Skin resident T cells

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General discussion
GENERAL DISCUSSION

The skin is no longer viewed merely as a physical barrier tissue; rather, it is considered to be an organ with an active immune system which is involved in homeostasis as well as host defense. The successful control of pathogens by the skin’s immune surveillance and its effector functions are likely mediated by a variety of migratory and resident cells (chapter 1). The presence of intraepidermal lymphocytes was probably first described in 1922 by Kondo1, but subsequent reports on the absence of T cells in healthy human skin shed doubt on the existence of these cells. The article by Bos et al. from 1987 (The skin immune system (SIS): Distribution and immunophenotype of lymphocyte subpopulations in normal skin) set the record straight, once and for all, about the presence of T cells;2 these authors showed that all lymphocytes present in healthy skin are T cells, and that the vast majority are clustered around vessels; also that these perivascular cells are either CD4+ or CD8+, and are generally activated. Only fewer than 10% of lymphocytes are intraepidermal, directly subepidermal, or not perivascular, and these are mostly CD8+. This conceptual breakthrough contributed to a new generation of lymphocyte-focused dermatology research (chapter 2). Over the past decades, evidence accumulated favoring a population of memory T cells that do not recirculate, but stay resident in peripheral tissues such as the skin. These resident memory T (T\textsubscript{RM}) cells function as alarming sensors, cytotoxic killers and provide a long-term local memory that can spread widely when re-infected with the same antigen.4-6

It was not known what T cell subsets have the ability to differentiate into T\textsubscript{RM} in peripheral tissues. This question has never been studied in humans and only limited information was available in mice (chapter 3). There are three major circulating CD45RO\textsuperscript{+} memory T cell subsets in humans that could potentially give rise to T\textsubscript{RM} in peripheral tissues. Central memory T cells (T\textsubscript{CM}) co-express L-selectin and CCR7 and actively recirculate between the blood and lymph nodes, while effector memory T cells (T\textsubscript{EM}) lack expression of L-selectin and CCR7 and are tropic for peripheral tissues. Migratory memory cells (T\textsubscript{MM}) that express CCR7 but lack L-selectin, recirculate between the blood and the skin but appear to be excluded from central lymph nodes.7 To identify which subsets can give rise to T\textsubscript{RM}, we isolated these three memory T cell subsets from healthy human peripheral blood and differentiated them into T\textsubscript{RM} both in vitro and in vivo, using human blood and skin grafted mice. We find that T\textsubscript{CM} are the most effective precursor cells of T\textsubscript{RM} cells in vivo. Although T\textsubscript{EM} have a higher conversion rate to T\textsubscript{RM}, the ability of T\textsubscript{CM} to persist in the circulation and give rise to additional T\textsubscript{CM}, T\textsubscript{MM} and T\textsubscript{EM} in vivo allows them to populate the skin in higher numbers and generate more T\textsubscript{RM}. This behavior of human T\textsubscript{CM} is consistent with findings in animal models of disease in mice and macaques.8,9 T\textsubscript{CM} can provide effective long-term...
immunity against infections such as leishmaniasis in mice. Part of this protection may arise from the ability of $T_{CM}$ to seed peripheral tissues with highly protective $T_{RM}$. Although $T_{CM}$ were the most effective precursors, $T_{RM}$ were generated by all circulating T cell subsets after entry into the skin.

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. We made clear that $T_{CM}$ express tissue-homing addressins at levels similar to $T_{EM}$, and indeed, these cells are present in multiple healthy human peripheral tissues (chapter 4). 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 $T_{EM}$. 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 $T_{CM}$.

Acknowledging that human skin contains a combination of resident and recirculating memory T cells, we set to characterize the relative frequencies and functional activities of the distinctive subsets (chapter 5). We developed a human engrafted mouse model and used alemtuzumab, a humanized anti-CD52 antibody capable to deplete recirculating T cells from skin in order to discriminate between recirculating and resident memory T cell populations. We find that two discrete populations of $T_{RM}$ (CD103$^+$ and CD103$^-$) are stationed in human skin and two distinct populations of T cells recirculate through skin ($T_{MM}$ and $T_{CM}$). Each of these subsets have distinct recirculation and functional capacities and each is likely to play a unique role in protection against known pathogens. The majority of human skin $T_{RM}$ are dermal CD4$^+$ T cells that lack CD103 expression. CD103$^+$ T cells, both CD4$^+$ and CD8$^+$, are enriched in the epidermis. CD103$^+$ $T_{RM}$ had more potent effector functions but a poorer proliferative capacity. With respect to their functional capacities, $T_{MM}$ were intermediate between $T_{CM}$ and $T_{RM}$ in their production of inflammatory cytokines. Interestingly, we found that Th2 cytokine production was enriched in recirculating T cells but that the production of Th1, Th2 Th17 and Th22 associated inflammatory cytokines were enriched in effector memory T cells ($T_{EM}$). It is worth noting that the antigen specificities of human skin $T_{RM}$ are a valuable immunologic record of what infectious pathogens and antigens an individual has been exposed to during his or her lifetime. Moreover, encoded within the cytokine and effector functions of these T cells is a history of how these individuals overcame each pathogen. There are currently no techniques that allow us to extrapolate antigen specificity from the
sequences of the TCR receptor. However, once such techniques are developed, we will be able to investigate the immunologic history of an individual from the T cells remaining in the skin.

