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Review

Molecular and cellular basis of T cell lineage commitment

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ABSTRACT

The thymus forms as an alymphoid thymic primordium with T cell differentiation requiring the seeding of this anlage. This review will focus on the characteristics of the hematopoietic progenitors which colonize the thymus and their subsequent commitment/differentiation, both in mice and men. Within the thymus, the interplay between Notch1 and IL-7 signals is crucial for the orchestration of T cell development, but the precise requirements for these factors in murine and human thympoeisis are not synonymous. Recent advances in our understanding of the mechanisms regulating precursor entry and their maintenance in the thymus will also be presented.

1. Introduction

During embryogenesis, the generation of the prospective thymic epithelium in the third pharyngeal pouch endoderm and the vascularisation of the resulting thymus anlagen is dependent upon the activity of the Foxn1 transcription factor [1,2]. The expansion and maturation of this embryonic thymic epithelium is regulated by neural crest-derived mesenchyme [3]. Immigration of the first T precursors occurs only upon proper fabrication of this thymic anlage; their differentiation within the thymus results in the establishment and maintenance of a mature peripheral T cell pool with a wide self-MHC-restricted non-autoimmune TCR repertoire.

The crucial role of the thymus in T cell generation was demonstrated by seminal studies conducted by the Australian scientist Francis Albert Pierre Miller [4]. The thymus was already acknowledged by the Greeks and its name may be derived from the Greek word "thymos" which denoted life force or soul. Despite this rich history and even though Miller's pioneering work on the thymus was performed in 1961, it was long thought that the thymus was obsolete with its function having become redundant during the course of evolution. Indeed, in 1963, Sir Peter Medawar, recipient of the 1960 Nobel prize for his work on graft rejection and the acquisition of immune tolerance, stated; "We shall come to regard the presence of lymphocytes in the thymus as an evolutionary accident of no very great significance" (Medawar, 1963) [5]. During the past half century, remarkable progress has been made, and it is no longer possible to study T cell development without recognizing the critical importance of the thymus in this process.

At what point in its development does a hematopoietic precursor cell become destined to pursue a T lineage fate? The phenotype and characteristics of hematopoietic progenitors have been extensively studied during the past decade, resulting in the identification of multiple bone marrow and peripheral blood subsets that are capable of differentiating into T lineage cells in the thymus. Despite enormous advances in the field, there are still considerable issues that remain unresolved. The phenotypes of hematopoietic precursor cells in mice and humans differ significantly, adding yet another layer of complexity to research aimed at understanding the regulation of T lineage commitment. Further complexities are the result of recent findings showing that the destinies of apparently identical hematopoietic precursors differ based on the milieu in which they are localized; i.e. bone marrow versus thymus. Here, we review recent findings arising from studies of hematopoietic precursor phenotypes in mice and humans, regulation of thymocyte differentiation and thymic importation of hematopoietic precursors.

2. Thymocyte differentiation: which cells are competent to pursue a T lineage fate?

In the murine thymus, the immature early T lineage progenitor (ETP) has high T and only limited B potential [6]. These thymocyte precursors, characterized as $lin^{-}CD44^{+}CD25^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$ (LSK) with only low CD127 (IL-7Rα) expression, are derived from hematopoietic stem cells (HSC) in the bone marrow. However, HSC themselves do not appear capable of seeding the thymus under physiological conditions. The most immature progenitors in the thymus do not harbor stem cell potential as shown by their inability to support long-term thymopoiesis; intrathymic transfer of progenitors already present in the thymus results in only a single wave of thymopoiesis [7–9]. Based on these experimental data, it has been concluded that long-term thymocyte differentiation requires

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an on-going migration of donor progenitors from the BM to the thymus. However, the precise nature of the precursor or precursors responsible for seeding the thymus under physiological conditions has not yet been well elucidated [10].

Within the BM, two subsets of hematopoietic precursors cells harbor the potential to generate thymocytes; multipotent progenitors (MPP) as well as the more committed common lymphoid progenitors (CLP). There is still some controversy concerning which cell subsets are the immediate precursors to thymocytes. MPP, retaining both myeloid and lymphoid potential, are generated from HSC and are characterized by the upregulation of the Fms-like tyrosine kinase receptor 3 (Flt3/CD135; resulting in LSK/CD135$^+$ cells) [11]. Subsets of MPP, characterized by expression of molecules such as RAG-1 [12] and P-selectin [13] can also give rise to thymic precursors. This is also the case for the more committed CLP that, in contrast to MPP subsets, lack myeloid potential [14]. At least two major CLP subsets have been identified; CLP-1 (lin$^-$Sca-1$^+$CD117$^{+/lo}$CD127$^+$CD135$^+$) [15] and CLP-2 (lin$^-$Sca-1$^+$CD117$^-$CD127$^+$CD135$^+$B220$^+$ (CLP-2) [16], with the latter thought to be the most differentiated population with T cell potential before commitment to the B cell lineage [16]. The recent finding that immature thymocytes retain myeloid potential [17,18] supports a model wherein MPP directly enter the thymus. Furthermore, MPP have been shown by numerous laboratories to be responsible for a more extended thymopoiesis than CLP [12,13,19–22]. Nevertheless, recent reports have raised new questions as they implicated CLPs as the only immediate source of ETPs, with MPPs allowing an extended thymopoiesis because of their renewal in the BM before further differentiation into CLPs [23,24]. Thus, further experiments are necessary to elucidate the stages directly linking HSC in the bone marrow to a T cell-committed progenitor in the thymus.

