REGULAR ARTICLE

blood advances

A primary role for human central memory cells in tissue immunosurveillance

Ahmed Gehad,[1] Jessica E. Teague,[1] Tiago R. Matos,[1-3] Victor Huang,[1] Chao Yang,[1] Rei Watanabe,[1] John T. O'Malley,[1] Cornelia L. Trimble,[4] Thomas S. Kupper,[1] and Rachael A. Clark[1]

[1]Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Lisbon, Portugal; [3]Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and [4]Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, Baltimore, MD

Key Points

- Human T_{CM} are tissue tropic, have impressive effector functions, and are found in non-inflamed human tissues.
- T_{CM} can act alone to induce inflammation in human skin–grafted mice; results suggest a role for human T_{CM} in primary immunosurveillance.

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_{CM}) coexpress L-selectin and CCR7 and provide central immunosurveillance by patrolling the lymph nodes draining peripheral tissue sites.[1,2] In mice and nonhuman primates, T_{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_{EM}) after antigen reencounter.[3-6] Although they can be drawn into inflamed tissues, T_{CM} have not been identified in animals as providing primary tissue-based immunosurveillance.[7] We report that human T_{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.

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,

Submitted 4 August 2017; accepted 1 January 2018. DOI 10.1182/bloodadvances.2017011346.

The full-text version of this article contains a data supplement.

