Editorial

Information-dense analysis for information-dense understanding

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Using mass cytometry coupled with cellular barcoding, Wong *et al.* (1) report a high-dimensional map of human T-cell trafficking receptors and cytokine profiles in several different tissues. Specifically, the authors analyzed T cells from eight human organs, including healthy donors and patients with different underlying conditions, with a range of three to five individual samples per organ. Perhaps unsurprisingly, their findings highlight the dynamic and complex nature of T cells in different organs and stress the importance of looking beyond peripheral blood when analyzing human T cell phenotype and function. The large amount of data generated by mass cytometry will undoubtedly serve as a reference point for future information-dense studies of human lymphocytes, but the main finding in this paper, the astonishing heterogeneity of T-cell trafficking markers and cytokine expression, suggests that regulation of T cell distribution and function in humans is more complex than previously recognized. Finally, this study complements and corroborates several previous studies, most notably those by Thome *et al.* (2,3) and Sathaliyawala *et al.* (4), which demonstrated differential distribution of human memory T cells in different organs and across decades of life. This resource should be especially useful for researchers who aim to engineer T cells for disease interventions, such as cancer immunotherapy.

In accordance with the bulk of literature, Wong *et al.* show that leukocytes vary in composition in different human organs (5). Cord blood and peripheral blood mononuclear cells (PBMCs) mainly consist of T cells; with most of cord blood T cells being naïve in phenotype, while T cells in PBMCs exhibiting either naïve or memory phenotype. The secondary lymphoid organs tonsil and spleen share a similar make-up consisting predominantly of B cells. In the four non-lymphoid tissues examined (liver, colon, lung, and skin), T cells predominate with most of the T cells displaying an effector memory phenotype ($CD45RA^{-}$ $CCR7^{-}$) as would be expected for adult tissues. While B cells from all anatomical sites consistently expressed CCR6, CCR7 and CXCR5, T cells from different organs had distinct expression of trafficking receptors. Interestingly, no consensus set of T cell trafficking markers were discernible in any of the organs examined.

The heterogeneity of T cell surface receptors, especially trafficking markers, is striking. Unlike B cells that have the ability to secrete antibodies and act at a distance, T cells need to make a direct contact with target cells to exert their effector functions. One would expect a common set of trafficking receptors expressed on different T cell subtypes from the same tissue. Wong *et al.* convincingly show that this is not the case. There are several mutually non-exclusive interpretations of this result. One possibility is that T cell subsets differ intrinsically in tissue trafficking. Other possibilities relate to data analysis and the small sample size (3 to 5 donors) per organ from patients with different underlying conditions. In the current study, most of the data (except for trafficking receptors in Supplemental Figure 2) is shown as grouped analysis of all individual samples. The comparison of T cells from various tissues from healthy and diseased donors with different underlying conditions inevitably increased the heterogeneity of T cell markers. In support of this possibility, studies have shown gender-specific differences in the T cell repertoire (6) and

the strength of the immune response (7). Similarly, multiple antigen stimulations are known to up-regulate distinct programs in the same T cells, which would contribute to donor-to-donor differences (8). It would be informative to visualize individual donor data for all parameters and analyze how this relates to the averages presented here. In addition, although Wong *et al*. analyzed 41 trafficking markers, the number may still be insufficient to capture the "area-codes" for different T cell subsets in different tissues (9).

Consistent with previous reports in mice (10), Wong *et al*. found that most of the tissue-resident memory T cells (T_{RM}) express CD103 and CD69 as well as integrin β7, a subunit of the CD103 complex. Similarly, both human and mouse skin-resident T_{RM} cells express CLA and CCR4 but not CCR10. Interestingly, Wong *et al*. also found $CD69^+CD103^-$ T_{RM} cells in different organs, which may be migratory T cells from the circulation that are not completely restricted by the local microenvironment or they may be in the process of conversion to T_{RM} cells outside of their resident tissues. Furthermore, Wong *et al*. were able to divide both $CD4^+$ and $CD8^+$ T_{RM} cells into four subtypes based on the expression of CLA, HLA-DR and CD25 for $CD4^+$ T cells and CCR5, CD49a, CD45RA, CD45RO, and CD161 for $CD8^+$ T cells. The proportion of these subtypes varied in different human organs and exhibited different cytokine expression, suggesting heterogeneity of T_{RM} in humans.