3. Of mice and men: phenotypes of human thymocyte precursors and subsets

In humans, the phenotype of the primitive hematopoietic precursor capable of giving rise to differentiated lineages is distinct from that detected in mice. In contrast to the lin$^-$Sca-1$^+$c-kit$^+$ (LSK) progenitors found in mice, human hematopoietic precursors harbor the CD34 marker. The CD34$^+$ cells that seed the thymus can differentiate into multiple lineages, giving rise not only to T, B and NK cells of the lymphoid compartment but also to myeloid cells such as DCs and erythrocytes [25,26]. T cell commitment thus occurs after entry of precursors into the thymus. It is generally agreed that human HSC differentiate into either myeloid or lymphoid lineage restricted precursors (CLP). Notably, a CD34$^+$ subset with a CD10$^+$CD24$^-$ phenotype has recently been shown to possess the characteristics of a CLP, retaining the ability to generate B, T and NK lineages but having lost myeloid potential [27]. These progenitors are present in the cord blood and in the BM but can also be found in the blood throughout life. As such, they may constitute the proximate thymic precursors in man.

In the human thymus, precursors with the CD10$^+$CD7$^-$ phenotype express genes that are common to both the T and B cell differentiation pathways but they appear to be committed to T cell differentiation [27]. Thus, in contrast to early murine thymocyte progenitors that have been reported to maintain myeloid differentiation potential (as discussed above, see [17,18]), this does not appear to be the case for human precursors. In humans, thymocyte differentiation of early precursors proceeds by the stepwise acquisition of CD7 followed by CD1a, a member of the CD1 family of MHC-like glycoproteins. CD34$^+$CD1a$^+$ pre T cells, in contrast to their upstream CD34$^+$CD1a$^-$ precursors, are rearranged at the TCRβ, γ and δ loci [28] and are unable to develop into non-T lineages (see [25,26,29]).

The phenotype of human thymocytes undergoing β-selection has not been completely explicited, with several lines of evidence indicating that β-selection can occur in distinct populations of cells differing in their CD4/CD8 expression profiles. A very low frequency of productive TCRβ V-DJ rearrangements has been detected in the double negative (DN) CD34$^+$CD1a$^+$ population, suggesting that a few cells may undergo β-selection before the expression of either CD4 or CD8 [30]. Directly downstream of the CD34$^+$CD1a$^+$ phase, cells express CD4, but not yet CD8, and they are referred to as CD4$^+$ intermediate single positive (ISP) cells. Intracytoplasmic (ic) TCRβ$^-$ populations have been found in CD4$^+$ISP cells and in the CD4$^+$CD8α$^+$β$^-$ early double positive thymocytes, thus demonstrating that not all populations beyond the CD34$^+$CD1a$^+$ stage are post-β-selection cells [28,31]. Blom et al. reported that β-selection can occur at the CD4$^+$ISP stage, based on their detection of TCRβ V-D-J recombination in a low level of CD4$^+$ISP cells and cytoplasmic expression of TCRβ protein in 5% of CD4$^+$ISP cells [28]. The group of Toribio, however, located the β-selection checkpoint at a later stage, namely, in CD4$^+$CD8α$^+$CD8β$^+$ DP cells that express a complex of TCRβ and pTα on the cell membrane [31]. On the basis of these studies, it appears that expression of a TCRβ protein and subsequent β-selection occurs within a certain developmental window and is not tightly coupled to regulation of CD4, CD8α and CD8β expression. In contrast, it appears that β-selection in mice occurs at a precise stage of differentiation, within DN3 (CD25$^+$CD44$^-$) thymocytes [32]. The timing of the rearrangement has a functional consequence; in mice, it is those DN4 cells that have undergone a productive TCRβ rearrangement that show a proliferative burst [33–35], whereas in humans, we have found that it is the CD4ISPs and CD4^{hi}DP immature thymocytes (characterized as TCRαβ^{lo}) thymocytes that are proliferating and express high levels of the ubiquitous glucose transporter Glut1 [36]. In mice, it is known that β-selection and subsequent proliferation correlate with a reversal in the polarity of thymocyte migration from the subcapsullar zone back into the cortex [37,38], and this requires an adherence to the extracellular matrix [39]. In humans, the data indicate that it is the Glut1$^+$CD4^{hi}TCRαβ^{lo} DP population that is associated with this critical phase of migration within the thymus [36]. It will therefore be important to study the functionality of the corresponding murine and human thymocyte populations based on their differentiation stage rather than based solely on the presence of cell surface markers.

4. Of mice and men: Notch and IL-7

Within the thymic microenvironment, the interplay between the Notch1 transcription factor and the IL-7 cytokine is crucial in supporting and orchestrating T cell development. However, the precise requirements for these factors in murine and human thympoeisis, as monitored by differentiation and proliferation, are not synonymous. The role of Notch1 in commitment to the T cell lineage was elegantly demonstrated by two ground-breaking studies showing that a constitutively active Notch1 results in αβ T cell development in the bone marrow while conversely, the conditional ablation of Notch1 expression causes an early block in T cell development with the development of phenotypically normal immature B cells in the thymus [40,41]. Indeed ETP, in contrast with BM HSC (LSK), are dependent on Notch signals for their generation and/or maintenance [42,43]. The transduction of Notch signals in the murine thymus has recently been shown to be mediated by the delta-like 4 (DL4) ligand; interaction of thymic progenitors with DL4-expressing thymic epithelial cells (TEC) suppresses B lineage potential and is an essential and nonredundant step in T cell lineage commitment [44,45].