath-1G Ab (Supplemental Figure 6). In addition, the ear thickness changes of mice treated with control rat IgG were comparable to those treated without Campath-1G Ab (data not shown). These results demonstrate that Tregs play an important role in the challenge phase in terminating the CHS response.

Suppressive activity of Kaede-red and Kaede-green Tregs on T cell proliferation. To further determine the suppressive function of the Tregs migrating from the skin during the cutaneous immune response, Kaede-red and Kaede-green CD4$^+$ Tregs in the skin DLN were prepared as in Figure 3A and cocultured with regional LN cells from DNFB-sensitized mice. Antigen-specific T cell proliferation induced by 2,4-dinitrobenzene sulfonic acid (DNBS), a water-soluble compound with the same antigenicity as DNFB, was significantly inhibited by addition of 6×10^3 Kaede-red Tregs (Figure 4C). On the other hand, 8 times the number of Kaede-green Tregs was required to achieve a similar magnitude of inhibitory effect of the Kaede-red Tregs (Figure 4C). These data indicate that the skin-

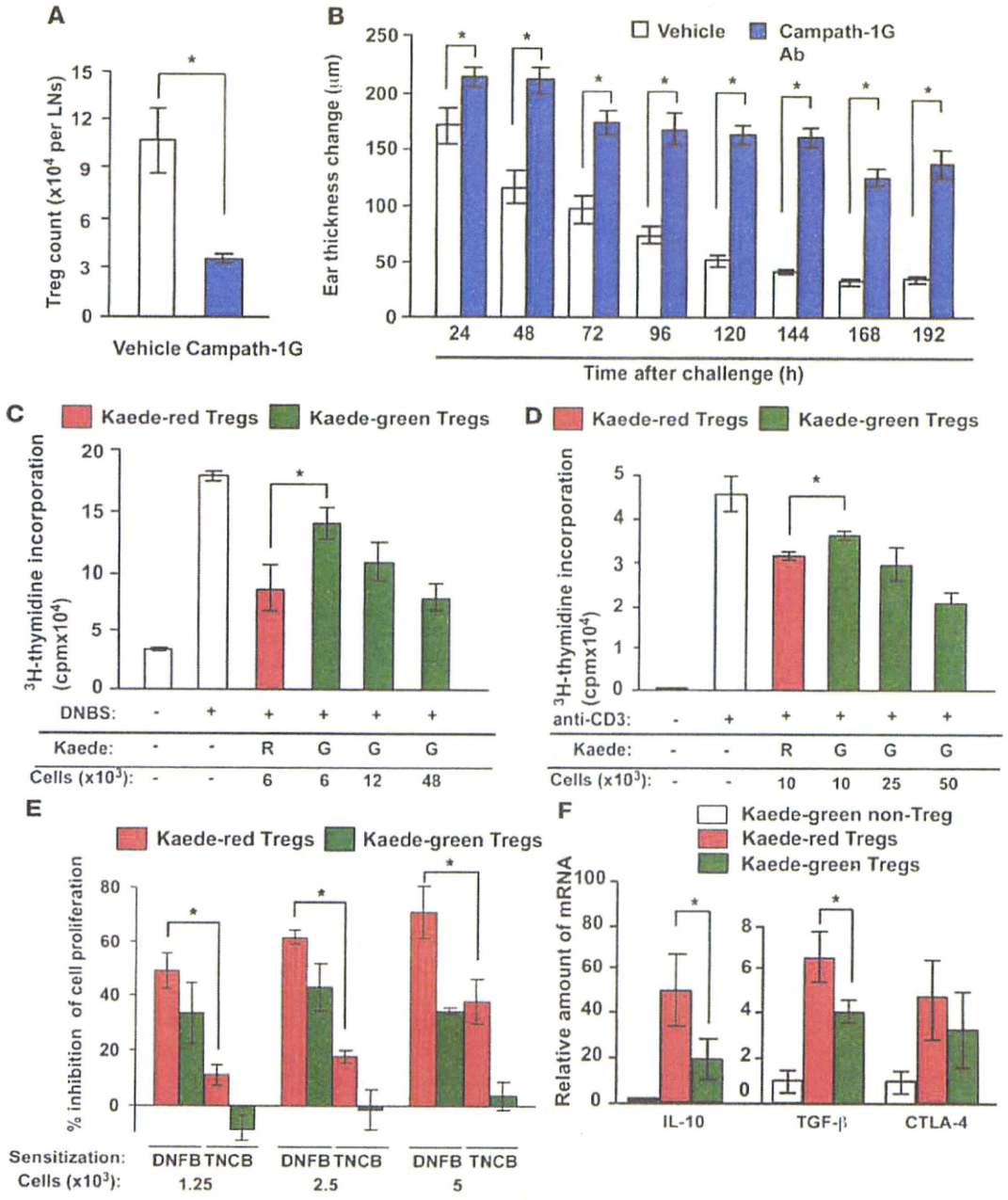

Figure 4

Enhanced ear swelling response by Treg depletion and immunosuppressive activity of Treg subsets on T cell proliferation in vitro. (**A**) The number of Tregs in the LNs after administration of Campath-1G Ab. (**B**) CHS: the Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized, and injected with vehicle or Campath-1G Ab before challenge (n = 8 for each group). (**C–F**) Immunosuppressive activity of Tregs. Kaede-red and Kaede-green Tregs were sorted from the Kaede/Foxp3$^{hCD2/hCD52}$ mice, sensitized, challenged, and photoconverted. (**C**) Skin DLN cells of mice sensitized with DNFB were stimulated with DNBS in the presence or absence of Kaede-red Tregs or Kaede-green Tregs in vitro (n = 3). (**D**) Suppressive effect of Tregs in vitro. Kaede-red and Kaede-green Tregs were prepared as above and added to T cells stimulated with plate-bound anti-CD3 Ab. (**E**) Antigen specificity of Treg functions. LN cells from DNFB-sensitized or TNCB-sensitized mice were stimulated with DNBS or TNBS in vitro. Kaede-red and Kaede-green Tregs were added, and percentage inhibition of cell proliferation was evaluated as follows: (cell proliferation with DNBS or TNBS) – (cell proliferation with DNBS or TNBS in the presence of Tregs)/(cell proliferation with DNBS or TNBS) – (cell proliferation with vehicle) × 100. (**F**) Quantitative RT-PCR analysis on mRNA for *Il10* (IL-10), *Tgfb1* (TGF-β), and *Ctla4* (CTLA-4) of Kaede-red Tregs and Kaede-green Tregs. The expression of each gene was normalized by the expression of *Gapdh*, and those in Kaede-green non-Tregs were normalized to 1 (n = 3). Data are representative of 3 independent experiments and presented as means ± SD (**A–F**). *$P < 0.05$ between the indicated groups (Student's t test, **A**, **B**, **E**, and **F**; 1-way ANOVA followed by Dunnett multiple comparison test, **C** and **D**).

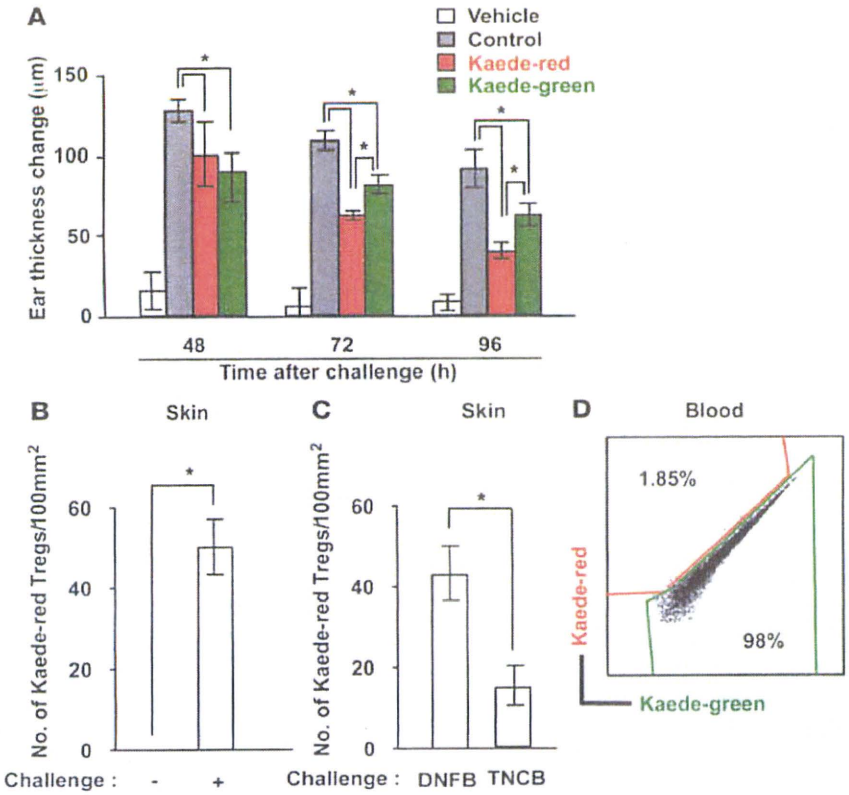

Figure 5
Immunosuppressive effect of Kaede-red Tregs in the skin. (**A**) Suppression of CHS response by Kaede-red Tregs. Kaede-red or Kaede-green Tregs (4×10^3 cells/ear) of Kaede/Foxp3$^{hCD2/hCD52}$ mice sensitized, challenged, and photoconverted as in Figure 3A were injected into ear skin of mice sensitized with DNFB 5 days prior. Immediately after injection, the mice were challenged, and the ear thickness change was measured at 48, 72, and 96 hours after challenge. (**B–D**) The mice were sensitized, challenged, and photoconverted as in Figure 3A. Twenty-four hours after photoconversion, 20 μl of 0.3% DNFB (challenge; +) or vehicle (challenge; –) (**B**) or 20 μl of 0.3% DNFB or 20 μl of 1% TNCB (**C**) was painted onto the ear. Twenty-four hours later, the ear skin and blood (**D**) were collected and dissociated for flow cytometry. The number of Kaede-red Tregs in the skin and the frequency of Kaede-red Tregs in CD4$^+$ T cell subset of the blood were evaluated ($n = 3$, each group). Data are presented as means ± SD and representative of 3 independent experiments (**A–C**). Student's t test was performed between the indicated groups. $^*P < 0.05$. Numbers within plots indicate percentage of cells in the respective areas (**D**).

derived Tregs have a stronger inhibitory effect on hapten-specific T cell proliferation than LN-resident Tregs. It should be noted that we might underestimate the inhibitory capacity of skin-migratory T cells relative to resident Tregs, since Kaede-green cells should have included the cells migrated from the skin before photoconversion and the cells that infiltrated to the skin after photoconversion and migrated to DLN.

