Skin resident T cells

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A primary role for human central memory cells in tissue immunosurveillance


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Central memory T cells (T_{CM}) patrol lymph nodes, providing central immunosurveillance against known pathogens, but have not been described as conducting primary tissue immunosurveillance. We analyzed the expression of tissue-homing addressins in human T_{CM} vs effector memory T cells (T_{EM}) from the same donors. In humans, the majority of human T_{CM} were tropic for either skin or gut, and the overall tissue tropism of T_{CM} was comparable to that of T_{EM}. T_{CM} were present in healthy, noninflamed human skin, lung, colon, and cervix, suggesting a role for T_{CM} in the primary immunosurveillance of peripheral tissues. T_{CM} also had potent effector functions; 80% of CD8^{+} T_{CM} produced TC1/TC2/TC17/TC22 cytokines. T_{CM} injected into human skin-grafted mice migrated into skin and induced inflammatory eruptions comparable to T_{EM}-injected mice. In summary, human T_{CM} express peripheral tissue-homing receptors at levels similar to their effector memory counterparts, are found in healthy human tissues, have impressive effector functions, and can act alone to induce skin inflammation in human engrafted mice. Our studies support a novel role for human T_{CM} in primary immunosurveillance of peripheral tissues and highlight the important role of this long-lived cell type in tissue-based immune responses.
INTRODUCTION

Central memory T cells (T\textsubscript{CM}) co-express L-selectin and CCR7 and provide central immunosurveillance by patrolling the lymph nodes draining peripheral tissue sites\textsuperscript{1,2}. In mice and nonhuman primates, T\textsubscript{CM} provide effective long-term protection because they persist long term in the circulation, have a high proliferative potential, and can give rise to both effector and effector memory T cells (T\textsubscript{EM}) after antigen reencounter\textsuperscript{3-6}. Although they can be drawn into inflamed tissues, T\textsubscript{CM} have not been identified in animals as providing primary tissue-based immunosurveillance\textsuperscript{7}. We report that human T\textsubscript{CM} express tissue-homing receptors, are found in noninflamed human tissues, and have potent effector functions, supporting a role for these cells in primary tissue immunosurveillance.

RESULTS

We compared the expression of tissue-homing addressins in T\textsubscript{CM} vs T\textsubscript{EM} from healthy human blood donors by CyTOF (Figure 1). T\textsubscript{EM} are generated in response to tissue-based infections and should all be tropic for peripheral tissues\textsuperscript{10,11}. Skin (cutaneous lymphocyte antigen, CLA\textsuperscript{12}) and gut (α4β7 integrin)\textsuperscript{13,14} homing receptors have been identified, but receptors that direct T cells to lung, brain, and other peripheral tissues remain uncharacterized. We therefore measured the expression of skin and gut-homing addressins in T\textsubscript{CM} vs T\textsubscript{EM} from healthy human donors. Subsets of T\textsubscript{CM} and T\textsubscript{EM} expressed either gut-homing or skin-homing addressins, with very few cells expressing both (Figure 1A). Both CD8\textsuperscript{+} and CD4\textsuperscript{+} T\textsubscript{CM} contained skin-tropic and gut-tropic populations (Figure 1A-B, G). The expression of skin and gut-homing receptors was comparable in T\textsubscript{CM} and T\textsubscript{EM}, except that significantly more CD4\textsuperscript{+} T\textsubscript{EM} expressed gut-homing receptors and significantly more CD8\textsuperscript{+} T\textsubscript{CM} expressed skin-homing receptors (Figure 1C-D). When the total number of T cells expressing skin or gut-homing receptors were compared, T\textsubscript{CM} and T\textsubscript{EM} expressed tissue-homing addressins at similar levels (Figure 1E-F).