Intravital imaging studies in mouse models have demonstrated that CD8$^+$ T$_{RM}$ in skin tissue actively crawl in between keratinocytes in search of newly infected cells, a property termed tissue patrol. While there is a growing appreciation of the relevance of human skin-T$_{RM}$, the in situ behavior of these cells in humans had not been analyzed. We wondered if human skin-T$_{RM}$ cells remained static in a single location or if they were capable to patrol their surroundings and respond upon antigen encounter, similarly to mice T$_{RM}$ cells. To allow this, we established an ex vivo imaging system for the in situ labeling and real-time tracking of CD8$^+$ T$_{RM}$ in human skin (chapter 6). Using this approach, we demonstrate that human CD8$^+$ cells actively migrate in both the epidermal and dermal layers of the skin, with median speeds in the same range as those of murine CD8$^+$ skin-T$_{RM}$. These CD8$^+$ cells reflect tissue-resident memory T cells, as all CD8$^+$ cells isolated from both skin compartments express CD69$, the principal defining feature of T$_{RM}$. These data establish that tissue patrol is a property of human CD8$^+$ skin-T$_{RM}$, and fit with the model that relatively rare CD8$^+$ T$_{RM}$ can act as local sentinels to provide a rapid and tissue-wide anti-pathogen response.$^{13,14}$ The observation of T$_{RM}$ patrol in both the dermis and epidermis, two sites with a different tissue architecture, combined with the notion that tissue patrol has been observed for murine T$_{RM}$ in multiple organs$^{15,16,17}$, makes it reasonable to postulate that a continuous migratory behavior forms a shared property of all human CD8$^+$ T$_{RM}$ populations. In future studies in healthy human skin it will be interesting to investigate whether the CD4$^+$ CD103$^-$ memory T cells that are present at high density in the dermis$^{7,18}$ show a similar patrolling behavior as CD8$^+$ T$_{RM}$, and whether these cells co-localize with either CD8$^+$ T$_{RM}$ or defined antigen-presenting cell populations (APCs). To our knowledge, this is the first longitudinal analysis of the behavior of resident memory T cells in human tissue.

We followed by studying the pathogenic role of T$_{RM}$ cells in certain skin diseases, such as in psoriasis. Our work shows that $\alpha\beta$ T cell clones enriched in active and clinically resolved psoriatic skin lesions, some of which had TCR antigen receptors unique to psoriasis, were also found in nonlesional skin from the same patients, although at lower frequencies (chapter 7). T$_{RM}$ are by nature long lived and difficult to kill. Psoriasis and mycosis fungoides, a lymphoma arising from skin T$_{RM}$,$^{19}$ are both characterized by inflammatory skin lesions that appear to completely resolve with therapy but then often recur in the same anatomic locations once therapy is discontinued. This behavior suggests that most conventional therapies probably do not eradicate pathogenic T cells but instead suppress the activity of these cells. Withdrawal of active therapy allows these T cells to reactivate
and re-initiate inflammatory lesions. We found that putative pathogenic T cell clones in clinically resolved lesions were still actively producing IL-17A, even in the absence of clinical inflammation. We believe that these cells represent a smoldering nidus of inflammation that can lead to reactivation of overt inflammation and recurrence of psoriatic skin lesions. A better understanding of the signaling pathways that allow long term survival of T<sub>RM</sub> in skin could leave to novel therapies capable of eradicating these cells and leading to long term remissions in psoriasis.

Schlapbach et al. (2014) previously found that production of IL-9 by human T cells was transient, preceded the up-regulation of other inflammatory cytokines and enhanced cytokine production from other T cell subsets in vitro. It suggest that one possible role for IL-9 may be to enhance cytokine production by pathogenic T cells, acting as an early amplifier of inflammation. We find that IL-9 is produced at significant levels by T cells in human psoriasis and that IL-9 acts on T<sub>RM</sub> cells to enhance IL-17 and IL-22 production, enhancing inflammation in two IL-17/IL-23 dependent mouse models of psoriasiform dermatitis (chapter 8). We propose that IL-9 can enhance cytokine production by multiple T cell subsets and thereby may act as an early amplifier of inflammation in diverse inflammatory states.