We tested the effect of the Tregs on antigen-nonspecific T cell proliferation stimulated with membrane-bound anti-CD3 Ab. Kaede-red Tregs inhibited T cell proliferation more potently than did Kaede-green Tregs, and again a higher number of Tregs were required (Figure 4D) to obtain an extent of inhibition similar to that seen in Figure 4C.

To further evaluate the antigen specificity of Tregs in T cell proliferation, we isolated the DLN cells 5 days after DNFB or 2,4,6-trinitro-1-chlorobenzene (TNCB) sensitization and restimulated them with DNBS or trinitrobenzene sulfonic acid (TNBS), respectively, and added Kaede-red Tregs or Kaede-green Tregs prepared from the DLNs as in Figure 3A. Kaede-red Tregs inhibited DNBS-induced T cell proliferation more than Kaede-green Tregs (Figure 4E), as shown in Figure 4C. However, this antiproliferative effect was not seen when these Kaede-red or Kaede-green Tregs were added to TNBS-stimulated LN cells from the mice sensitized with TNCB (Figure 4E). In addition, in the criss-cross comparison, similar antigen-specificity was observed on TNCB-immunized Kaede-red Tregs (data not shown). We also analyzed mRNA expressions of inhibitory cytokines and surface molecules by quantitative RT-PCR. Kaede-red Tregs expressed higher mRNA levels of *Il10* and *Tgfb1* than Kaede-green Tregs (2, 3, 23) (Figure 4F). On the other hand, although there was no significant difference, Kaede-red Tregs tended to express higher mRNA levels of cytotoxic T lymphocyte-associated molecule-4 (*Ctla4*) than did Kaede-green Tregs (2, 3, 23) (Figure 4F). These results suggest that Tregs migrating from the skin have a more efficient suppressive potency on T cell proliferation with abundant inhibitory mediators and that this antiproliferative effect shows some antigen specificity.

Tregs recirculating from the skin inhibit local cutaneous immune response in situ. The strong ability of Kaede-red Tregs to suppress in vitro T cell proliferation prompted us to determine whether Kaede-red Tregs can inhibit a local cutaneous immune response in situ. Kaede-red or Kaede-green Tregs prepared as described (Figure 3A) were injected subcutaneously into the ears of mice sensitized with DNFB 5 days before, and the ears were challenged with DNFB. The DNFB-induced ear thickness change was suppressed by the injection of Kaede-red and Kaede-green Tregs at all time points (Figure 5A). It was noted, however, that Kaede-red Tregs suppressed CHS more than Kaede-green Tregs at 72 and 96 hours after challenge (Figure 5A).

Considering that Tregs function as a regulator for primed T cells, they should serve as suppressors at the challenged site. The above late-phase inhibitory action of Kaede-red Tregs raised the possibility that Tregs migrating from the skin can return to the skin and exert suppressive activity. Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized, challenged, and photoconverted as in Figure 3A. Twenty-four hours after photoconversion, the left and right ears were rechallenged with DNFB and vehicle (Figure 5B) or TNCB (Figure 5C), respectively. Another 24 hours later, Kaede-red Tregs were observed in the ears challenged with DNFB, but not in those challenged with vehicle (Figure 5B). The ear rechallenged with a different hapten, TNCB, contained Kaede-red Tregs, but its number was lower than

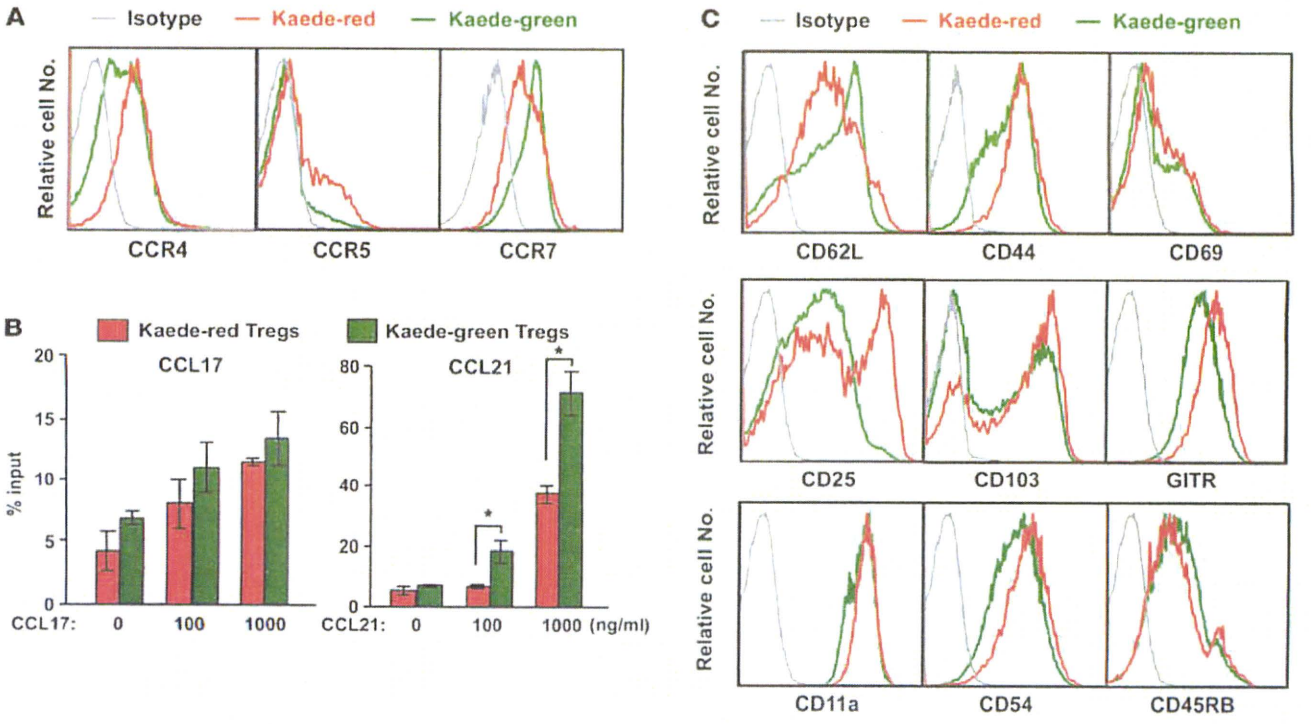

Figure 6

Surface molecule expressions on Kaede-red and Kaede-green cells. (**A**) Chemokine receptor expression. Skin DLN cells were prepared from the mice sensitized, challenged, and photoconverted as in Figure 3A. These LN cells were stained with isotype-matched control, CCR4, CCR5, and CCR7 mAbs, and the expression levels of Kaede-red and Kaede-green Tregs were evaluated by flow cytometry. (**B**) Transwell assay. DLN cells were transferred to the upper chamber of the transwell, and CCL17 or CCL21 was added to the lower chamber. The cells were incubated for 3 hours, and the numbers of Kaede-red and Kaede-green cells that migrated to lower chamber were analyzed by flow cytometry. Data are presented as means ± SD and representative of 2 independent experiments. Student's *t* test was performed between the indicated groups. $^*P < 0.05$. (**C**) Surface molecule expression. LN cells were stained with isotype-matched control, CD62L, CD44, CD69, CD25, and CD103 mAbs, and the expression levels were evaluated by flow cytometry. These data are representative of 3 independent experiments.

that of the ear rechallenged with DNFB (Figure 5C). In addition, Kaede-red Tregs were detected in $CD4^+$ cells of the blood 24 hours after rechallenge (1.79% ± 0.07%, average ± SEM, $n = 3$) (Figure 5D). Moreover, a previous report has suggested that LN cells migrate to the skin (24). We conducted an evaluation of this report by photoconverting DLNs. We sensitized the dorsal skin of mice with DNFB and challenged the abdominal skin with DNFB 4 days later. Two days after challenge, the DLNs of the mice were photoconverted and the ears were rechallenged with DNFB. Twenty-four hours later, the ears of the skin were analyzed by flow cytometric analysis. We found that a substantial fraction of $CD4^+$ $hCD2^-$ non-Tregs and $CD4^+$ $hCD2^+$ Tregs were Kaede-red positive (Supplemental Figure 7). These results suggest that the Tregs that egressed from the skin had a capacity to remigrate to the skin upon challenge.

It has been reported that the representative chemokine receptors essential for migration of lymphocytes into the skin and LNs are CCR4 and CCR7, respectively (9, 14, 25). In addition, CCR5 may be an important chemokine receptor for Tregs to migrate into the skin (26). Kaede-red Tregs expressed higher levels of CCR4 and CCR5 and a lower level of CCR7 than Kaede-green Tregs (Figure 6A). When the skin DLN cells prepared as in Figure 3A were applied to a transwell assay, Kaede-red Tregs showed good chemotactic responses to both CCL17, a ligand for CCR4, and CCL21, a ligand for CCR7, but the chemotaxis of Kaede-red Tregs to CCL21 was weaker than that of Kaede-green Tregs (Figure 6B).

We further analyzed the surface molecules of Kaede-red Tregs in the DLNs of Kaede/Foxp3$^{hCD2/hCD52}$ mice treated as in Figure 3A. Kaede-red Tregs expressed a lower level of CD62L but higher levels of CD44 and CD69 than Kaede-green Tregs (Figure 6C), suggesting that the skin-derived Tregs show a more memory-related T cell phenotype. Interestingly, Kaede-red Tregs contained a $CD25^{hi}$ fraction, which was barely perceptible in Kaede-green Tregs. In addition, Kaede-red Tregs expressed higher levels of CD103, an integrin important for T cell migration into the skin as well as CD11a and CD54, integrins induced upon activation, and a glucocorticoid-induced TNFR family–related gene/protein (GITR), another marker of Tregs (2, 27, 28) (2). However, the expression level of CD45RB was comparable between the Kaede-red and Kaede-green Tregs. These results suggest that Kaede-red Tregs are of the memory/effector phenotype (29) and have a higher potential to migrate to the skin than LN-resident Tregs.