These results suggest human T\textsubscript{CM} have intrinsic tissue tropism and may play a role in primary immunosurveillance of peripheral tissues. To evaluate this possibility, we isolated T cells from noninflamed human skin, lung, colon, and cervix. We observed populations of T\textsubscript{CM} in each of these healthy peripheral tissues, confirming that T\textsubscript{CM} do gain access to noninflamed human tissues (Figure 1H-I). T\textsubscript{CM} are known for their ability to persist in the circulation, to proliferate, and to give rise to effector T cells in animal models, but their production of cytokines that can directly combat infection has not been characterized in humans.
Figure 1. Human TCM express tissue-homing addressins and are found in healthy human peripheral tissues. (A-B) Comparison of the expression of skin-homing (CLA) and gut-homing (α4β7) addressins in TCM and TEM by CyTOF analyses from a representative human donor. The gating strategy used to identify TCM and TEM among human peripheral blood CD45RO+ CD45RA- FOXP3+ memory T cells is shown. (C-D) Aggregate data of skin and gut addressin expression by (C) CD4+ and (D) CD8+ TCM (white bars) and TEM (black bars). T cells that expressed neither CLA nor α4β7 were designated as unassigned. The mean and standard error of the mean (SEM) of 4 donors are shown. (E-G) TCM and TEM have comparable expression of skin- and gut-homing addressins. The mean and SEM of the total assignable tissue-tropic populations (total tropism, the sum of skin and gut-tropic populations) for (E) CD4+ and (F) CD8+ T cells are shown. The mean and SEM of 6 donors are shown. (H) TCM are present in noninflamed human peripheral tissues. T cells were isolated from noninflamed human tissues and analyzed by flow cytometry. TCM as a percentage of the total T-cell population are shown for (H) individual representative samples and (I) pooled data. The mean and SEM of 5 skin, 2 lung, 3 colon, and 6 cervix samples are shown.
We studied cytokine production of T<sub>CM</sub> and T<sub>EM</sub> and found that both CD4 and CD8 T<sub>CM</sub> had impressive effector functions (Figure 2A-J). Interferon-γ (IFN-γ) was the most highly produced signature cytokine in T<sub>CM</sub>, as it was in T<sub>EM</sub>. CD8<sup>+</sup> T<sub>CM</sub> were particularly cytokine rich, with a mean 70% and 97% of CD8<sup>+</sup> T<sub>CM</sub> producing signature cytokines among the skin-tropic and gut-tropic populations, respectively. The proportion of CD8<sup>+</sup> T<sub>CM</sub> producing signature cytokines was not significantly different from T<sub>EM</sub> from the same donors (Figure 2G). Gut-tropic CD8<sup>+</sup> T<sub>CM</sub> produced significantly more IL-2 than CD8<sup>+</sup> T<sub>EM</sub>, but levels produced by all other subsets were comparable (Figure 2C, H).

Between 40% and 55% of CD4<sup>+</sup> T<sub>CM</sub> also produced signature cytokines, and IFN-γ was the most frequently produced cytokine in both CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>CM</sub> (Figure 2A-B, F). Tumor necrosis factor-α (TNF-α) was produced by the majority of T cells tested, and IL-10 was produced at low levels in all subsets (Figure 2D-E, I-J). These results demonstrate that all T<sub>CM</sub>, and CD8<sup>+</sup> T<sub>CM</sub> in particular, have considerable effector functions that endow them with the capacity to contribute to frontline anti-pathogen responses in peripheral tissues.

We used a human engrafted mouse model to measure the relative ability of T<sub>CM</sub> and T<sub>EM</sub> to enter skin and induce inflammation. NSG mice were grafted with neonatal human foreskin, a tissue that lacks T cells, and infused IV with allogeneic purified peripheral blood T<sub>EM</sub>, T<sub>CM</sub>, or naive T cells isolated from healthy adult human donors (Figure 2K; supplemental Figure 1).<sup>9</sup> A robust inflammatory dermatitis was observed in both T<sub>EM</sub>- and T<sub>CM</sub>-injected mice (Figure 2L-S). T<sub>CM</sub> effectively entered human skin and induced inflammatory changes, including interface dermatitis, epidermal spongiosis, and epidermal necrosis (Figure 2Q-S). One caveat is that T<sub>CM</sub> may differentiate into effector cells within the skin graft, and we cannot rule out a contribution of newly generated effector cells to skin inflammation. In contrast, injection of naive T cells led to minimal infiltration of T cells into skin and little if any visible inflammation (Figure 2P). Very few human T cells migrated into mouse skin adjacent to the grafts, and no inflammation in mouse skin was appreciated (supplemental Figure 2). Although this is not a model of immunosurveillance per se, it does measure in vivo the ability of human T cells to enter human skin and induce inflammation.