We followed by studying the involvement of T<sub>RM</sub> cells into other immunological contexts, such as after allogeneic hematopoietic stem cell transplantation. Our hypothesis was that tissue-resident T cells of the recipient patient could survive the conditioning regimens prior to transplantation, and therefore contribute to the pathophysiology of acute GVHD (chapter 9). It would contradict the existing dogma, which assumed that aGVHD is triggered by donor T cells. The limitations of this study include the small sample sizes and the retrospective nature of the human acute GVHD studies necessitating use of FFPE specimens. Archival FFPE tissue is more challenging to stain via IF (due to crosslinking of epitopes by formalin) and can suffer from DNA degradation thus hampering FISH. More extensive analysis of host T cells in patient samples was therefore precluded. Moving forward, a large prospective human study in which fresh tissue is collected will allow for deeper interrogation of host T cell phenotype and function. Coupled with humanized mouse models, such studies will be able to evaluate the true contribution of host T cells to acute GVHD. Though challenging, these studies should be pursued given the possible significant implications for clinical care.

The growing appreciation that T<sub>RM</sub> cells are central players in immunity to vitiligo and melanoma has increased interest in T<sub>RM</sub> cells as promising targets for future vaccines and immunotherapies. Therefore, we wrote a comprehensive and up-to-date literature review aimed to raise awareness about T<sub>RM</sub> cell involvement in both vitiligo and melanoma.
(chapter 10). We discuss how blocking the proliferation, maintenance and coordination of $T_{RM}$ cells can efficiently inhibit melanocyte apoptosis in mice models. Future trials in patients will provide important insights into targeting $T_{RM}$ cells for the treatment of human vitiligo. Vaccination strategies have successfully generated $T_{RM}$ cell populations, that have effectively suppressed tumor growth in mouse models of melanoma. However, developing these therapies will require additional experimental studies to obtain more insight into the exact phenotype and function of $T_{RM}$ cells in mice and humans.

In the following 5 chapters we explored one of the most of the most use therapies to $T_{RM}$-associated skin diseases: Phototherapy. The main limitation of these manuscripts is not being systematic reviews; instead these are peer-reviewed literary reviews. We aimed to demystify phototherapy by exploring in a comprehensive, complete and up-to-date fashion, its applications and immunological effects. Phototherapy is perhaps the most ancient form of treatment in dermatology, being responsible for the management of a wide variety of skin diseases and it remains an extremely effective, safe and broadly used form of treatment (chapter 11). Still, contradictory attitudes towards UV radiation remain present among the medical community. The fear of phototherapy-induced skin cancer and reports of fatal overexposure results in a significant geographic variation of the use of phototherapy. Additionally, there is also a wide discrepancy on protocols prescribed for the same medical indication despite the many studies published on these therapies. We aimed to provide an updated comprehensive overview about photoimmunology, photocarcinogenesis and the use of phototherapy in psoriasis and vitiligo. The impact of UV radiation on the immune system and thus in human health is evident, even though its mechanisms of action remain unclear. It induces a complex series of events, affecting both molecular and cellular structures that can lead not only to local but also systemic and long-lasting effects. The sequelae involving photocarcinogenesis can simultaneously contribute to the development of cutaneous cancers. Nevertheless, the unique immunosuppressive capability of UV radiation can be effectively and safely used as a therapeutic modality for several human diseases (chapter 12). Skin carcinogenesis is a highly complex process, where UVR is a recognized complete carcinogen. The myriad of molecular changes induced by UVB and UVA ultimately trigger mutagenic events that lead to altered skin cell proliferation and differentiation as well as immunosuppression, two key conditions for the development of cutaneous neoplasms. The accumulation of irreversible skin cell photodamage, under impaired tumor immunosurveillance, explains the increased risk of skin cancer associated with natural UV light exposure and justifies the concern about the carcinogenic potential of phototherapy using artificial UVR (chapter 13). Nevertheless, phototherapy remains an essential therapeutic option in the management of psoriasis, remaining a first-line treatment for many patients (chapter 14). It is efficacious, safe, cost-effective, one of the most preferable and convenient therapies for
patients and avoids the issue of systemic immunosuppressive effects seen with biologic and traditional systemic therapies. NBUVB should be offered to people with plaque or guttate-type psoriasis that cannot be controlled with topical treatments alone, keeping in mind patient specific-considerations.