Kinetics and surface phenotype of $CD25^{hi}$ Kaede-red Tregs. The above data (Figure 5A) suggest that Tregs migrating from the skin have a highly potent immunosuppressive capacity even in situ. One of the features of these skin-derived Tregs is the presence of a $CD25^{hi}$ subset (Figure 6C) that has not, to our knowledge, been thoroughly described before. Initially, we sought to characterize the localization of $CD25^{hi}$ Tregs and found that $CD25^{hi}$ cells were substantially detected in Kaede-red Tregs of the DLNs of mice pretreated as in Figure 3A but were only somewhat or marginally detected in

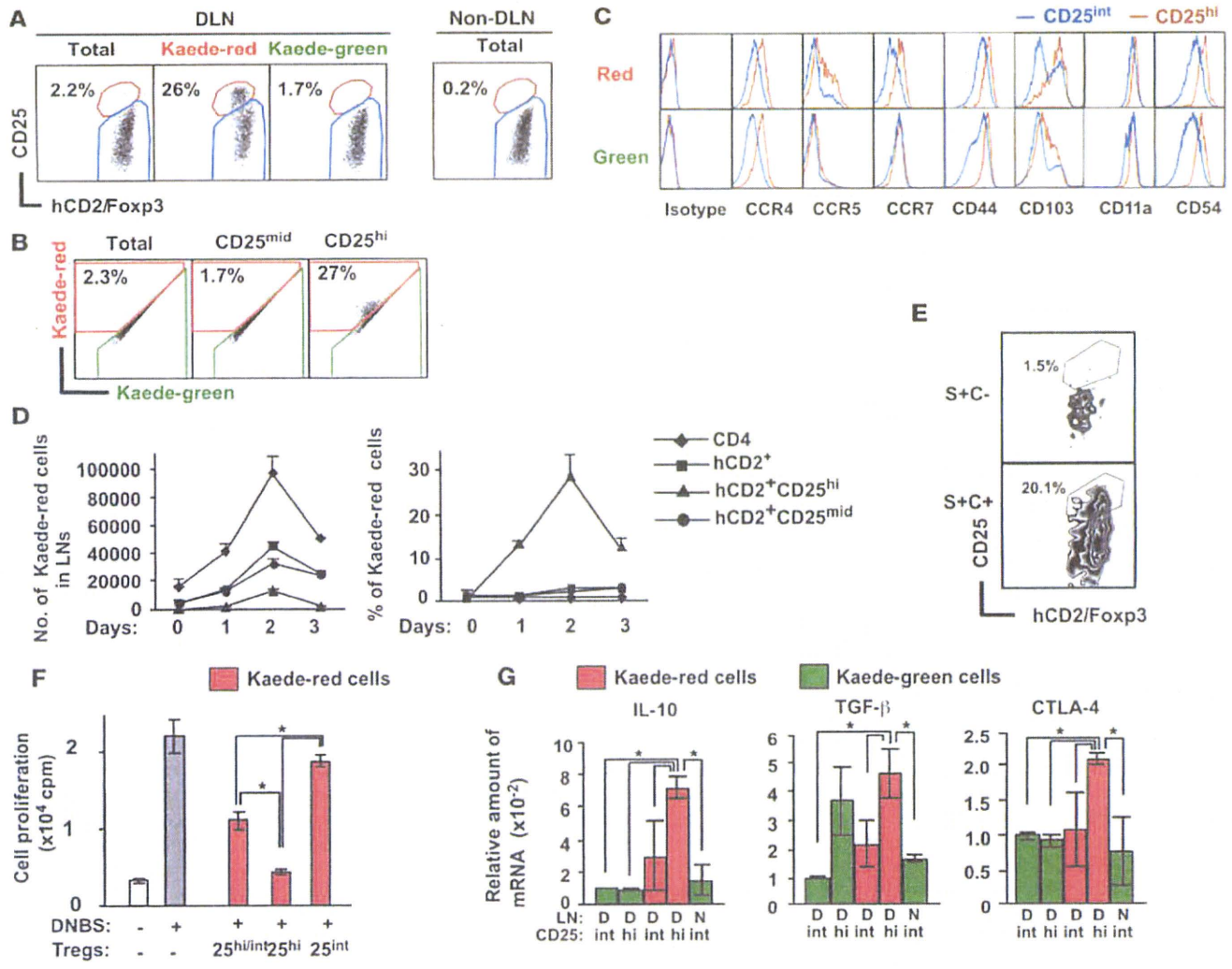

Figure 7

Kinetics and suppression activity of CD25^{hi} Kaede-red migratory Tregs. (**A–C**) Characterization of CD25^{hi} subset. Kaede/Foxp3$^{hCD2/hCD52}$ mice were treated as in Figure 3A, and the expression levels of hCD2/Foxp3 and CD25 on CD4^{+}hCD2/Foxp3^{+} Tregs in total, Kaede-red, and Kaede-green DLN cells and in non-DLN cells (**A**), the frequency of Kaede-red populations in each population (**B**), and the expression levels of surface markers on Kaede-red or Kaede-green Tregs in the DLNs (**C**) were analyzed. (**D**) Kinetics of T cell migration. Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized and challenged as in Figure 3A and photoconverted immediately (day 0), 1 (day 1), 2 (day 2), or 3 (day 3) days after challenge. The number of each subset migrating for 24 hours after photoconversion and the frequency of Kaede-red cells among each subset were measured. (**E**) Foxp3$^{hCD2/hCD52}$ mice were sensitized with DNFB (S+) and challenged with DNFB (C+) or vehicle (C–). Skin suspensions were evaluated for the expression of hCD2/Foxp3 and CD25. (**F**) Skin DLNs cells of sensitized B6 mice were stimulated in the absence or presence of Kaede-red total hCD2^{+} Tregs (25$^{hi/int}$), CD25^{hi} Tregs (25^{hi}), or CD25^{int} Tregs (25^{int}). (**G**) mRNAs for *Il10* (IL-10), *Tgfb1* (TGF-β), and *Ctla4* (CTLA-4) of Kaede-green CD25^{int} or CD25^{hi} Tregs, Kaede-red CD25^{int} or CD25^{hi} Tregs, or Kaede-green CD25^{int} Tregs in DLNs (D) or non-DLNs (N) were evaluated. The expression level in Kaede-green CD25^{int} Tregs was normalized to 1. Data are presented as means ± SD (n = 3) (**D**, **F**, and **G**). $^{*}P < 0.05$ between indicated groups. (**F** and **G**). Numbers within plots or histograms indicate percentage of cells (**A**, **B**, and **E**).

Kaede-green Tregs of the DLNs or in non-DLNs (Figure 7A). Consistently, the frequency of the Kaede-red population in the CD25^{hi} population was greater than that in the CD25^{mid} population (Figure 7B). These CD25^{hi} Tregs showed higher levels of CCR4, CCR5, CCR7, CD44, CD103, CD11a, and CD54 than CD25^{int} Tregs in the Kaede-red subset (Figure 7C). On the other hand, the expression levels of CCR5 and CD103 of the CD25^{hi} subset in the Kaede-green cells tended to be lower than that in the Kaede-red cells, and the expression of CCR7 in Kaede-green Tregs was similar between CD25^{int} and CD25^{hi} subsets (Figure 7C).

We then examined the kinetics of T cell migration from the skin. Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized and challenged as in Figure 3A and photoconverted immediately, 1, 2, or 3 days after challenge. The DLN cells were collected 24 hours after each photoconversion, and the number of Kaede-red CD4^{+}, CD4^{+}hCD2^{+}, CD4^{+}hCD2^{+}CD25^{hi}, and CD4^{+}hCD2^{+}CD25^{mid} cells migrating for 24 hours after photoconversion was determined (Figure 7D). The peak response of cell migration from the skin occurred on day 2 (between 48 and 72 hours after challenge) when the frequency of Tregs among CD4^{+} T cells migrating from

the skin was high (Figure 7D). In addition, CD4$^+$hCD2$^+$CD25^{hi} cells were detected only at this time point (Figure 7D) and showed a high frequency of Kaede-red positivity, especially on day 2 (Figure 7D), suggesting that this subset is replaced by the skin-derived cells more readily than other subsets.

Strong immunosuppressive activity of CD25^{hi} Kaede-red migratory Tregs. To evaluate whether CD25^{hi} Tregs are localized in the skin during immune responses, Kaede/Foxp3$^{hCD2/hCD52}$ mice were sensitized and challenged as in Figure 3A. We detected a significant number of CD25^{hi} Tregs in the challenged local skin, but few in the nonchallenged skin 48 hours after the challenge (Figure 7E), suggesting that CD4$^+$hCD2$^+$CD25^{hi} cells are induced in the skin and migrate into the DLNs.

To determine the role of skin-derived CD25^{hi} Tregs, Treg subsets were isolated from the DLNs of mice pretreated as in Figure 3A and cocultured with DLN cells from DNFB-sensitized mice. The CD25^{hi} Tregs showed much stronger suppressive activity on T cell proliferation than the CD25^{int} subset (Figure 7F).

We further examined the mRNA expression profiles of cytokines in the CD25^{hi} Treg subsets. In agreement with the above in vitro result, Kaede-red CD25^{hi} Tregs contained significantly higher amounts of *Il10*, *Tgfb1*, and *Ctla4* than Kaede-red CD25^{int} Tregs in the DLNs, Kaede-green CD25^{hi} or CD25^{int} Tregs in the DLNs, or Kaede-green CD25^{int} Tregs in the non-DLNs, except in the case of *Tgfb1* expression level between Kaede-red CD25^{hi} Tregs and Kaede-green CD25^{hi} Tregs in DLNs (Figure 7G). These results suggest that CD25^{hi} Tregs migrating from the skin play a major suppressive role in cutaneous immune response.

Discussion

In this study, we found that memory/effector phenotype Foxp3$^+$ Tregs as well as Foxp3$^-$ non-Tregs migrated from the skin to DLNs in the steady state. The number of CD4$^+$ T cells in the skin and their migration to DLNs were prominently increased during a cutaneous immune response. Among the migrating T cells, Foxp3$^+$ Tregs constituted one of the major populations. Notably, the Tregs that migrated from the skin returned to the skin upon exposure to an antigen. The migrating Tregs held strong immunosuppressive effect and expressed high levels of mRNA for inhibitory mediators compared with LN-resident Tregs. Moreover, depletion of endogenous Tregs in vivo prolonged the CHS response. Finally, these circulating Tregs specifically included the CD25^{hi} subset that showed an activated phenotype and a very strong inhibitory function on T cell proliferation, with high levels of mRNA for inhibitory mediators. These data suggest that Tregs circulate between blood, skin, and lymphoid tissues to regulate peripheral immune responses.