NanoString-based gene expression profiling of the skin grafts demonstrated comparable levels of T cells in the skin of T<sub>CM</sub>- and T<sub>EM</sub>-injected mice (Figure 2T). Moreover, the levels of TNF-α, IFN-γ, IL-17A, IL-22, perforin, granzyme A, granzyme B, and inflammatory chemokines were similar in the skin of T<sub>CM</sub>- and T<sub>EM</sub>-injected mice (Figure 2U-W). These studies demonstrate and confirm the ability of T<sub>CM</sub> to enter the skin and initiate inflammation in the absence of other T-cell subsets.
Figure 2. Human TCM have potent effector functions, home to skin, and induce dermatitis in human engrafted mice. (A, F) Both CD4+ and CD8+ TCM produced T-cell signature inflammatory cytokines. Cytokine production was assayed by CyTOF following stimulation with phorbol 12-myristate 13-acetate/ionomycin. Production of TH1/TC1 (IFN-γ), TH2/TC2 (IL-4, IL-13), TH17/TC17 (IL-17A), TH22/TC22 (IL-22) signature cytokines by TCM and TEM is shown. Figures represent the mean of 6 donors. (B, G) Total production of signature cytokines by TCM (white bars) compared with TEM from the same donors (black bars) for (B) CD4+ and (G) CD8+ T cells. (C-E, H-J) Production of IL-2, TNF-α, and IL-10 by (C-E) CD4+ and (H-J) CD8+ T cells is shown. The mean and SEM of 6 donors are shown. (K-W) Human TCM home to skin and induce an inflammatory dermatitis comparable to TEM in human engrafted mice. (K) The human engrafted mouse experimental model. (L-P) Hematoxylin and eosin evaluation of human skin grafts 3 weeks after injection of (L-M) saline, (N) TEM, (O) TCM, or (P) naive T cells.
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TCM homed to human skin grafts and induced T-cell–mediated inflammatory dermatitis. (P) Injection of naive T cells led to minimal inflammation. (Q-S) The inflammatory patterns induced by purified TCM included (Q) interface dermatitis, (R) spongiotic dermatitis, and (S) epidermal necrosis. Results shown are representative of those obtained with 6 different human blood cell donors. (T) T-cell migration into the skin as assessed by NanoString CD3/CD4/CD8 gene expression analysis was comparable in TCM- and TEM-injected mice. (U-V) The production of inflammatory T-cell cytokines in skin (TNF-α, IFN-γ, IL-17A, and IL-22) and cytotoxic effector molecules (PRF1, perforin; GZMA, granzyme A; GZMB, granzyme B) was comparable in TCM- and TEM-injected mice. (W) Production of inflammatory chemokines in skin was comparable in TCM- and TEM-injected mice. For panels T-W, the mean and SEM of messenger RNA copies detected by NanoString analyses from 6 different human TCM/TEM donors are shown. Scale bars, 100 mm. GVHD, graft-versus-host disease; NS, normal skin; ns, not significant.
DISCUSSION

T_{CM} provide effective long-term memory responses because they have the capacity to persist long term in the circulation, have a high proliferative capacity, and can replenish other memory T-cell subsets, including T_{EM}.^{3-6,15} The role of T_{CM} in immunosurveillance has been assumed to be limited to patrolling the lymph nodes for evidence of pathogen exposure. The initial description of human T_{CM} characterized these cells as having poor effector functions and little tissue tropism.^{1} However, these studies did not evaluate the expression of the gut-homing addressin α4β7, used a different antibody to detect CLA than the one that identifies cutaneous T cells (HECA-205 vs HECA-45212), and did not study the cytokine production of polyclonally stimulated T_{CM}. With the benefit of updated and more comprehensive approaches, it is clear that T_{CM} express tissue-homing addressins at levels similar to T_{EM}, and indeed, these cells are present in healthy human peripheral tissues. This is consistent with a prior report that CCR7 is expressed by the majority of CLA- and α4β7-expressing T cells in human blood.^{16}

Human T_{CM}, particularly CD8^{+} T_{CM}, also have significant effector functions. T_{CM} alone were capable of entering human skin and initiating inflammation comparable to that induced by T_{EM}. These findings demonstrate that T_{CM} express tissue-homing receptors, are found in healthy human peripheral tissues, have potent effector functions, and can migrate into and initiate tissue-based inflammation. Our findings suggest that human T_{CM}, much like T_{EM}, are imprinted with both tissue-homing addressin expression and specialized programs of cytokine production and likely participate directly in the immunosurveillance of peripheral tissues.

Tissue-tropic T_{CM} have not yet been described in animal models, perhaps because young mice kept in pathogen-free conditions, the animals used in most experiments, lack the large numbers of pathogen-specific recirculating T_{CM} that human patients have accumulated over decades of pathogen exposures. Alternatively, there may be key differences in homing of human vs mouse T_{CM}.

Our work demonstrates that human T_{CM} enter and have the capacity to provide primary immunosurveillance of peripheral tissues. Added to their known abilities to persist long term in the circulation, proliferate, and give rise to additional memory T-cell subsets, our work supports a critical role for T_{CM} in providing long-term protection against known pathogens.
MATERIALS AND METHODS

Samples
All studies were performed in accordance with the Declaration of Helsinki. Approval of the Partners Institutional Review Board committee was obtained for all studies. Deidentified blood, skin, foreskin, lung, and colon were obtained from Brigham and Women’s Hospital, and cervix was obtained from Johns Hopkins. Lung and colon were obtained from distant, uninvolved tissue obtained from patients undergoing resection of small isolated tumors. Normal cervix was obtained from patients undergoing hysterectomy for nonmalignant disorders. T cells were isolated by collagenase digestion or short-term explant culture.