© 2018 by The American Society of Hematology

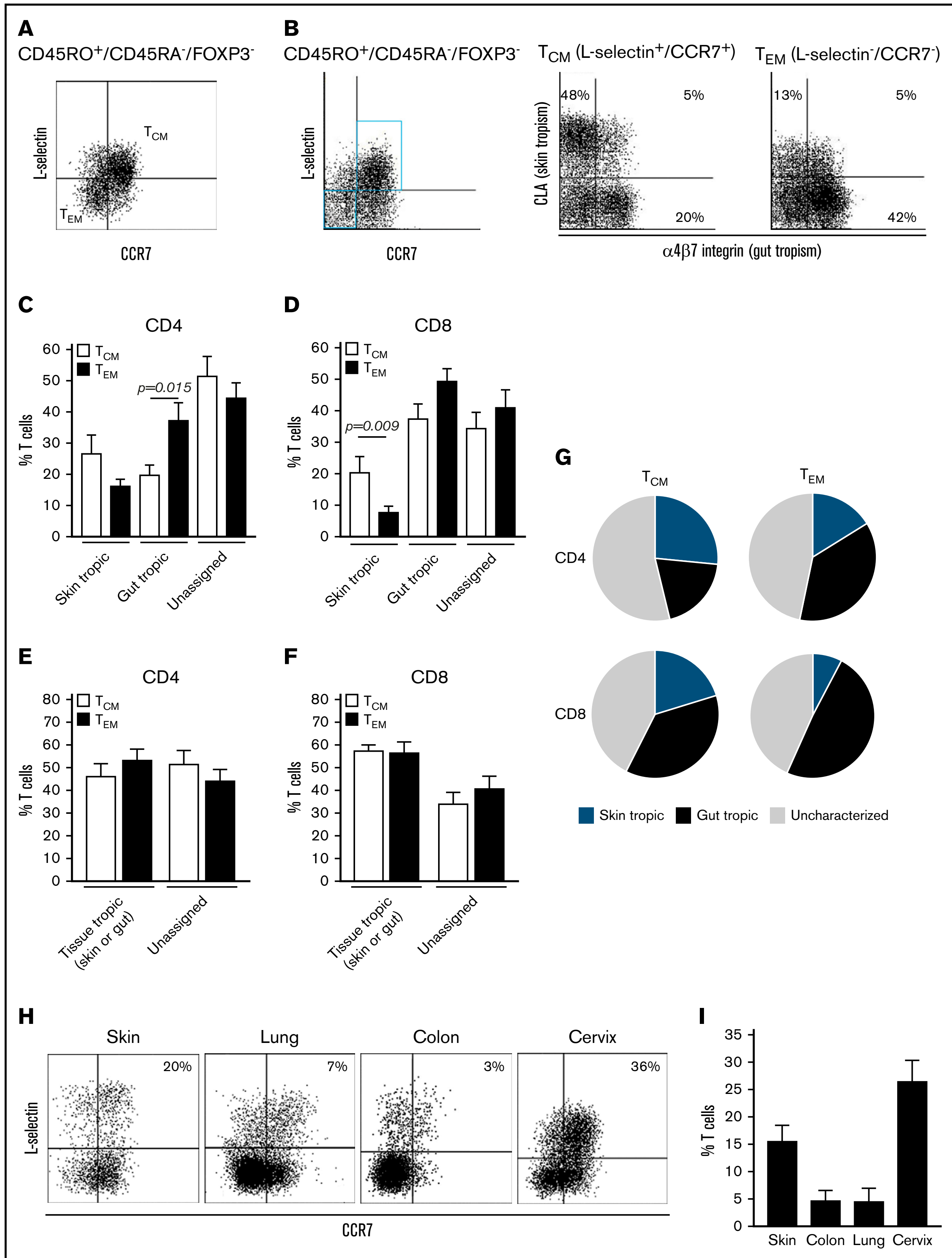

Figure 1. Human T_{CM} express tissue-homing addressins and are found in healthy human peripheral tissues. (A-B) Comparison of the expression of skin-homing (CLA) and gut-homing (α4β7) addressins in T_{CM} and T_{EM} by CyTOF analyses from a representative human donor. The gating strategy used to identify T_{CM} and T_{EM} 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$^+$ T_{CM}

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.[8,9]

Flow cytometry and CyTOF

A list of antibodies used to immunostain cells is included in the supplemental Methods. 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.

Human engrafted mouse model

Human engrafted mice were prepared as described previously.[9] Briefly, neonatal foreskins were grafted onto the backs of 6- to 8-week-old nonobese diabetic/severe combined immunodeficiency/interleukin-2 (IL-2) receptor γ chain^{null} mice (Jackson Laboratories). One week later, 3×10^6 flow-sorted T_{CM}, T_{EM}, 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, $\alpha = 0.05$. 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, $\alpha = 0.05$.

Results

We compared the expression of tissue-homing addressins in T_{CM} vs T_{EM} from healthy human blood donors by CyTOF (Figure 1). T_{EM} are generated in response to tissue-based infections and should all be tropic for peripheral tissues.[10,11] Skin (cutaneous lymphocyte antigen, CLA)[12] and gut ($\alpha_4\beta_7$ integrin)[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_{CM} vs T_{EM} from healthy human donors. Subsets of T_{CM} and T_{EM} expressed either gut-homing or skin-homing addressins, with very few cells expressing both (Figure 1A). Both $CD8^+$ and $CD4^+$ T_{CM} contained skin-tropic and gut-tropic populations (Figure 1A-B,G). The expression of skin and gut-homing receptors was comparable in T_{CM} and T_{EM}, except that significantly more $CD4^+$ T_{EM} expressed gut-homing receptors and significantly more $CD8^+$ T_{CM} expressed skin-homing receptors (Figure 1C-D). When the total number of T cells expressing skin or gut-homing receptors were compared, T_{CM} and T_{EM} expressed tissue-homing addressins at similar levels (Figure 1E-F).

These results suggest human T_{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_{CM} in each of these healthy peripheral tissues, confirming that T_{CM} do gain access to noninflamed human tissues (Figure 1H-I).