There have been a few studies that sought to address the possibility of T cell migration from the periphery to LNs. In their experiments, one report suggested that the memory/effector subset of CD4$^+$ T cells is the major constituent in the afferent lymph by cannulation of sheep (6, 11–13), and the other suggested the naive subset is dominant using subcutaneous injection of fluorescent-labeled lymphocytes (14). Recently, effector/memory phenotype of Tregs has been reported to migrate from blood to islet and to DLNs sequentially using an islet allograft model with transfer of in vitro-induced Tregs (30). However, since all the above experiments require traumatic or artificial procedures to label T cells in the periphery, it remains unknown whether endogenous T cells egress from the periphery into DLNs under pathophysiological conditions. In this study, using the Kaede-Tg system, we have clearly demonstrated that a subset of T cells with memory/effector phenotype migrates to DLNs in the steady state and during a cutaneous immune response. During the immune response, Tregs are the major constituents and they return to the skin upon exposure to an antigen. Therefore, as naive T cells circulate between blood and LNs, cells of the memory/effector T cell phenotype, especially Tregs, seem to circulate between blood and the skin. In this study, we used the skin as a representative of the peripheral tissues, but it would be of interest to explore this issue in other peripheral tissues, such as lungs and intestines.

To date, the roles of externally transferred Tregs in CHS have been reported (31); however, the regulatory activity of endogenous Tregs has not been fully assessed. In this study, we found that depletion of Tregs during the elicitation phase prolonged the CHS response. In addition, CHS-induced migratory Tregs suppressed the proliferation of DNFB-sensitized LN cells in a ratio as low as 1:100 (Tregs to LN cells), but such an inhibitory effect was not observed in non-antigen-specific mitogen-induced T cell proliferation systems. Therefore, Tregs circulating between the skin and LNs may inhibit not only T cells, but also antigen-presenting cells, such as dendritic cells, or antigen-presenting cell–T cell interactions. Moreover, subcutaneous injection of migratory Tregs into the skin suppressed CHS more markedly than that of LN-resident Tregs. Similar findings were observed when these Tregs were transferred intravenously (data not shown), suggesting that Tregs migrating from the skin hold a high immunosuppressive potential.

The CD25^{hi} subset that migrated from the skin seems to have an activated phenotype, indicated by the positivity of CD25 and CD103. It has been reported that transfer of preactivated CD25$^+$CD103$^+$ cells strongly suppressed T cell proliferation (32) and CD25$^+$CD103$^+$ cells are the main producer of IL-10 after TCR stimulation (29). The CD25^{hi} subset in our finding expresses high levels of CD103 and IL-10 and strong suppressive capacity and phenotype, consistent with an activated effector/memory Treg subset (28, 33). It should be noted that we demonstrate that the CD25^{hi} subset was localized in the skin and only transiently migrated from the skin after CHS elicitation. Thus, the role of skin in generation, education, and spatiotemporal regulation of this CD25^{hi} subset during immune responses needs to be elucidated in the future, which may lead us to understand the role of peripheral tissues in regulation of immune responses.

Notably, Treg cell circulation was remarkably induced during cutaneous immune responses. Therefore, we have focused on the roles of Tregs instead of effector/memory T cells migrating from the skin. In fact, the administration of migratory Tregs strongly suppressed CHS response at the later phase after a challenge (Figure 5A), and in vivo depletion of Tregs prolonged the CHS response, particularly during the later phase (Figure 4B). These results suggest that these circulating Tregs might be involved in the termination of immune responses. However, immune responses and homeostasis are regulated and maintained by the balance between Tregs and effector/memory T cells, and it has been thought that CHS occurs by the dominance of effector/memory T cells over Tregs. Hence, it is intriguing that the elicitation of CHS induces Tregs despite their possible antagonistic role for the development of acquired immune response. In this sense, it will be of interest to explore more the roles of effector/memory T cells and Tregs migrating from the skin in regulating immune response. Clarification of these issues will lead not only to understanding of the novel mechanism of cutaneous immune responses but also to control of systemic immune responses through modulating cutaneous immunity.

Methods

Mice and photoconversion. Tg mice carrying Kaede cDNA under the CAG promoter were established previously (17). These mice with B6 genetic background expressed photoconvertible Kaede in all of their cell types. It should be noted that the use of violet light (436 nm) rather than harmful UVA (320–400 nm) or UVB (290–320 nm) allowed us to photoconvert Kaede in the cells with no detectable damage (17).

Because of the moiety of its wavelength, violet light exposure penetrates through the skin to subcutaneous tissue, but not further (data not shown). Although the exposure of Kaede to violet light permanently changes its structure and photoconverted Kaede has a very long biological half-life in lymphocytes, cell proliferation dilutes photoconverted Kaede with newly synthesized nonphotoconverted Kaede, and after several cell divisions, the detection of red fluorescence becomes difficult (17). Moreover, exposure of the cells to violet light for 10 minutes has no effect on T and B cell proliferation (17). To exclude the immunomodulatory effect of photoconversion in vivo, we used the CHS model. Photoconversion of the abdominal skin immediately after sensitization on the abdomen did not affect CHS response (data not shown). When mRNA levels of *Il1b* were examined 6 hours after photoconversion (436 nm) or low-dose (3 kJ/m^2) UVB exposure, a significant increase of mRNA levels of *Il1b* was observed by UVB but not by photoconversion (Supplemental Figure 8). Therefore, we assume that photoconversion of the skin does not provoke significant inflammation in the skin or inflammatory stimuli in keratinocytes.

B6 Foxp3$^{hCD2/hCD52}$ mice were generated by homologous recombination in a B6-derived ES cell line using a targeting construct in which cDNA encoding a human CD2 and human CD52 fusion protein along with an intraribosomal entry site was inserted into the 3′ untranslated region of the endogenous Foxp3 locus (18). All CD4$^+$Foxp3$^+$ cells expressed hCD2, but CD4$^+$ Foxp3$^-$ cells did not (data not shown), indicating that the expression of the human CD2 reporter faithfully reflects the intracellular expression of Foxp3 in Foxp3$^{hCD2/hCD52}$ mice. Foxp3$^{hCD2/hCD52}$ mice (18) were intercrossed with Kaede-Tg mice to generate Kaede/Foxp3$^{hCD2/hCD52}$ mice for further evaluation. These mice were bred in specific pathogen–free facilities at Kyoto University or RIKEN. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Kyoto University Faculty of Medicine and RIKEN.

Antibodies and flow cytometry. Fluorochrome-conjugated or biotinylated anti-human CD2, anti-mouse CD4, CD11a, CD11c, CD25, CD44, CD45RB, CD62L, CD69, CD103, GITR, CCR4, CCR5, and CCR7 mAbs were obtained from BD Biosciences, eBioscience, or Biolegend. Data were acquired using the JSAN system (Bay bioscience) or FACSCanto II flow cytometry system (BD Biosciences) and analyzed with FlowJo (TreeStar).

Cell preparation from the skin and cell sorting. Briefly, the ears were removed and split into dorsal and ventral halves, and cartilage was removed. The skin of the ears was floated on 0.25% trypsin/EDTA for 30 minutes at 37°C. Then the epidermis was peeled from the dermis, and both epidermis and dermis were minced with forceps. The minced tissues were incubated for 1 hours in collagenase II (Worthington Biochemical) containing hyaluronidase and DNaseI (Sigma-Aldrich). The cell suspensions were filtered with 40 μm of cell strainer.

For cell sorting, Kaede-red Tregs or Kaede-green Tregs were purified from inguinal and axillary LNs of Kaede/Foxp3$^{hCD2/hCD52}$ mice. Briefly, the mice were sensitized and challenged with DNFB in the same way as the B6 mice for DNBS-induced cell proliferation. Two days after the challenge, cells of abdominal skin were photoconverted, and single-cell suspensions were prepared from inguinal and axillary LNs 24 hours after photoconversion. The cells of each population were sorted by the FACSAria II flow cytometry system (BD Bioscience).

Photoconversion, CHS model, in vivo Treg depletion, and cell proliferation assay. Photoconversion of the skin was performed as described previously (17). Briefly, mice were anesthetized, shaved, and exposed to violet light at 95 mW/cm^2 with a 436-nm bandpass filter using Spot UV curing equipment (SP500; USHIO).

For the CHS model, mice were immunized by application of 25 μl of 0.5% DNFB (Nacalai Tesque) in 4:1 (wt/vol) acetone/olive oil to their shaved abdomens on day 0 and challenged on the right ear on day 5 with 20 μl of 0.3% (wt/vol) DNFB (34). Ear thickness was measured before and after challenge, and ear-thickness change was calculated.

For Treg depletion in vivo, mice were injected with Campath-1G Ab through the tail vein (0.5 mg/body) 1 day before the CHS challenge (22). The injection was repeated every 4 days throughout the experiment. The same amount of vehicle or rat IgG (0.5 mg/body; Sigma-Aldrich) was used as a control.

For DNBS- or TNBS-dependent cell proliferation, mice were sensitized with 50 μl of 0.5% DNFB (wt/vol) or 50 μl of 5% TNCB (Tokyo Kasei) (wt/vol) in acetone/olive oil (4/1; vol/vol) on the dorsal skin, and 5 days later, single-cell suspensions were prepared from inguinal and axillary LNs. CD25-positive cells were depleted from the cells by Auto-MACS (Miltenyi Biotec) using PE-labeled anti-mouse CD25 antibody (eBioscience) and magnetic microbeads coated with anti-PE (Miltenyi Biotec). Less than 1% of Foxp3$^+$ cells were present in the remaining LN cells. 7×10^5 LN cells/well in a 96-well plate were cultured in RPMI 1640 containing 10% FBS with or without 50 μg/ml DNBS (Alfa Aesar) for 3 days. For TNBS stimulation, the LN cells were incubated in 2.5 mM TNBS (Tokyo Kasei) in PBS for 20 minutes at 37°C and subsequently washed 3 times in PBS, and 7×10^5 cells/well in a 96-well plate were cultured in RPMI 1640 containing 10% FBS for 3 days. Cells were pulsed with 0.5 μCi ^{3}H-thymidine for the last 24 hours of culture and subjected to liquid scintillation counting.