Mass cytometry can analyze close to 100 different markers of individual cells with essentially no spectral overlap. The closest information-dense experimental approach is single-cell RNA-seq. How do the results from the two methods compare? Unfortunately, single T cell RNA-seq has only been reported for melanoma-infiltrating T cells in human (11). A comparison of the frequency of T cells positive for the 31 trafficking markers and cytokines shows no correlation between $CD8^+$ T cells from skin analyzed by mass cytometry and 889 single $CD8^+$ T cells from melanoma analyzed by RNA-seq. This comparison is probably inadequate as T cells from the two studies are likely different. While a more appropriate comparison has to await future studies, it is worth considering the advantages and disadvantages of each approach. Compared to mass cytometry, single cell RNA-seq provides transcriptome information at the whole genome level. However, an important advantage of mass cytometry analysis is the avoidance of false-positive results that stem from post-transcriptional and post-translational modifications that cannot be captured by transcriptome analysis.

A recent study reported human T cell receptor (TCR) repertoire across decades of life by comparing data from cord blood to PBMCs from donors of increasing age (6). The results show the highest diversity of TCR in cord blood and reduced TCR diversity in PBMCs with age. A separate study reported a difference in TCR repertoire of $CD4^+$ and $CD8^+$ T effector memory (T_{EM}) cells in secondary lymphoid organs with increased clonal expansion of $CD8^+$ but not $CD4^+$ T cells (2). It would be interesting to investigate the relationship between diverse T cell phenotypes and function from different tissues and TCR repertoire diversity. Do the same T cell clones in different locations display heterogeneity in trafficking receptors and cytokine profiles? Are antigen-specific TCR clones specifically retained in tissues by up-regulation of trafficking receptors such as CD103? Does expression of CD103 dictate the retention and subsequent expansion of specific clones?

An improved understanding of tissue-specific T cell phenotypes, trafficking receptors and cytokine profiles is extremely valuable for the development of T cell based immunotherapies. For instance, this knowledge can aid in the development of engineered T cells that can be targeted to specific organs based on T cell trafficking receptor combinations identified by Wong *et al*. Most chimeric antigen receptor T cells (CAR-T) to-date are not designed with tissue targeting moieties based on T cell trafficking and significant on-target off-tumor and neurological toxicities (12) have been observed with these T cells, some of which can be attributed to the whole-body distribution of systemically delivered CAR-T cells. Based on data provided by Wong *et al*., although complex, expression of combinations of trafficking receptors may aid in the targeting of engineered T cells to specific tissues. For instance, in applications where engineered T cells are to be targeted to the colon, expression of CCR5 and CD103 should aid in directing these cells to the colon. Such tissue targeting could potentially minimize unwanted side effects due to systemic distribution. A similar approach can be utilized in antibody therapies with bi-, tri- or multivalent antibodies, with one arm of the antibody recognizing specific trafficking receptors, such as CLA for skin homing, for tissue-specific targeting.

Additionally, recognizing the diverse nature of T cells from different organs will be important in the selection of the source of T cells for specific applications. Cord blood T cells which are predominantly naïve (Supplementary Figure S3A and B, Wong *et al*.) may be an appropriate

source for T cell engineering approaches in which antigen specific memory T cells are not desired. CAR-T therapies may benefit from using PBMCs as the source of T cells based on recent observations that administration of a defined ratio of CD4:CD8 CAR-T cells results in superior therapy efficacy (13). On the flip side, the use of T_{RM} cells from specific organs in applications such as adoptive T cell immunotherapy (ACT) could assist in limiting the distribution of these cells to other organs when local administration is not an option.

It is reported here that a subset of helper $CD4^+$ cells that produce interleukin-9 (IL-9) is mainly found in human skin. These IL-9^+ Th9 cells were not readily detectable in other tissues analyzed. IL-9 is a pleiotropic cytokine with a multitude of effects on numerous immune cells (14). T cells are the main source for IL-9 and accordingly, IL-9 was originally identified as a T cell growth factor (15). More recently, Th9 cells were implicated in anti-tumor immunity against melanoma in mouse models (16,17). Purwar *et al*. showed that loss of IL-9 promotes melanoma development, and that higher numbers of Th9 cells are detected in normal human skin in comparison to patient melanoma metastases supporting an anti-tumor role for these T cells in patients. These findings along with the observations presented by Wong *et al*. suggest that using Th9 cells in adoptive T-cell therapy may provide robust anti-tumor immunity in melanoma. Moreover, since IL-9 producing T helper cells are concentrated in the skin as suggested here, and the effect of IL-9 in preventing tumor growth was found to be independent of B and T cells by Purwar and colleagues (17), delivery of IL-9 via nanoparticles or liposomes decorated with skin homing receptors such as CLA, could be a novel approach for local delivery of IL-9 in melanoma. Similarly, different cytokine combinations may boost the immune system in different tissues and help against cancer as an addition to standard immune modulatory therapies.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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