T_{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. We studied cytokine production of T_{CM} and T_{EM} and found that both CD4 and CD8 T_{CM} had impressive effector functions (Figure 2A-J). Interferon-γ (IFN-γ) was the most highly produced signature cytokine in T_{CM}, as it was in T_{EM}. $CD8^+$ T_{CM} were particularly cytokine rich, with a mean 70% and 97% of $CD8^+$ T_{CM} producing signature cytokines among the skin-tropic and gut-tropic populations, respectively. The proportion of $CD8^+$ T_{CM} producing signature cytokines was not significantly different from T_{EM} from the same donors (Figure 2G). Gut-tropic $CD8^+$ T_{CM} produced significantly more IL-2 than $CD8^+$ T_{EM}, but levels produced by all other subsets were comparable (Figure 2C,H). Between 40% and 55% of $CD4^+$ T_{CM} also produced signature cytokines, and IFN-γ was the most frequently produced cytokine in both $CD4^+$ and $CD8^+$ T_{CM} (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_{CM}, and $CD8^+$ T_{CM} in particular, have considerable effector functions that endow them with the capacity to contribute to frontline antipathogen responses in peripheral tissues.

We used a human engrafted mouse model to measure the relative ability of T_{CM} and T_{EM} 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_{EM}, T_{CM}, or naive T cells isolated from healthy adult human donors (Figure 2K; supplemental Figure 1).[9] A robust inflammatory dermatitis was observed in both T_{EM}- and T_{CM}-injected mice (Figure 2L-S). T_{CM} effectively entered human skin and induced inflammatory changes, including interface dermatitis, epidermal spongiosis, and epidermal necrosis (Figure 2Q-S). One caveat is that T_{CM} 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

Figure 1. (continued) (white bars) and T_{EM} (black bars). T cells that expressed neither CLA nor $\alpha4\beta7$ were designated as unassigned. The mean and standard error of the mean (SEM) of 4 donors are shown. (E-G) T_{CM} and T_{EM} 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) T_{CM} are present in noninflamed human peripheral tissues. T cells were isolated from noninflamed human tissues and analyzed by flow cytometry. T_{CM} 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.