For the proliferation assay of anti-CD3 stimulation, spleen CD4$^+$ cells deprived of CD25$^+$ cells were sorted by auto-MACS. Then, 5×10^4 cells/well were cultured in a 96-well plate coated with 1 μg/ml of anti-CD3 antibody for 72 hours. For the last 24 hours, cells were pulsed with 0.5 μCi ^{3}H-thymidine, and its incorporation was measured.

Quantitative RT-PCR analysis. Total RNA from purified cells was isolated with the RNeasy Mini Kit (QIAGEN). Quantitative RT-PCR with the Light Cycler real-time PCR apparatus was performed according to the instructions of the manufacturer (Roche) by monitoring the synthesis of double-stranded DNA during the various PCR cycles using SYBR Green I (Roche). For each sample, duplicate test reactions were analyzed for expression of the gene of interest, and results were normalized to those of the *Gapdh* mRNA.

In vivo immunosuppression assay. A total of 4×10^5 cells of isolated Kaede-red Tregs or Kaede-green Tregs in 20 μl PBS were subcutaneously injected into the ventral surface of each ear. Ear thickness was measured for each mouse before and at the indicated time point after elicitation with a micrometer, and the difference was expressed as ear swelling (n = 4–6 in each group).

Chemotaxis assay. Skin cell suspensions of Foxp3$^{hCD2/hCD52}$ mice were tested for transmigration across uncoated 5-μm transwell filters (Corning Costar Corp.) for 3 hours to CCL21 (R&D Systems) or medium in the lower chamber, and the numbers of cells that migrated to the lower chamber were determined by flow cytometry (35). The migration index was shown as a percentage of input by dividing with total input cells in upper chamber.

Statistics. Data were analyzed with the unpaired Student's 2-tailed t test unless otherwise stated. A P value of less than 0.05 was considered to be significant.

Acknowledgments

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and the Ministry of Health, Labor, and Welfare of Japan.

Received for publication August 24, 2009, and accepted in revised form December 16, 2009.

Address correspondence to: Kenji Kabashima, Department of Dermatology and Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University, Yoshida-Konoe, Kyoto, 606-8501, Japan. Phone: 81.75.753.9502; Fax: 81.75.753.9500; E-mail: kaba@kuhp.kyoto-u.ac.jp. Or to: Michio Tomura, Laboratory for Autoimmune Regulation, Research Center for Allergy and Immunology, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama City, Kanagawa 230-0045, Japan. Phone: 81.45.503.9699; Fax: 81.45.503.9697; E-mail: tomura@rcai.riken.jp.

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TCLs from subjects with allergy tended to produce higher levels of IL-5 than those from subjects without allergy, although this did not reach a statistical significance. Interestingly, the highest levels of IL-4 and IL-5 production were observed in the higher-avidity TCLs ($EC_{50} \leq 2$ μg/mL) from subjects with allergy (see this article's Fig E3 in the Online Repository at www.jacionline.org). No differences in the production of IL-10 could be observed between the TCLs from subjects with and without allergy, and only a few TCLs produced IFN-γ or IL-17. Collectively, these results demonstrate that some of the TCLs from subjects with allergy, in particular those with a higher functional TCR avidity, are clearly T_H2-biased, whereas the TCLs from subjects without allergy exhibit mostly an undifferentiated T_H0 phenotype.

Taken together, we have demonstrated in this study that subtle differences in the frequency, functional TCR avidity, and phenotype of Can f 1–specific memory $CD4^+$ T cells exist between subjects with dog allergy and subjects without allergy. However, the mechanisms leading to these differences remain to be elucidated. Importantly, we found no evidence for differences in either the frequency or function of $CD4^+CD25^+Foxp3^+$ Treg cells or IL-10–producing Tr1 cells between the subject groups with and without allergy. This finding is in line with the emerging evidence that immune tolerance to allergens in healthy individuals without allergy cannot be explained solely by Treg cell function.[10] One intriguing possibility that has not been examined so far in detail is that the nature of the nonallergic response may depend on the type of allergen: for some allergens, active suppression by regulatory T cells may be important for maintaining tolerance, whereas for other allergens, T_H1 deviation or immune ignorance may be the prevalent mechanism. Moreover, it is possible that the mechanisms conferring protection against a particular allergen may be different in truly nonatopic individuals than in those sensitized to other allergens. Obviously, studies comparing $CD4^+$ T-cell responses to different allergens in different subject groups are needed to investigate these hypotheses further.

The skillful technical assistance of Virpi Fisk and Raija Tukiainen is gratefully acknowledged.

Suvi Parviainen, MSc[a]
Antti Taivainen, MD, PhD[b]
Aino Liukko, BM[a]
Anssi Nieminen, MSc[a]
Marja Rytkönen-Nissinen, PhD[a]
Tuure Kinnunen, MD, PhD[a]*
Tuomas Virtanen, MD, PhD[a]*

From [a]the Department of Clinical Microbiology, Institute of Clinical Medicine and Biocenter Kuopio, University of Eastern Finland; and [b]the Department of Pulmonary Diseases, Kuopio University Hospital, Kuopio, Finland. E-mail: Tuure.Kinnunen@uef.fi.

Supported by Kuopio University Hospital (project no. 5021605) and the Academy of Finland (contract no. 115160).

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

*These authors contributed equally to this work.

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Available online July 12, 2010.
doi:10.1016/j.jaci.2010.06.005

Fluctuation of blood and skin plasmacytoid dendritic cells in drug-induced hypersensitivity syndrome

To the Editor:

Drug-induced hypersensitivity syndrome (DIHS), also known as drug reaction with eosinophilia and systemic symptoms, is a severe adverse systemic reaction that usually occurs about several

TABLE I. Clinical and laboratory data in DIHS

Case	Age (y)	Sex	Drugs	Latency period (d)	Fever	LA	WBC (/μL)	Eos (/μL)	Atypical lymph (/μL)	ALT (U/L)	Serum IgG (mg/dL)	Viral reactivation (d)
1	49	F	PB	27	+	+	18,700	5,423	935	338	ND	HHV-6 (T,30)
2	67	F	CBZ	24	+	+	21,500	645	1,505	166	648	HHV-6 (T,32)
3	33	M	CBZ	34	+	+	14,600	146	1,314	147	498	HHV-6 (T,25)
4	62	M	CBZ	28	+	+	22,900	3,893	3,435	159	680	HHV-6 (T,29)
5	46	M	CBZ	24	+	+	10,600	307	106	79	766	HHV-6 (T,21)
6	65	M	CBZ	58	+	+	10,300	ND	ND	39	857	HHV-6 (D,14)
7	48	F	PB, CBZ	449	+	+	17,200	4,128	ND	43	ND	HHV-6 (T,28)(D,14), CMV, EBV
8	39	M	CBZ	37	+	+	31,500	2,205	6,615	263	ND	HHV-6 (T,19)(D,11)

Clinical characteristics of the 8 patients, including age, sex, causative drugs (as determined by a drug-induced lymphocyte stimulation test), latency period, fever, and lymph adenopathy, are shown. Laboratory data were gathered at the peak of illness. Viral reactivation was confirmed serologically, based on increased antibody titers *(T)* from the baseline or high DNA levels *(D)*, and the date (days after the onset of skin rash) of reactivation is included.

Atypical lymph, Atypical lymphocyte count; *ALT*, alanine aminotransferase; *CBZ*, carbamazepine; *CMV*, cytomegalovirus; *Drugs*, causative drugs; *Eos*, eosinophil count; *F*, female; *LA*, lymph adenopathy; *M*, male; *ND*, not done; *PB*, phenobarbital; *WBC*, white blood cell count.

weeks after exposure to certain drugs such as anticonvulsants.[1] It is characterized by multiorgan involvement, high fever, hypogammaglobulinemia,[2] and sequential reactivation of various latent herpesviruses, such as human herpesvirus (HHV)–6, HHV-7,[1] cytomegalovirus, or EBV. However, the mechanism underlying the reactivation of these herpesviruses remains unknown.

Human plasmacytoid dendritic cells (pDCs) are a subset of leukocytes capable of producing large amounts of IFN-α/β, which enables neighboring cells to differentiate into mature dendritic cells or mature IgG-producing B cells, thereby resisting viral infection.[3] pDCs have been identified by using the lineage (CD3, CD14, CD15, CD16, CD19, and CD56)$^-$ CD123$^+$ phenotype[4] in the blood and inflamed secondary lymphoid organs.[3,5] It has been hypothesized that pDCs are specialized for viral recognition and antiviral responses and that activated pDCs are involved in psoriasiform and lichenoid drug reactions.[6,7] In this study, we sought to determine the distribution of pDCs in the blood and skin of patients with DIHS and to discuss the possibility that pDCs are involved in the reactivation of viruses in DIHS.

The diagnosis of all patients enrolled in this study was confirmed according to the criteria for DIHS proposed by a Japanese consensus group.[1] Clinical characteristics and laboratory data of the 8 patients are shown in Table I. After diagnosis was made, the causative drugs were withdrawn, and treatment with prednisolone (0.5-1 mg/kg daily) was initiated. As controls, age-matched and sex-matched healthy donors and patients with generalized maculopapular drug eruption (MPE) were enrolled.

After informed consent was obtained, PBMCs were obtained from the subjects at the indicated time points. Frequencies of lineage$^-$ CD123$^+$ pDCs were evaluated by flow cytometry. Patients with DIHS showed low percentages and numbers of circulating pDCs, especially on viral reactivation at 3 to 4 weeks after the onset of DIHS (Fig 1, *A and B*). These values were significantly lower than those in healthy subjects and patients with MPE (Fig 1, *B*). We considered a possibility that pDC counts were affected by treatment with oral corticosteroids, but patients with MPE who were treated with 0.5 to 1 mg/kg prednisolone did not show decreased circulating pDC levels (Fig 1, *B*).

We obtained biopsy specimens from patients with DIHS or MPE and healthy donors. These frozen specimens were stained with rat antihuman CD123, CD16, and isotype-matched control antibodies (BD Biosciences, San Jose, Calif). Significantly high numbers of CD123$^+$CD16$^-$ pDCs were detected in the dermis of patients with DIHS (Fig 1, *C*); the infiltration occurred to a much lesser extent in patients with MPE (Fig 1, *D*), and only rarely in healthy donors (Fig 1, *D*).