T cell isolation from tissues
T cells were isolated by collagenase digestion or from short term explant cultures. Collagenase digestion: Tissue samples were extensively minced and then incubated for 2 h at 37°C in RPMI-1640 containing 0.2% collagenase type I (Invitrogen) and 30 Kunitz Units/ml DNase I (Sigma Aldrich). Cells were then collected by filtering the collagenase-treated tissue through a 70 μm cell strainer (Fisher Scientific). Short term explant cultures: 9mmx9mmx1.5mm Cellfoam matrices (Cytomatrix Pty Ltd, Australia) were autoclaved, then incubated in a solution of 100 mg/ml rat tail collagen I (BD Biosciences, Bedford, MA) in phosphate buffered saline for 30 minutes at 37° C, followed by two rinses in phosphate buffered saline. Skin was cut into approximately 2mmx2mmx2mm explants and approximately 10 explants were placed on the surface of each matrix. Skin on the matrices was cultured submersed in Iscove’s modified medium (Mediatech, Herndon, VA) with 20% heat-inactivated fetal bovine serum (Sigma, St Louis, MO), penicillin and streptomycin, and 1.75 μl/l 2-mercaptoethanol. T cells were collected 3 weeks later by aspiration of medium in the wells and by flushing of the matrices.

Flow cytometry and CyTOF
Flow cytometry samples were run on a Becton Dickinson FACSCanto instrument, and CyTOF samples were analyzed on a Fluidigm CyTOF 2 mass cytometer. Data were analyzed with FCS Express 5.0 or FACSDiva 8.0. Cells were sorted on a FACSARia cell sorter.

Antibodies used: Flow cytometry antibodies used in this study were CD3 (UCHT1, Biolegend), CD45RA (HI100, Biolegend), L-selectin (DREG-56, Biolegend), and CCR7 (150503, R&D Systems). CyTOF antibodies used in this study from Fluidigm were CD45RA (HI100), FOXP3 (PCH101), IL-2 (MQ1-17H12), IL-10 (JES9D7), IL-17A (BL168), IFNγ (B27), anti-biotin (1D4-C5, to detect CLA), and anti-APC (APC003, to detect α4β7). CyTOF antibodies used in this study from the Harvard Medical Area CyTOF Antibody Resource Core were CCR7 (G043H7), CD3 (UCHT1), CD4 (RPA-T4), CD8b (SIDI8BEE), L-selectin...
(DREG-56), CD45RO (UCHL1), TNFα (Mab11), IL-4 (8D4-8), IL-13 (JES10-SA2), and IL-22 (BG/IL22). CyTOF antibodies used in this study from Biolegend were CLA-biotin (HECA-452) and anti-mouse IgG1-APC (RMG1-1, to detect α4β7). The α4β7 (ACT-1) antibody used for CyTOF was a kind gift from Dr. Eugene Butcher.

Human engrafted mouse model

Human engrafted mice were prepared as described previously. Briefly, neonatal foreskins were grafted onto the backs of 6- to 8-week-old non-obese diabetic/severe combined immunodeficiency/ interleukin-2 (IL-2) receptor γ chainnull mice (Jackson Laboratories). One week later, 3 x10⁶ flow-sorted Tcm, TEM, or naive T cells from an unrelated adult human blood donor were injected IV. The gating strategy and flow cytometry profiles of infused cells are included in supplemental Figure 1. Skin grafts were harvested after 3 weeks for analysis.

Statistical analyses

Primary methods of data analysis included descriptive statistics (means, medians, and standard deviation). Differences between 2 sample groups were detected using the 2-tailed Wilcoxon-Mann-Whitney test, α= 0.05. For comparisons of multiple groups, a Kruskal-Wallis 1-way analysis of variance with a Bonferroni-Dunn’s posttest for multiple means test was used, α= 5 0.05.

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REFERENCES

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Gating strategy and flow cytometry profile of T cells infused into human engrafted mice. (A) The gating strategy used to isolate T cell subsets is shown. (B) Flow cytometry histograms of sorted cells.

Supplementary Figure 2. Human T cells home specifically to the human skin grafts and do not cause inflammation of host mouse skin. (A, B) H&E and (C, D) anti-human CD3 T cell stains demonstrate that infused human T cells home specifically to the human skin graft. Human skin grafts can be identified by their lack of hair follicles. Little if any human T cell infiltration into the skin of host mice was observed. The (A, C, D) mouse human junction and (B) a sample of mouse skin adjacent to the graft are shown. Scale bar is equivalent to 100 microns.