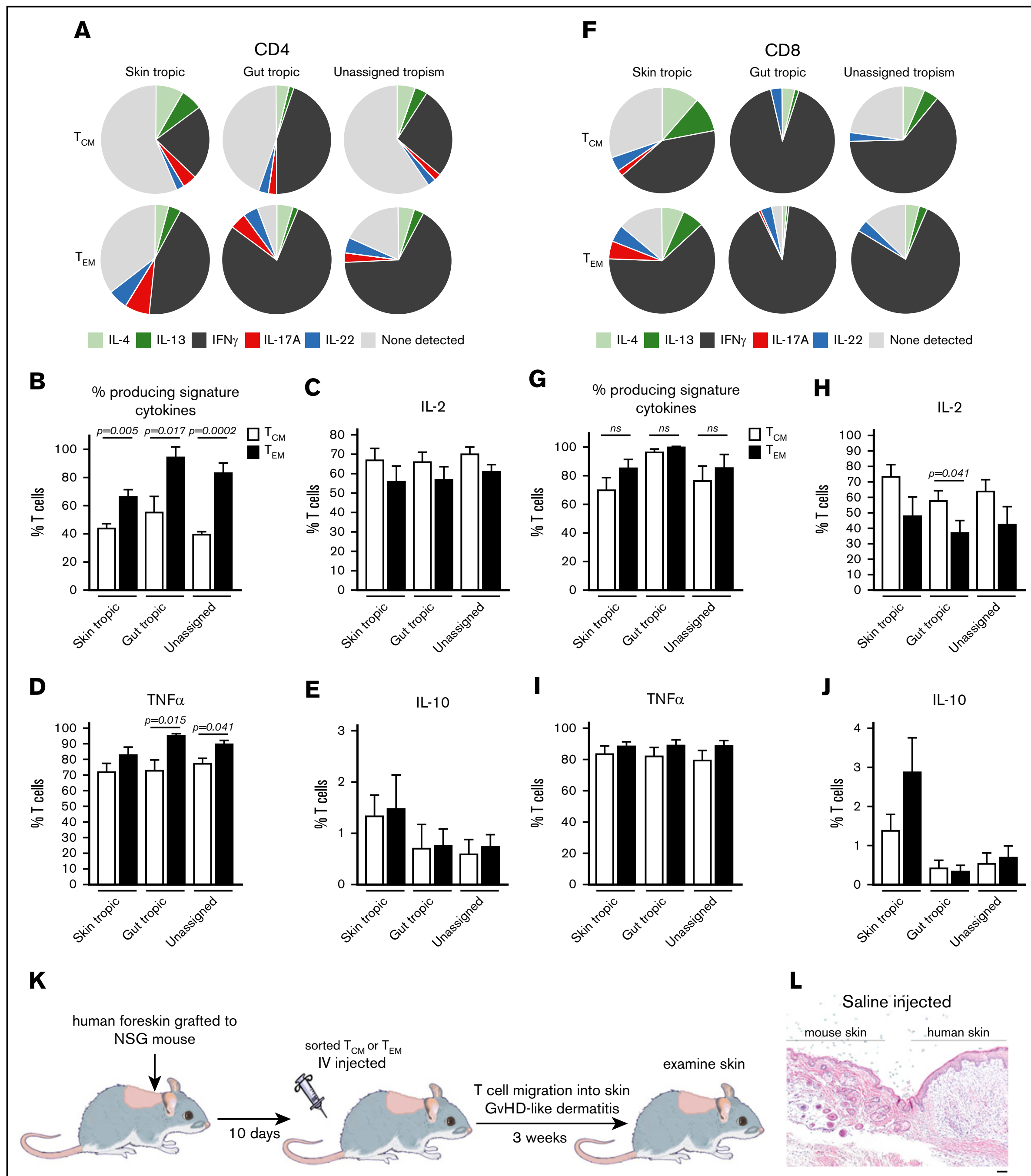

Figure 2. Human T_{CM} have potent effector functions, home to skin, and induce dermatitis in human engrafted mice. (A,F) Both $CD4^+$ and $CD8^+$ T_{CM} produced T-cell signature inflammatory cytokines. Cytokine production was assayed by CyTOF following stimulation with phorbol 12-myristate 13-acetate/ionomycin. Production of T_H1/T_C1 (IFN-γ), T_H2/T_C2 (IL-4, IL-13), T_H17/T_C17 (IL-17A), T_H22/T_C22 (IL-22) signature cytokines by T_{CM} and T_{EM} is shown. Figures represent the mean of 6 donors. (B,G) Total production of signature cytokines by T_{CM} (white bars) compared with T_{EM} 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 T_{CM} home to skin and induce an inflammatory dermatitis comparable to T_{EM} 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) T_{EM}, (O) T_{CM}, or (P) naive T cells. T_{CM} 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 T_{CM} 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 T_{CM}- and T_{EM}-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 T_{CM}- and T_{EM}-injected mice. (W) Production of inflammatory chemokines in skin was comparable in T_{CM}- and T_{EM}-injected mice. For panels T-W, the mean and SEM of messenger RNA copies detected by NanoString analyses from 6 different human T_{CM}/T_{EM} donors are shown. Scale bars, 100 μm. GVHD, graft-versus-host disease; NS, normal skin; *ns*, not significant.