Recently, pDCs have been revealed to play a defensive role against viruses.[8] In this study, we focused on the distribution of pDCs in DIHS and found that pDCs accumulate in the skin of DIHS, and that the number of pDCs in circulation decreases significantly around the time point of viral reactivation. These findings suggest that the numerical reduction of circulating pDCs might be caused by their accumulation to the skin. Because we could not define the exact date of viral reactivation, it is difficult for us to state whether the decrease of pDCs precedes or follows viral reactivation. However, we speculate that the paucity of circulating pDCs could depress the antiviral activity in patients with DIHS. This hypothesis is supported by the observation that circulating IgG levels are low in DIHS, because pDCs induce B-cell maturation to produce IgG.[2] As to pDC dynamics, it still

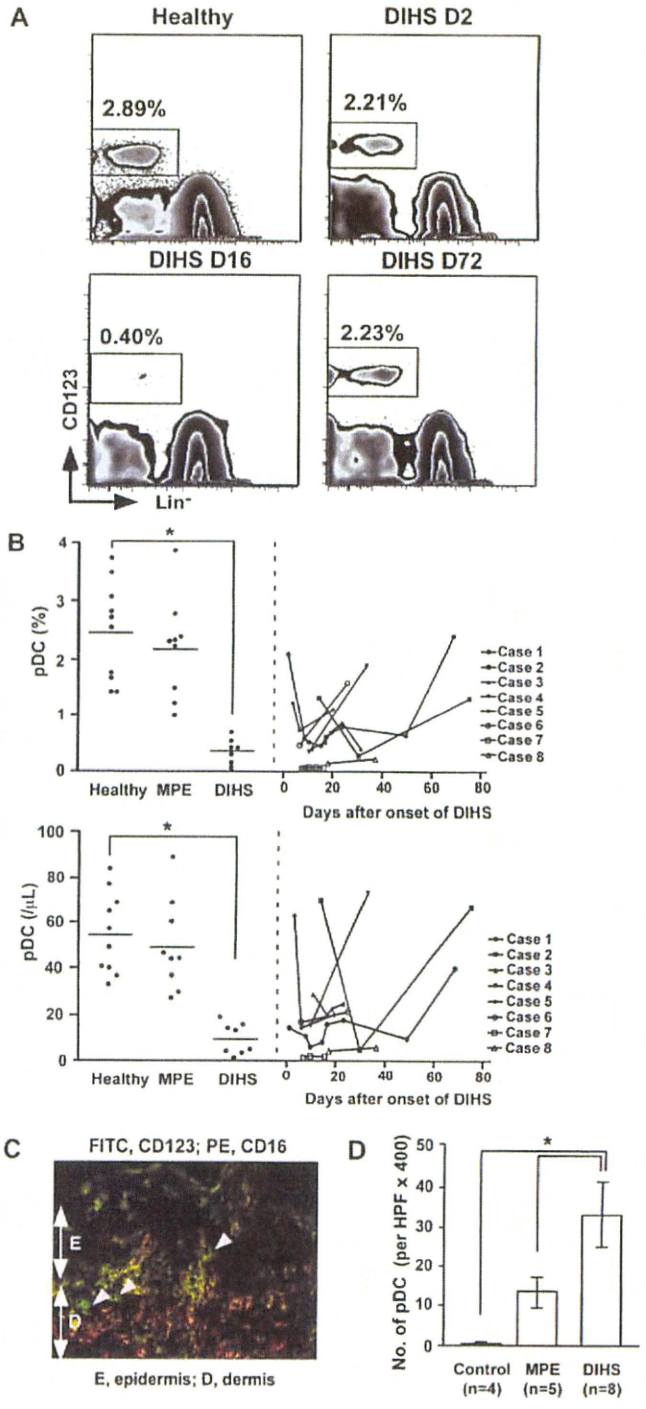

FIG 1. pDC distribution in the blood and in the skin. **A and B,** Fluorescence-activated cell sorting plots of PBMCs in a healthy donor and a patient (case 1) with DIHS 2, 16, and 72 days after the onset *(A)*. The percentages *(upper panel)* and the numbers *(lower panel)* of lineage$^-$ *(Lin$^-$)* CD123$^+$ pDCs in healthy donors, patients with MPE, and patients with DIHS at viral reactivation and at other indicated time points. **C,** Immunohistochemistry of the skin from a patient with DIHS. Arrowheads indicate pDCs. Skin materials were obtained around the peak of illness. **D,** Numbers of pDCs in the skin of healthy donors, patients with MPE, and patients with DIHS per high power fields *(HPF)*. Data are presented as means ± SDs. $^*P < .05$ (Student *t* test). *PE,* Phycoerythrin.

remains unknown how pDCs accumulate in the skin preferentially in patients with DIHS. Because HHV-6 transcripts were detected in the skin of patients with DIHS,[9] pDCs may accumulate to attack the herpesviruses. In addition, the causative drugs such as anticonvulsants might stimulate fibroblasts and/or endothelial cells to release chemoattractants for pDCs in the skin.

pDCs are known to induce autoimmune diseases.[10] The rapid increase in the frequency of pDCs in the blood after treatment for DIHS (Fig 1, *B*) may be associated with the occurrence of certain autoimmune diseases, such as type 1 diabetes mellitus, which are more likely to occur after DIHS.[11] It has been reported that the reduced number of circulating pDCs in patients with SLE can be caused by their accelerated migration to the sites of inflammation including skin lesions.[12] It would be of interest to examine whether administration of type I IFN prevents viral reactivation of DIHS and whether blockade of type I IFN suppresses the occurrence of autoimmune diseases after DIHS. Although we still have much to learn, our studies on pDCs may shed light on the mechanisms underlying DIHS.

Kazunari Sugita, MD, PhD[a]*
Mikiko Tohyama, MD, PhD[b]*
Hideaki Watanabe, MD, PhD[c]
Atsushi Otsuka, MD, PhD[d]
Saeko Nakajima, MD[d]
Masafumi Iijima, MD, PhD[c]
Koji Hashimoto, MD, PhD[b]
Yoshiki Tokura, MD, PhD[a]
Yoshiki Miyachi, MD, PhD[d]
Kenji Kabashima, MD, PhD[d]

From [a]the Department of Dermatology, University of Occupational and Environmental Health, Kitakyushu; [b]the Department of Dermatology, Ehime University School of Medicine, Toon; [c]the Department of Dermatology, Showa University School of Medicine, Tokyo; and [d]the Department of Dermatology, Kyoto University Graduate School of Medicine, Kyoto, Japan. E-mail: kaba@kuhp.kyoto-u.ac.jp.

Supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor, and Welfare of Japan.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

*These authors contributed equally to this work.

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Available online July 12, 2010.
doi:10.1016/j.jaci.2010.06.004

Man's best friend? The effect of pet ownership on house dust microbial communities

To the Editor:

The prevalence of asthma among children has been steadily increasing in westernized nations. Although host genotype likely plays a role in predisposition to allergic disease, the rate at which asthma has increased and the geographically distinct location of this phenomenon implicate environmental factors as being important. Epidemiologic studies have suggested that contact with animals provides protection against allergic disease development[1]; childhood farm exposure, specifically to livestock, is associated with a significant decrease in the risk of atopic sensitization, a protective effect that persists into early adulthood. More recently, in our own birth cohort, maternal prenatal exposure to household pets, particularly dogs, has been suggested to affect fetal immune response development.[2] Higher concentrations of cord blood IgE were associated with mothers unexposed to pets,[2] a notable finding given that previous studies have demonstrated a link between increased cord blood IgE levels and the risk for subsequent development of allergic disorders.[3]

In this study we examined whether the presence of dogs or cats or the absence of a furred pet affected the microbial composition of house dust. Using a high-density phylogenetic microarray, the 16S rRNA PhyloChip,[4] and fungal automated rRNA intergenic spacer analysis, we examined 16 dust samples collected from households with 1 or more dogs (D households; n = 6), 1 or more cats (C households; n = 5), or no furred pets (NP households; n = 5). Five (83.3%), 5 (100%), and 3 (60%) samples from D, C, and NP households, respectively, had sufficient material for microbial analysis. Bacterial community richness (number of bacterial taxa detected), evenness (relative distribution of taxa in communities), and diversity (calculated by using richness and evenness indices) were notably increased in all dog-owning and a subset of cat-owning households. Dust from households with dogs was significantly richer ($P = .04$) and more diverse ($P = .04$) compared with dust from households without pets (Fig 1, *A* and *B*); community evenness was not significantly different between these 2 groups ($P = .14$; Fig 1, *C*, and see Table E1 in this article's Online Repository at www.jacionline.org). These data suggest that dog ownership increases house dust diversity, driven largely by introduction of additional types of bacteria.

Significant differences in taxon relative abundance between the D and NP groups identified 337 taxa significantly increased in abundance in dog-owning houses (see Table E2 and the Methods section in this article's Online Repository at www.jacionline.org). They primarily belonged to the Proteobacteria (112 taxa), Actinobacteria (63 taxa), Firmicutes (47 taxa), Verrucomicrobia (7 taxa),

The Role of Regulatory T Cells in Contact Hypersensitivity

Tetsuya Honda[1], Yoshiki Miyachi[1] and Kenji Kabashima[1,2,*]

[1]*Department of Dermatology and* [2]*Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan*

Received: January 26, 2010; Accepted: February 19, 2010; Revised: March 5, 2010

Abstract: Regulatory T cells (Tregs) are one of the T cell subsets that have strong immune-suppressive activity. Contact hypersensitivity (CHS), clinically manifested as contact dermatitis, is one of the most frequently used mouse models to address cutaneous immune responses, and is composed of two phases: sensitization and elicitation. Recently the role of Tregs in CHS has been investigated using newly generated genetically engineered mice. In this review, we will provide an overview of recent patents and the mechanism of Treg-mediated immunosuppression especially in terms of IL-10, CD39, CTLA-4, and RANKL, and discuss the role of Tregs in CHS during the sensitization and elicitation phases

Keywords: Foxp3, regulatory T Cells, Contact Hypersensitivity.

INTRODUCTION

Regulatory T cells (Tregs) are one of the T cell subsets which have strong immune-suppressive activity. Tregs were originally identified as $CD4^+$ $CD25^+$ T cells [1, 2]. When mice were depleted of $CD4^+$ $CD25^+$ cells, they spontaneously developed autoimmune diseases and allergies [1, 2], indicating that CD4+ CD25+ T cells are essential for the maintenance of self-tolerance. Later on, the fork head box p3 (FOXP3) gene was identified as the master transcriptional factor of Tregs [3]. As the regulation of Tregs function are potential target for the regulation of various kinds of immune diseases, many patents have been carried out to generate Tregs [4, 5]. For example, Cheroutre *et al.* [4]. have intimated a method for stimulating regulatory T cells to increase or decrease immune responses. They reported that addition of TGF-beta or a TGF-beta analogue and a retinoic acid receptor agonist, or an amount of a retinoid X recepotor or peroxisome proliferator activated receptor-gamma agonist were sufficient to stimulate or increase differentiation of Tregs.

