# Thymus Transcriptome and Cell Biology

Geraldo A. Passos
Editor

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# Foreword

The importance of the thymus as the primary lymphoid organ responsible for the generation and selection of T lymphocytes is now obvious. Nevertheless, the thymus has long been a mysterious organ. It was not until 1961 that J. F. Miller showed in seminal studies that "the thymus at birth can be essential to life," quickly followed by its role in immunological tolerance by skin grafting experiments in mice. It is always surprising that this key discovery for any immunologist, physician, or biologist did not happen sooner. The skepticism surrounding this discovery, from such eminent immunologists as Burnet, Medawar, or Mitchison, may also be astonishing, Sir Peter Medawar even going so far as to declare in 1963 "we will come to look at the presence of lymphocytes in the thymus as an evolutionary accident with little significance." This controversy remains exemplary and instructive for our ways to get out of accepted dogmas. This was a "golden age" of immunology, the 1960s being particularly remarkable with the discovery of the major histocompatibility complex (MHC), the H-2 system in mice and HLA in humans, by Jean Dausset (1980 Nobel Prize with Baruj Benacerraf and George Snell), Jon van Rood, and many others. These two major discoveries paved the way for the demonstration of thymic selection, a major physiological function of the thymus in shaping the T-cell adaptive immunity. They also provided the basis of our current understanding of how the immune system works. With time, T-lymphocyte subpopulations, T- and B-cell cooperation, mechanisms of allogeneic MHC restriction, T-cell receptor structure and T-cell selection mechanisms, and identification of regulatory T cells have been gradually described. Each of these steps leads us back to thymopoiesis, ranging from the identification of factors required for the entry of hematopoietic progenitors into the T-lymphocyte development program to the factors regulating the expression of "tissue-restricted antigens" within the thymic epithelium. Those are key in establishing the central tolerance, among which *AIRE* and *Fezf2* are the best known, others still being to be described. This has been remarkably studied in murine models, and several chapters of this book are devoted to this central issue.

Although the concepts are very similar, the data concerning human thymopoiesis still need to be further developed. However, they are progressing rapidly thanks to methodological approaches and to large-scale studies. It is now clear

that, contrary to conventional wisdom still present in textbooks, the thymus remains functional in adults. This is important to better understand the parameters that govern the thymic function under physiological conditions, during aging, or in pathologies, especially lymphopenic situations after hematopoietic cell grafts, during HIV disease, or in autoimmunity. We are thus evolving from studies purely focusing on thymocytes to a broader view considering the thymus as an organ in its complexity. Excellent chapters of this book deal with what is called the "cross-talk" between thymocytes and cell populations housed in the thymus whose heterogeneity and complexity are gradually being uncovered. They include of course the different subpopulations of cortical and medullary thymic epithelial cells but also dendritic cells, macrophages, and so-called innate lymphoid cells, important in the process of thymic regeneration, without putting aside endothelial cells, key in the entry of lymphoid progenitors and also in the egress of recently generated naive T cells or "recent thymic emigrants." All this global knowledge will enable to consider future thymic regeneration strategies that will be personalized according to age, gender, and clinical contexts. This is to say the importance of this book and its timely content. The thymus still has a lot to teach!

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# Preface

If I had to choose an organ that in humans and mice symbolizes the intersection between immunology, endocrinology, molecular biology, and genetics, this organ would certainly be the thymus gland.

Galen in Greece had anatomically described this organ more than twenty centuries ago but only had its function scientifically attributed in the second half of the twentieth century by Jacques Miller. In his experiments of neonatal thymectomy of mice, Miller observed that the operated pups were suffering from susceptibility to infections with concomitant lymphopenia. These experiments were instrumental in finally assigning to the thymus its function in the immune system.

The fact that the thymus was the last of the major organs of the body to have its function assigned promptly incites exclamation.

Even intersected as said, the main function of the thymus is immunological and strongly associated to the development and positive/negative selection of T cells and induction of central immune tolerance.