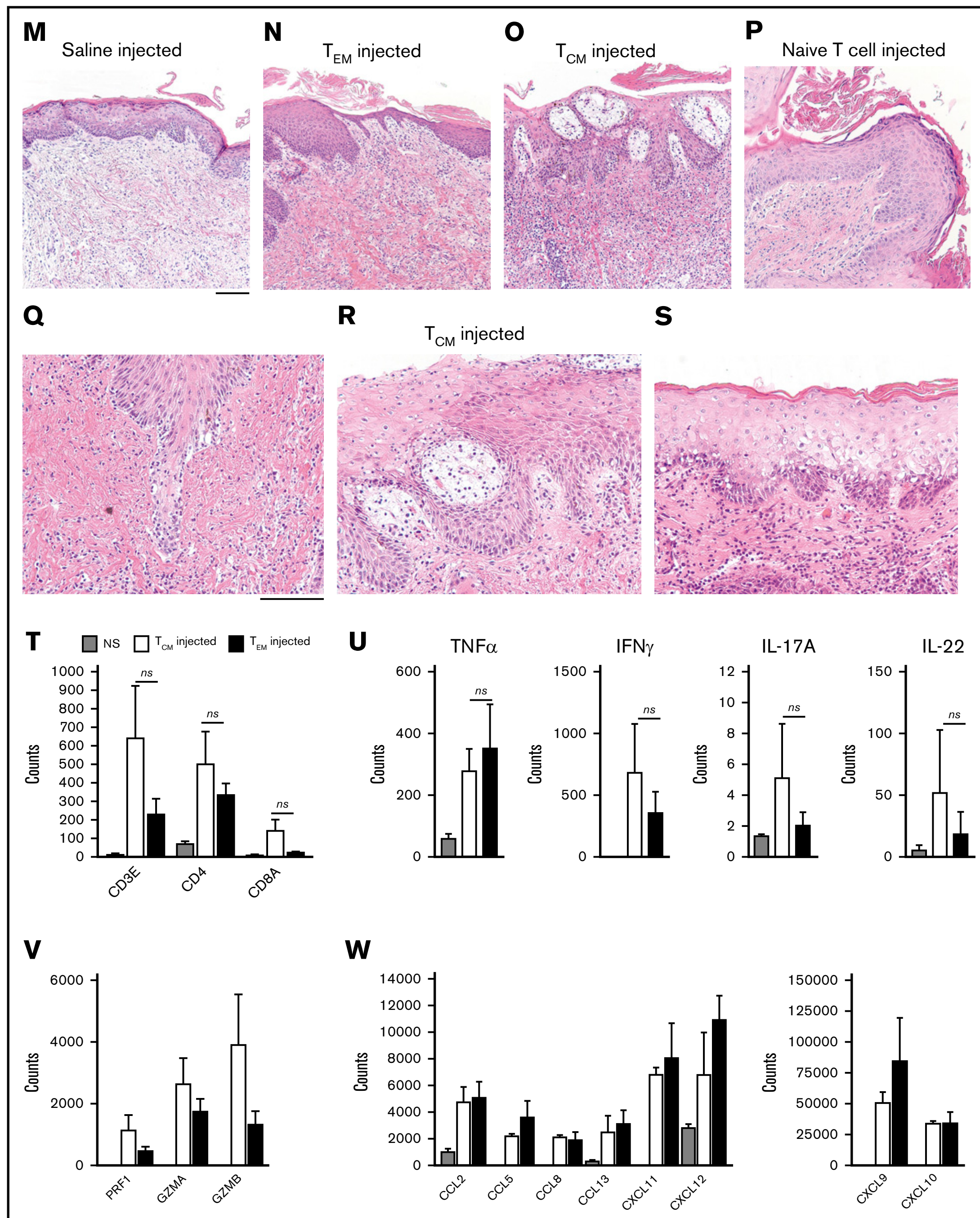

Figure 2. (Continued).

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_{CM}- and T_{EM}-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_{CM}- and T_{EM}-injected mice (Figure 2U-W). These studies demonstrate and confirm the ability of T_{CM} to enter the skin and initiate inflammation in the absence of other T-cell subsets.

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 $\alpha_4\beta_7$, used a different antibody to detect CLA than the one that identifies cutaneous T cells (HECA-205 vs HECA-452[12]), 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 $\alpha_4\beta_7$-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.

Acknowledgments

Michael Yaremchuk, Jeffrey Darrow, George Volpe, Dax Guenther, and Raffi Der Sarkissian of the Boston Center for Plastic Surgery and Bohdan Pomahac and Simon Talbot of Brigham and Women's Hospital generously provided adult human skin samples. CyTOF samples were acquired with the assistance of Nicole Paul of the Dana-Farber Cancer Institute. Eugene Butcher kindly provided the ACT-1 antibody.