We conclude this thesis with a collection of five explanatory manuscripts aimed to bridge the gap between the work of the lab-based researchers and clinicians: The research techniques made simple (RTMS). The challenge of effective communication persists among scientists, in part because of uneven knowledge of fundamental basic science concepts and laboratory techniques (chapter 15). The unparalleled rapid development of science has resulted in innovative treatments and cutting-edge technologies being already used in health care today. Thus, basic common science literacy is essential to help individuals successfully communicate their ideas and to foster state-of-the-art dermatologic care. Writing these RTMS manuscripts forced us to be creative in order to translate these complex techniques into a language understandable by a diverse readership. Mass cytometry and high-throughput sequencing (HTS) of the T-cell receptor (TCR) represent two novel and complex techniques that we untangle in two subsequent chapters. The success of mass cytometry-based experiments is dependent on well-thought-out goals, detailed experimental design, and knowledge of potential technical pitfalls and limitations (chapter 16). A methodical approach is essential to control for experimental noise when conducting precise comparisons between samples. Importantly, this approach facilitates the ability to harness the full potential of mass cytometry to characterize complex biological systems at single cell resolution. CyTOF may lead to key discoveries in investigative dermatology, including identification of signaling phenotypes with predictive value for early diagnosis, prognosis, or relapse, and a thorough characterization of intratumor heterogeneity and disease-resistant cell populations, that may ultimately unveil novel therapeutic approaches. The continuous development and enhancement of analysis tools for mass cytometry expands our ability to study complex and heterogeneous biological systems at the level of individual cells (chapter 17). This enables our understanding of the progression and development of healthy and pathologic cells, such as in psoriasis, atopic dermatitis, and vitiligo. For example, why do some lesions only reoccur in the same anatomical site? Why are some areas of the body more commonly affected? What distinguishes pathological cells in a stable versus a progressive disease? Disease-specific cell subsets can be identified, characterized, monitored during treatment, and perhaps screened for early biomarkers predictive of relapse risk. Differences may be revealed among cells responsible for the clinical heterogeneity of cutaneous T cell lymphoma, ultimately unveiling disease-specific biomarkers and personalized novel therapeutic approaches. Innovative therapies can be studied to specifically target malignant cell populations resistant to conventional treatments, such as

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in melanoma. We aimed to demystify the developing tools for mass cytometry and its data analysis so these technologies may be adopted and results understood to address these and other important research questions in the coming years.

HTS of the TCR is a highly sensitive and precise technique, allowing quantification of the relative frequency of each clone within the full T-cell repertoire (chapter 18). It enables to study concomitantly each unique T-cell and all clonal populations over time, comparing different biologic tissues of the same individual or among different individuals that may be either healthy or suffering from a certain disease. HTS technology is based on the specific detection of the CDR3 region on just one of the two chains of the TCR heterodimer. An important next step forward would be establishment of technology that is able to simultaneously define both chains of the TCR. HTS is meaningfully expanding our understanding of the complex and exquisite role of T cells in the immune system and may lead us in groundbreaking personalized medicine by accurately monitoring and improving therapeutic interventions and possibly uncovering diagnostic and prognostic biomarkers.

The RTMS format of these manuscripts includes not only the manuscript but also bullet points highlighting the pros and cons, examples of how to use the technique for diverse research questions, a quiz to test mastery of the information provided, and a PowerPoint presentation allowing readers to promptly share and teach the techniques. Hence, in this chapter we challenge the reader to test the knowledge acquired about mass cytometry and TCR sequencing (chapter 19).
CONCLUSIONS

Until recently, it was commonly thought that all T cells recirculate between blood and lymph in their search for and clearance of pathogens and malignant cells. However, over the past decades, evidence accumulated favoring a population of memory T cells that do not recirculate, but stay resident in peripheral tissues such as the skin. These T_{RM} cells function as alarming sensors, cytotoxic killers and provide a long-term local memory that can spread widely when re-infected with the same antigen. However, when dysfunctional, skin located T_{RM} cells can have a profound role in various skin disorders, including psoriasis and vitiligo. T_{RM} cell-mediated skin disorders often show lesions with fixed cut-offs from healthy skin. Typically, these lesions resolve when treated, but tend to recur at the exact same location when treatment is discontinued. In the context of transplantation, T_{RM} cells can react to allogeneic cells promoting tissue inflammation. Targeting T_{RM} cells could lead to efficient treatment for these skin disorders. Yet, we still lack therapies capable of affecting only T_{RM} cells, and which are effective for prolonged periods of time. Phototherapy is perhaps the most ancient form of treatment in dermatology, but remains a valuable treatment option for T_{RM}-associated diseases, such as psoriasis and vitiligo. More research has to be done on safety of blocking and inducing T_{RM} cells in human. Thankfully, novel research technologies and methodologies are emerging. Much can be learned about this new subset of T cells. Using this knowledge in therapeutic and preventive therapy development could lead to a better quality of life of many patients suffering from skin disorders.
REFERENCES