There are at least two kinds of FOXP3+ Tregs: naturally occurring Tregs (nTregs) and inducible Tregs (iTregs) [6] nTregs develop in the thymus, and play an important role in the maintenance of self tolerance and immune homeostasis. Scurfy mice, that possess a defective *FOXP3* gene, exhibit hyperactivation of CD4+ T cells and overproduction of proinflammatory cytokines, and typically die within a month after birth [7]. Patients with IPEX syndrome (immune dysregulation polyendocrinopathy, enteropathy, X-linked syndrome) have a mutation in the human *FOXP3* gene, and are therefore regarded as the human counterpart of the Scurfy mice [8]. On the other hand, iTregs are induced from naïve T cells in the presence of transforming growth factor (TGF)-β, and develop in the periphery. Retinoic acid facilitates the differentiation of naïve T cells to *FOXP3*+ cell [9, 10] and may be related to the establishment of oral tolerance, although it remains to be determined whether iTregs are functionally stable and to what extent they contribute under physiological conditions. On the other hand, type I regulatory T (Tr1) cells, which can be induced by antigenic stimulation of naïve T cells in the presence of IL-10 *in vitro*, possess suppressive activity *in vitro* by inducing large amounts of IL-10 and TGF-β, but intriguingly they are Foxp3 and CD25 negative [11, 12].

Besides self-tolerance, evidence has accumulated regarding the regulatory roles of Tregs in a variety of pathophyiological immune responses, such as gastritis, arthritis, encephalomyelitis, inflammatory bowel disease (IBD) and insulin-dependent diabetes, all of which were improved by the administration of Tregs and accelerated by the dysfunction of Tregs [6, 13-16]. On the other hand, Tregs can play negative role for host defense, such as anti-tumor immunity or host defense for bacteria during septic shock [6, 17]. Therefore, many patents have been published for the regulation of Tregs function, such as blocking antibody for CTLA-4, or production of sCTLA4[18-20].

Contact hypersensitivity (CHS), clinically manifested as contact dermatitis, is one of the most frequently used mouse models to address cutaneous immune responses. Contact dermatitis is a common skin disorder caused by many agents such as metals and plants. CHS is mediated by type 1 helper T (Th1) cells and cytotoxic T (Tc1) cells [21, 22] . Recently the role of Tregs in CHS has been investigated using newly generated genetically engineered mice [23].

In this review, we will provide an overview of the mechanism of Treg-mediated immunosuppression, and discuss the role of Tregs in CHS.

Suppression Mechanism by Tregs

Tregs potently suppress proliferation of T cells. When Tregs are co-cultured with responder cells that have been

*Address correspondence to this author at the Department of Dermatology, Kyoto University Graduate School of Medicine, 54 Shogoin-Kawara, Sakyo, Kyoto 606-8507, Japan; Tel: + 81-75-751-3310; Fax: + 81-75-761-3002; Email: kaba@kuhp.kyoto-u.ac.jp

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stimulated with a specific antigen or a polyclonal T cell receptor stimulator in the presence of antigen presenting cells (APCs) s *in vitro*, Tregs suppress proliferation of responder T cells. Using these *in vitro* assays, multiple suppression mechanisms have been proposed. For example, IL-10 [16], TGF-β [24], indoleamine 2,3-dioxygenase (IDO) [25, 26], IL-35 [27], and CD39 [28], have been considered as soluble suppressive factors of T cell proliferation. Absorption of IL-2, one of the most important cytokine for T cell proliferation, by Tregs may also be involved in inhibiting T cell proliferation [29].

It has recently been reported that Tregs inhibited the T cell stimulatory capacity of APCs by down-regulating CD80 and CD86 expression through cytotoxic T-lymphocyte antigen (CTLA)-4 and lymphocyte function-associated antigen (LFA)-1 [30-32]. Using two-photon microscopic analysis, Tadokoro *et al.* [33] and Tang *et al.* [14] have revealed that Tregs inhibit the stable contact and interaction between APCs and effector T cells. However, it has yet to be determined how these *in vitro* findings correlate with *in vivo* suppression, because the suppressive functions of Tregs may differ depending on the disease model. For example, IL-10 plays an important role in the suppression of Tregs in IBD [16], since IL-10 deficient mice or Tregs that cannot produce IL-10 did not suppress IBD. On the contrary, both wild-type (WT) and IL-10 deficient Tregs are able to suppress autoimmune gastritis [15] , suggesting that suppressive mechanisms other than IL-10 are present in the gastritis model.

Development of CHS

CHS is composed of two phases: sensitization and elicitation [21]. In the sensitization phase, low molecular weight compounds called hapten are cross-linked to epidermal proteins and taken up by resident dendritic cells such as Langerhans cells (LC) and dermal dendritic cells. Subsequently, these cells are matured by proinflammatory cytokines such as TNF-α, IL-1β, and prostaglandin E_2, and migrate to the draining lymph nodes to present antigens in a CCR7 and CXCR4-dependent manner [34-36]. After antigen presentation, naive T cells are activated and differentiated into antigen-specific Th1 and Tc1 cells under the influence of polarizing signals such as IL-12 and other chemical mediators [37]. After the establishment of this sensitization, when the skin is re-exposed to the same hapten, an antigen-specific T cell-mediated inflammation is provoked, which represents the beginning of the elicitation phase. By re-exposure to the same hapten, keratinocytes and mast cells produce chemokines or pro-inflammatory cytokines such as TNF-α and IL-1β, which activate endothelial cells and induce the expression of E- or P-selectins [38-40]. Then, neutrophils and antigen specific T cells enter the dermis and release IFN-γ, which further stimulate keratinocytes, leading to massive leukocyte infiltration [41].

Effect of Tregs on the CHS Response

The effect of Tregs on CHS has mainly been investigated in the elicitation phase. Ring *et al.* purified CD4+ CD25+ Tregs from naïve mice and administered them into trinitrochlorbenzene (TNCB) -sensitized recipient mice intravenously one day before elicitation [42]. Administration of Tregs significantly suppressed the ear swelling response and inflammatory cell infiltration into the skin compared to those of vehicle-treated mice. They reported that these suppressive effects are mediated by soluble factors, especially IL-10, because administration of a culture supernatant of Treg suppressed the CHS response, which was reversed by an anti-IL-10 antibody. Furthermore, Tregs from IL-10-deficient mice failed to suppress the CHS response by inhibition of leukocyte influx into inflamed skin. The same group has recently reported that adenosine produced by Tregs in a CD39-dependent manner was critically involved in the influx of leukocytes through endothelial cells (EC) and the suppression mechanism of Tregs in CHS [43]. Adenosine triphosphate (ATP) is first degraded by CD39 to adenosine diphosphate (ADP) and then to adenosine monophosphate (AMP). The AMP is serially dephosphorylated by CD73 to adenosine. Tregs are strongly positive for CD39 and CD73 expression. They also reported that both adenosine and Tregs negated the adherence of effector T cells to ECs by downregulating E- and P- selectins on ECs, and injection of adenosine and Tregs abrogated the ear-swelling response in CHS, which was not seen using Tregs from CD39 deficient mice. Conventional T cells have only a low basal expression of CD39, and differ greatly from Tregs which are strongly positive for cell surface expression of CD39 and CD73. Moreover, Tregs further upregulate CD39 expression after activation, with this activation being a prerequisite for Tregs acquiring their suppressive capacity.

On the other hand, the effect of Tregs in the sensitization phase has only rarely been investigated. Dubois *et al.* [44] reported the involvement of Tregs in the induction of oral tolerance and inhibition of dinitrofluorobenzene (DNFB) - induced CHS. Oral tolerance was induced by feeding DNFB prior to DNFB-sensitization, which was not seen in CD4+ T cell-deficient mice. However, transfer of naïve CD4+ CD25+ T cells restores oral tolerance in those mice independent of IL-10. They also reported that administration of anti-CD25 mAb impairs oral tolerance in WT mice [44]. Intriguingly, administration of anti-CD25 mAb before sensitization had no affect on the ear swelling response, suggesting that CD4+ CD25+ T cells are responsible for oral tolerance induction at a time other than during the sensitization phase. However, the role of CD4+ CD25+ Tregs in sensitization of CHS remains not fully elucidated, and should be investigated in the future.

On the contrary, most of the studies on ultraviolet (UV)-induced Tregs have been focused on the sensitization phase. UV-irradiation before sensitization leads to immunosuppression, one of the mechanisms of which is induction of Tregs by UV. Schwartz *et al.* reported that administration of CD4+ CD25+ cells from UV-irradiated DNFB-sensitized mice impaired sensitization [45, 46]. Those UV-induced Tregs did not suppress the CHS response when administered before elicitation, which is in contrast to the effect of natural CD4+ CD25+ Tregs. However, direct injection of UV-induced Tregs into elicitation sites suppressed the CHS response. They concluded that UV-induced Tregs did not express skin-homing receptors for E- and P-selectins, which lead to the failure of inhibition of the elicitation phase. nTregs and UV-induced Tregs seem to have different phenotype and suppression mechanisms [47]. As for the

mechanism of Treg induction by UV, Loser *et al.* [48] reported that the receptor activator of NF-kappaB ligand (RANKL) was induced in keratinocyte by UV exposure, and RANKL-activated Langerhans cells (LCs) were responsible for the development of UV-induced Tregs. Similar findings were observed during skin grafting. Yoshiki *et al.* reported that the development of CHS is suppressed when mice are sensitized with a hapten through full-thickness grafted skin. CD4+ CD25+ but not CD4+ CD25- T cells in the draining LNs were responsible for this suppression [49]. In addition, a high expression of RANKL was observed in the grafted skin, and recombinant RANKL stimulated LCs to produce IL-10. These findings suggest that the hyposensitization of CHS through the grafted skin is attributable to Tregs induced by IL-10-producing LCs.