One of its major cellular components, the thymocytes, undergoes a maturation process that is dependent on the random recombination of DNA segments (V[D]J recombination) of T-cell receptor (TCR) leading to the generation of the diversity of T-cell clones. The experimental demonstration of V(D)J recombination by Susumu Tonegawa in the mid-1970s, initially involving immunoglobulin gene segments in B cells and later TCR in T cells, has opened a unique perspective, i.e., understanding the molecular basis of diversity of lymphocyte repertoire. For genetics, this represented a great impact, since it was demonstrated that the genome is dynamic and can recombine somatically.

Another very intriguing aspect about the thymus is the functioning of its stroma. Thymic stroma is not merely a connective tissue or a supporting structure. The thymic epithelial cells (TECs), which are part of the stroma, establish a close physical contact with developing thymocytes through TECs-thymocytes adhesion, which is crucial, both for the thymus as a whole and for the thymocytes themselves. This property is termed thymic crosstalk during which the medullary TECs (mTECs) present to the developing thymocytes a vast amount of self-peptides that represent virtually all the organs and tissues of the body.

This showed the intersection of the thymus with the molecular biology of large-scale gene expression or transcriptomics. The mTEC cells have become a very intriguing cell type because they express almost the entire functional genome without losing their characteristics. The meaning of this property is immunological, relative to self-representation and induction of central immune tolerance. Due to the enormous diversity of self-peptide antigens expressed by mTECs, this property was termed *promiscuous gene expression* (PGE), which is controlled by *Aire* and *Fezf2* genes.

The demonstration that the thymic stromal cells express the functional oxytocin hormone made possible an important relation of this organ with endocrinology.

The thymus is also closely associated with human genetics since mutations in *Aire* cause the APECED (APS-1) autoimmune syndrome, which is linked to chromosome 21q22.3, the exact physical location of the *Aire* gene in humans.

One curiosity that, perhaps, some researchers still do not know: the mouse genome project and science of transcriptomics have in the past benefited greatly from the thymus. To begin massive sequencing of mouse DNA in the early 1990s, researchers in fact made cDNA libraries from total RNA extracted from a mouse thymus. Briefly, thousands of expressed sequence tags (ESTs) were then generated and later positioned along the genome, which in turn was being assembled. The sequenced mouse EST libraries were then used in microarray technology that emerged in 1995 making the science of transcriptomics possible!

As we can see, the thymus is a fascinating organ. It is crucial for the maintenance of immune homeostasis and is the place where the self-non-self distinction occurs. Even so, it is still a neglected organ in immunological research. Immunology is one of the branches of biological sciences that has progressed the most in the last decades, and most of the works are directed to the peripheral effector cells, i.e., B and T cells, NK cells, dendritic cells, macrophages, etc. However, much still has to be better known about thymus maturation, its ontogeny and differentiation, the origin of the TEC cells, and the control of the thymus gene expression in health and autoimmune diseases.

A promising field of research that is still in its early stages is about the control of gene expression of “second” thymus (cervical thymus discovered in 2006) that is present in about 50% of humans and mice.

It is for these reasons that this book was organized, i.e., to review the main aspects of cell biology, gene expression, and clinical intervention of the thymus. It was conceived during the realization of the Third Meeting on Thymus Transcriptome and Cell Biology, held at the University of São Paulo, Ribeirão Preto Medical School, Ribeirão Preto, Brazil, on 21–22 November 2017. Many of the authors of this book participated in this fruitful meeting. The purpose of this book is also to attempt to motivate young scientists to research the thymus.

I am grateful to all the researchers who have dedicated a part of their time to writing their chapters and the Springer Nature Publishing to welcome us and give full support for this book to be published.

Finally, I would like to pay a homage to Dr. Bruno Kyewski (1950–2018) who was an excellent scientist and devoted much of his career at the German Cancer Research Center in Heidelberg, Germany, studying cell biology and promiscuous gene expression in the thymus.

Ribeirão Preto, Brazil
November, 2018

Geraldo A. Passos

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