This work was supported by National Institutes of Health (NIH), National Institute of Arthritis and Musculoskeletal and Skin Diseases grants R01 AR063962 (R.A.C.), R01 AR056720 (R.A.C.), P30 AR069625 (R.A.C.), T32 AR-07098-36 (T.S.K.; supplied salary for J.T.O.), NIH, National Institute of Allergy and Infectious Diseases grant R01 AI097128 (T.S.K. and R.A.C.), NIH, National Cancer Institute grant R01 CA203721 (R.A.C. and T.S.K.), and the AstraZeneca Foundation/Faculty of Medicine of the University of Lisbon Research Grant (T.R.M.).

Authorship

Contribution: A.G., J.E.T., and T.R.M. carried out the experiments and assisted in analyzing data; V.H., C.Y., and R.W. participated in developing the human engrafted mouse model; J.T.O. helped in analyzing gene expression data; R.A.C. designed experiments, analyzed data, and drafted the manuscript and figures; C.L.T. provided human cervix; and T.S.K. provided access to human lung and colon.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Rachael A. Clark, Department of Dermatology, Brigham and Women's Hospital, Room 501A, 221 Longwood Ave, Boston, MA 02115; e-mail: rclark1@partners.org.

References

1. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401(6754):708-712.

2. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annu Rev Immunol*. 2004;22(1):745-763.

3. Zaph C, Uzonna J, Beverley SM, Scott P. Central memory T cells mediate long-term immunity to Leishmania major in the absence of persistent parasites. *Nat Med*. 2004;10(10):1104-1110.

4. Wherry EJ, Teichgräber V, Becker TC, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol*. 2003;4(3):225-234.

5. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest*. 2008;118(1):294-305.

6. Gerlach C, Moseman EA, Loughhead SM, et al. The chemokine receptor CX3CR1 defines three antigen-experienced CD8 T cell subsets with distinct roles in immune surveillance and homeostasis. *Immunity*. 2016;45(6):1270-1284.

7. Wakim LM, Gebhardt T, Heath WR, Carbone FR. Cutting edge: local recall responses by memory T cells newly recruited to peripheral nonlymphoid tissues. *J Immunol*. 2008;181(9):5837-5841.

8. Clark RA, Chong B, Mirchandani N, et al. The vast majority of CLA+ T cells are resident in normal skin. *J Immunol*. 2006;176(7):4431-4439.

9. Watanabe R, Gehad A, Yang C, et al. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. *Sci Transl Med*. 2015;7(279):279ra39.

10. Campbell DJ, Butcher EC. Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. *J Exp Med*. 2002;195(1):135-141.

11. Robert C, Kupper TS. Inflammatory skin diseases, T cells, and immune surveillance. *N Engl J Med*. 1999;341(24):1817-1828.

12. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans. Preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol*. 1990;136(5):1053-1068.

13. Hamann A, Andrew DP, Jablonski-Westrich D, Holzmann B, Butcher EC. Role of alpha 4-integrins in lymphocyte homing to mucosal tissues in vivo. *J Immunol*. 1994;152(7):3282-3293.

14. Erle DJ, Briskin MJ, Butcher EC, Garcia-Pardo A, Lazarovits AI, Tidswell M. Expression and function of the MAdCAM-1 receptor, integrin alpha 4 beta 7, on human leukocytes. *J Immunol*. 1994;153(2):517-528.

15. Roberts AD, Ely KH, Woodland DL. Differential contributions of central and effector memory T cells to recall responses. *J Exp Med*. 2005;202(1): 123-133.

16. Campbell JJ, Murphy KE, Kunkel EJ, et al. CCR7 expression and memory T cell diversity in humans. *J Immunol*. 2001;166(2):877-884.