The Role of Endogenous Tregs in CHS

As described above, exogenously added Tregs have the ability to inhibit CHS. However, it remains unclear whether intrinsic Tregs play such suppressive roles under physiological conditions. To this end, specific depletion of Tregs *in vivo* is required. Although CD4+ CD25+ has been used as a marker for Tregs, CD25 is expressed in activated CD4 cells as well as in Tregs. Therefore, FOXP3 is the more definitive marker of Tregs, but Foxp3 is a transcriptional factor and exists intracellularly; therefore, purification of live Tregs has been technically difficult. To solve these problems, Dr Hori's group recently generated FOXP3 reporter mice expressing human CD2 and human CD52 chimeric protein, which have been designated as FOXP3$^{hCD2/hCD52}$ mice. Since *FOXP3*+ cells co-express hCD2 on the cellular surface, live *FOXP3*+ Tregs are sorted with anti-hCD2 monoclonal Ab, and depleted with neutralizing anti-hCD52 Ab (manuscript in preparation). Using these mice, we investigated the role of endogenous Tregs in CHS, especially focusing on Tregs in skin. Depletion of Tregs in elicitation phase caused a much more enhanced and prolonged ear swelling response compared with that of the control, indicating that Tregs play important roles in the termination of inflammation [23]. In addition, using Kaede-transgenic mice we have further examined the role of Tregs in CHS, especially focusing on skin Tregs. Kaede-transgenic mice are genetically engineered to ubiquitously express Kaede protein, which is a photoconvertible protein that changes its fluorescence from green to red under exposure to violet light [50]. Therefore, we can analyze the migration of cells from the skin under physiological conditions. Using these mice, we found that Tregs existed abundantly in the inflamed skin of CHS, and those skin Tregs further migrated back to draining LNs. Tregs from the skin showed significantly higher mRNA expression of Treg-associated molecules, such as IL-10, TGF-β and CTLA4. Consistently, Tregs from the skin showed significantly stronger suppressive activity both *in vivo* and *in vitro* [23] Fig. (**1**).

It has been reported that Tregs in the skin contribute to its homeostasis, because chronic depletion of skin Tregs leads to the development of spontaneous dermatitis [51, 52]. Our results suggest that Tregs in the skin also play important roles in the termination of dermatitis and possibly in the control of systemic immune responses. Schneider *et al.* [53]. Reported that, CCR7-deficient mice showed a reduced number of Tregs in draining LNs and an enhanced inflammatory

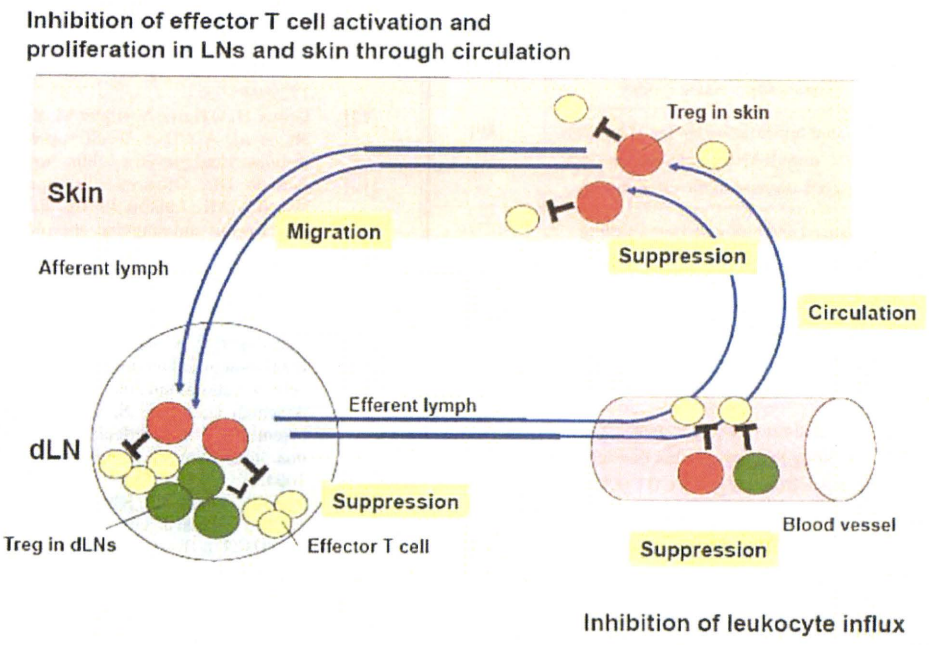

Fig. (1). Possible suppression mechanism of Tregs in cutaneous immune responses.
Tregs suppress effector T cells in the lymphnodes (LN) and inhibit leukocyte influx into the periphery. In addition, Tregs migrating into the skin could suppress the effector T cell functions in the skin, and a fraction of Tregs in the skin migrate back to the draining LNs (dLNs) through afferent lymphatic vessels and to circulation. Skin derived Tregs produce high amount of IL-10 and TGF-beta, which are supposed to be involved in the strong suppressive activity of the skin derived Tregs.

response in CHS after repeated hapten application, suggesting that Tregs homing to draining LNs through CCR7 is of significance in the function of Tregs.

CURRENT & FUTURE DEVELOPMENTS

We have reviewed the roles of Tregs in cutaneous immune responses based on recent studies Table **1**. Although a considerable amount of knowledge on Tregs has been accumulated, several critical issues still remain to be solved. It is important to further clarify the molecular mechanism involved in suppressing immune responses by exogenously added Tregs or intrinsic Tregs *in vivo*. From a clinical perspective, the precise mechanism by which Tregs function in the elicitation phase is an important issue to be addressed. However the details of its suppressive mechanisms are still unclear as is the role of intrinsic Tregs in sensitization. As described previously, multiple mechanisms and various molecules are involved in Treg-mediated immunosuppression. Further analysis using Foxp3-diphtheria toxin receptor knockin mice [54] or $Foxp3^{hCD2/hCD52}$ mice, which enable to deplete Tregs conditionally and specifically, could be useful to better investigate the molecular mechanism and physiological function of Tregs in cutaneous immune responses.

Table 1. An Overview of Recently Published Papers About Tregs and CHS

	Major Findings	Reference
Sensitization	Attenuated sensitization by Tregs induced by orally administered antigen in an oral tolerance model	[44]
	Attenuated sensitization by Tregs induced by RANKL activated LC in a UV-immunosuppression model	[48]
	Attenuated sensitization by Tregs induced by IL-10 from RANKL activated LC in a skin graft-immunosuppression model	[49]
Elicitation	Enhanced and prolonged ear swelling response by the depletion of endogenous Tregs	[23]
	Reduced ear swelling response by inhibiting leukocyte influx through IL-10 from Tregs	[42]
	Reduced ear swelling response by inhibiting leukocyte influx through adenosine from Tregs via CD39/CD73 (inhibition of E- and P-selectin expression in endothelial cells)	[43]

ACKNOWLEDGEMENTS

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and the Ministry of Health, Labor, and Welfare of Japan.

CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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J Environ Dermatol Cutan Allergol. 4 (3) : 137-142, 2010

シンポジウム

中毒性表皮壊死症（TEN）の発症機序

小豆澤宏明[1]

要　旨

薬疹は疾患の治療中あるいは検査中に投与された薬剤によって皮膚に起こる薬剤有害反応であり，患者にとっては有益な作用が期待できる薬剤であっても，その使用をあきらめなければならない厄介な副作用である．薬疹のなかで，比較的軽症の播種性紅斑丘疹型から重症のStevens-Johnson症候群（SJS）や中毒性表皮壊死症（TEN）といった病型でT細胞による免疫反応である遅延型過敏反応が関与すると考えられているが，その発症機序は必ずしも明らかではない．薬疹が重症化する要因は，原因薬剤の中止の遅れや，不慮の再投与により，免疫反応が増強されることが考えられるが，重症薬疹のなかには，急速に進行し，これらの要因のみでは重症化を説明できない症例もあり，SJSやTENが単に通常の薬疹の重症化したものではないと考えうる．われわれはこれまでにトランスジェニックマウスを用いてTENの病態を再現し，その病態を解析してきた．これまで薬疹の発症機序については表皮細胞を傷害するエフェクターT細胞の活性化とその傷害機序が注目されてきた．われわれの動物モデルでは表皮傷害性$CD8^+$T細胞がエフェクターT細胞となりうることが明らかであったが，意外なことにそれらが非生理的なほど多数生体内に存在しても表皮障害は比較的軽度でTENを発症しなかった．その理由として，生体には有害な免疫反応を抑制する胸腺由来の$CD4^+CD25^+Foxp3^+$制御性T細胞（regulatory T cell）が存在し，抗原提示細胞である樹状細胞の介在により，表皮障害を抑制しており，制御性T細胞の減少や機能異常が薬疹の重症化に関与することがわかってきた．制御性T細胞が，外来抗原である薬剤に対する免疫反応をいかにして抑制しているのかは明らかではないが，TEN患者における免疫学的背景を考える上でその役割は重要と考えられる

（J Environ Dermatol Cutan Allergol. 4 (3) : 137-142, 2010）

キーワード：toxic epidermal necrolysis, cytotoxic T lymphocyte, regulatory T cell, drug hypersensitivity, animal model

はじめに

免疫という防御システムは私たちの体を感染症から守っているが，同時に多彩な疾患の病態形成に関与する．大きく分けて自然免疫と獲得免疫の2つのシステムが知られるが，とくに後者ではT細胞免疫が重要な役割を担う．たとえば，急性移植片対宿主病（graft versus host disease, GVHD）では非自己である移植片（この場合はT細胞）が宿主内の自己抗原に対して細胞障害を引き起こす．また，自己免疫疾患は自己抗原に対して，自己反応性T細胞が免疫反応を起こすことで臓器障害につながることが知られる．それでは薬疹はどうであろうか．T細胞は自己免疫疾患と同じく自己の細胞であるが，抗原は非自己であり，自己免疫疾患とは異なる．一方，急性GVHDでは薬疹と区別が困難なほどよく似た臨床症状，組織障害がみられるが，抗原と反応性T細胞の関係が，自己，非自己という視点からみると全く逆である．そして最も異なる点は，急性GVHD，自己免疫疾患でT細胞が反応するのは自己のタンパク抗原であるのに対し，薬疹では外来の薬剤つまり低分子量の化学物質である．元来タンパク抗原を認識するT細胞が薬剤抗原により活性化し，結局は薬剤を排除しているのではなく，自己の細胞障害を

[1] 大阪大学大学院医学系研究科内科系臨床医学情報統合医学皮膚科学講座
〒565-0871　吹田市山田丘2-2
連絡先：小豆澤宏明
掲載決定日：2010年2月18日