Whole-Body Anatomy of Human T Cells

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Knowledge of the regional tissue distribution of T cell subsets is a prerequisite for understanding protective immunity and the pathophysiology of T cell-mediated diseases. In this issue of *Immunity*, Sathaliyawala et al. (2012) present a comprehensive human tissue T cell analysis.

In the 16th century, modern anatomy became the basic science with which to study the human body's function (O'Malley, 1964). Since then it has fundamentally contributed to our achievements in clinical—especially operative—medicine, where it has made surgeries of the highest complexity (e.g., organ transplantations) possible today. The introduction of microscopic anatomy in the 1800s allowed a deeper understanding of organ and tissue function, but histologic stainings also had their immanent restrictions: surface molecule combinations defining an individual cell's ontogeny and purpose are difficult to determine in (fixed) tissue sections. For this reason the role of "small lymphocytes" remained elusive (Gowans, 1996) for decades, until monoclonal antibodies and flow cytometry enabled the discrimination of more and more lymphocyte subsets on the single-cell level and helped to decipher their function for adaptive immune responses. Today state-of-the-art multicolor flow cytometry allows reliable discrimination of more than a dozen subset markers in one single sample, but this usually requires fresh ex-vivo-isolated leukocytes. Consequently, flow-cytometric analyses in humans have been mainly restricted to easily attainable sources for living lymphocytes such as peripheral blood, whereas systematic lymphoid or mucosal tissue examinations (except for examinations of occasional surgical specimens) have not been possible.

In mice, however, those systematic ex vivo organ-tissue analyses are both technically and ethically feasible, and recent studies on T cell memory have underlined their importance. For example, resident memory T cell subsets that might potentially be important mediators of local immunity protecting against reinfections of formerly encountered pathogens (as summarized in Masopust and Picker, 2012) have been identified. Because the confirmation of comparable distribution patterns of T cell subsets in humans is necessary (Davis, 2008) before potential implications for, as an example, vaccine-induced T cell responses can be fully assessed, comprehensive human analyses of tissue distribution of memory T cell subsets are urgently needed.

Sathaliyawala et al. (2012) have now addressed this issue by collecting lymphoid and mucosal tissues from 24 brain-dead organ donors. They received lung, small intestine, and colon tissue together with their respective draining lymph nodes in addition to blood, inguinal lymph nodes, and splenic tissue at the time of donor organ explantation. Those exceptional conditions allowed "ex vivo" flow-cytometry analyses with the highest cell vitality of the isolated lymphocyte samples, enabling the researchers to perform even functional analyses. All donors were younger than 60 years, free from chronic and immunological diseases, and had succumbed mostly to traumatic causes. Those characteristics allowed a unique snapshot of a presumably physiological T cell distribution.

Intriguingly, a general observation is that despite individual backgrounds and presumed variable infection or vaccination histories, distributions of common tissue-specific T cell subsets could be identified. Specifically, the early activation marker CD69 is highly expressed on memory T cells from mucosal and lymphoid tissues but could only be detected (with individual exceptions) on a minority of memory T cells in the blood compartment. Sathaliyawala et al. interpret this CD69 expression as a marker for resident memory T cells and thus as support for earlier observations describing CD69-expressing resident memory T cells in non-lymphoid tissues of mice (Jiang et al., 2012). However, CD69-expressing resident memory T cells are also found in secondary lymphoid organs in this study, contrasting with current analyses in mice.

With the help of well-established markers (CD45RA, CD45RO, and CCR7) for T cell subsets, the authors discriminate between naive, central memory (Tcm), effector memory (Tem), and CD45RA-re-expressing memory (Temra) T cells. In particular, the physiological role of Temra cells, which have been found to display an effector-like phenotype, is not yet completely understood. They are predominantly found among T cells directed against persisting viruses such as Cytomegalovirus (CMV) and accumulate with age (Buchholz et al., 2011). In the current study, Temra cells are nearly exclusively found within $CD8^+$, but not $CD4^+$, T cell subsets. Because detection of previously described $CD4^+$ $CD45RA^+$ memory T cells correlates with CMV seropositivity and advanced age (Henson et al., 2012), this discrepancy could be due to the overall young age of the organ donors. Nevertheless, incorporation of the routinely available results of virus serology of organ donors into the individual data sets might enable

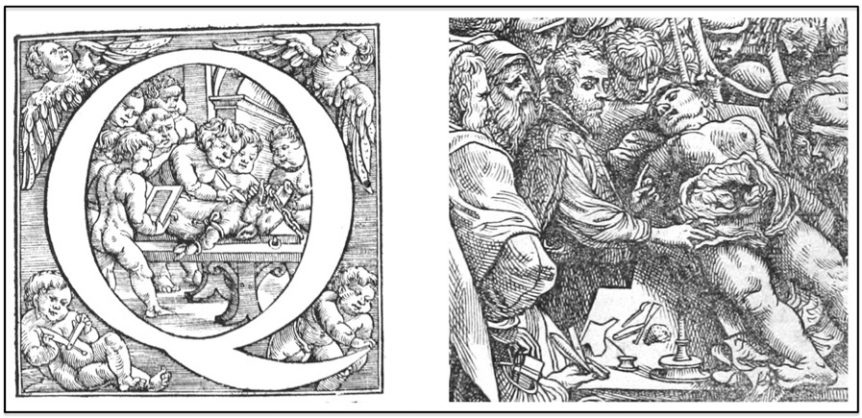

Figure 1. Woodcuts from *De Humani Corporis Fabrica libri septem* by Andreas Vesalius
(Left) Initial from the Fabrica's preface, which Vesalius dedicated to the emperor Charles V. Vesalius. In it, Vesalius states that he was trained during his medical studies only in a couple of animal preparations before he started to conduct human dissections himself ("ut ipse in brutorum aliquot sectionibus ... versatus ... sectionem, solito absolutius ... adductus publicè administrarem") and that by those experiences he had come to the conclusion that Galen had never dissected a human being for his classical anatomic studies ("nobis modò ex renata dissectionis arte ... constet ... nunquam ipsum [Galen] resecuisse corpus humanum).
(Right) Detail from the title page: Vesalius conducts a public human dissection.
Images are from Vesalius, A. (1543). De humani corporis fabrica libri septem. (Basileae: Ex officina Joannis Oporini). Photographs were kindly provided by the University Library of Munich (Ludwig-Maximilians-Universitaet), W 2 Med. 580.

correlation of phenotypic findings with highly prevalent latent herpes virus infections.

In comparison to $CD4^+$ Tcm cells, $CD8^+$ Tcm cells are found throughout all tested lymphoid and mucosal tissues at low frequencies. Even though the reason for this difference remains to be determined, the ubiquitous paucity of $CD8^+$ Tcm cells actually fits well into a concept of low-numbered but highly proliferative stem-cell-like memory T cells that endure self-renewal and long-time survival (Neuenhahn and Busch, 2009). In order to solidify this concept, however, it could be attractive to extend future analyses to bone marrow (Mazo et al., 2005) and other potential organ niches for $CD8^+$ Tcm cells.

In summary, the comprehensive organ-specific human lymphocyte phenotyping by Sathaliyawala et al. will be a valuable resource for human T cell analyses. Thus, the current data should be seen more as a starting point for further immunological in-depth analyses than as a completed human T cell evaluation. Staining protocols can be adapted and optimized (parameters were already changed during the current study), and more specific target populations (e.g., regulatory or antigen-specific T cells) should be addressed in the future. The immediate, gentle sample preparation is in this context an invaluable advantage because subset-defining functional multiparameter stainings can be optimally performed. Furthermore, immunization histories and results from infection serology should be correlated. Finally, additional tissues (e.g., thymus and bone marrow) could be included, although some organ-tissue recuperations might be incompatible with prioritized transplant use (liver) or irreconcilable with the integrity of the corpse's exterior (skin). With those restrictions in mind, a (nearly) whole-body human T cell anatomy could become reality.

Because such analyses performed as soon as possible postmortem could in principal be extended to other immunological and nonimmunological cytometric analyses, these manifold and highly valuable scientific examinations will probably need to be performed at more than one scientific center. This raises the question of whether the approach of ''ex vivo'' tissue analyses from brain-dead organ donors could also become a more widely accepted strategy in other institutions or countries. The ethical implications need to be discussed by the respective national medical societies and ethical committees, but an informed consent by the organ donor seems mandatory. In most western countries, whole-body donation for medical research and education is legally and structurally well established and ethically accepted, but it might be difficult to reconcile scientific interest in organ-donor tissues with national organ-procurement policies. In Europe, the responsible institutions often refrain from asking organ donors for general acceptance of postmortem research analyses in order to minimize the hurdle for donation in the context of today's dramatic organ shortage. Scientific study of donor tissues, in particular immunological analysis, could greatly increase the overall success of clinical therapies, including organ transplantation. If these benefits become clear to potential organ donors and responsible transplant authorities, acceptance of such scientific analysis could pave the way for a new era of understanding.

Nearly 500 years ago, Andreas Vesalius (1514–1564), physician and father of modern anatomy, found himself, admittedly in a more general way, in a comparable situation. Vesalius took advantage of the Renaissance Society's renewed acceptance of human dissections and, being a gifted anatomic dissector himself, discovered during a series of self-performed human dissections various misconceptions in Galen's medical doctrine, which was universally accepted at that time. Galen had performed most of his anatomic studies in animals, and a couple of faulty conclusions had been accepted because nobody questioned his authority. Vesalius summarized his findings in the revolutionary book on human anatomy, *De Humani Corporis Fabrica* (Figure 1), and paved the way for henceforth regular human dissections in anatomic research and medical education. The Vesalian conclusion that only postmortem examinations in humans could eventually provide a complete picture of the human body's functions can be seen as a strong argument for the need for more human postmortem studies such as that by Sathaliyawala et al. In that sense, the data set presented by Sathaliyawala et al. will become an important resource for clinical immunology.

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