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A short primer on early molecular and cellular events in thymus organogenesis and replacement

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Summary

Haematopoietic precursors have to undergo a complex series of maturational steps in the thymus before they exit into the periphery as functional T lymphocytes. Thymic stroma cells, the majority being of epithelial origin, provide the functional partners for the maturational progression along this differentiation pathway. Here we review some of the molecular and cellular mechanisms that account for thymus organogenesis and discuss a strategy to use thymic epithelial precursor cells for the regeneration of the thymic microenvironment.

Key words: development; stem cells; tymic epithelial cells; thymus

Introduction

The thymus constitutes the primary lymphoid organ where T cell precursors mature and acquire their specific functional competence prior to their exit to secondary and tertiary lymphoid organs. Importantly, the maturation of T cells is dependent on physical and functional interactions with several distinct types of stromal cells. Here the most abundant cell type is the thymic epithelial cell which forms a delicate three-dimensional cellular network spanning throughout both the cortex and the medulla. The interaction between lymphoid and stromal cells within the thymic microenvironment is also critical in order to shape the correct antigen specificity of the T cell repertoire so as to be able to respond to foreign antigens presented by Self MHC molecules but to be non-reactive to the body's own antigens (designated Self). This process of selection is referred to as central tolerance induction [1]. Although several mechanisms of peripheral tolerance have been identified, the thymic induction of T cell tolerance to Self remains of paramount importance in avoiding autoimmune pathologies. For this purpose, maturing T cells are not only selected against recognizing ubiquitous Self antigens but response to non-ubiquitous, i.e. tissue-specific antigens is also prevented. This latter aspect is critical to enable organ-specific immunological tolerance [2]. This promiscuous expression of peripheral, organ-specific antigens occurs typically in medullary thymic epithelial cells where the expression of the so-called Autoimmune Regulator (Aire) assures this important phenomenon [3, 4].

Generation of the thymus primordium

During embryonic development, the segmentation of the posterior pharynx constitutes the first step in the formation of the thymus. Subsequently, a series of as yet only incompletely understood developmental events are initiated that eventually result in the specification of epithelial cells of the 3rd pharyngeal pouch to adopt a thymic cell fate. This commitment takes place as early as day 10.5 of embryonic development (E10.5) in mice and analyses detailing the expression of the thymus-specific transcription factor Foxn1 have identified the ventral aspect of the 3rd pharyngeal pouch endoderm as the only site from where the thymus primordium emerges [5]. Although at first thought to be derived from epithelial cells of both endodermal and ectodermal origin, experiments in birds and more recently in mice have conclusively demonstrated that the thymic epithelial cells are exclusively of endodermal origin [6, 7].

In addition to the importance of endodermal epithelium, mesenchyme of the pharyngeal arches plays an equally critical role in the regular forma-

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tion of the thymus primordium. Some of the mesenchymal cells within the three caudal-most pharyngeal arches (i.e. 3, 4 and 6) are derived from neural crest cell expansions [8, 9]. These cells condense around the nascent aortic arch artery and eventually not only give rise to the smooth muscle cells of the great cephalic arteries and the aorto-pulmonary septum but also contribute to the connective tissue of both the thymus and the parathyroid glands [10]. The importance of mesenchyme for early thymus development has been investigated in vitro by culturing cell reaggregates that were composed of epithelium and mesenchyme separately isolated from E12.5 foetal thymus primordia. Alternatively, growing foetal thymic lobes were stripped of their mesenchymal capsule and subsequently grown in culture [11–14]. Taken together, these experiments revealed that thymic mesenchyme (but also mesenchyme from other organs) promotes epithelial cell development as assessed by the acquisition of several markers typical for differentiated thymic epithelial cells. In contrast, neural crest-derived mesenchyme does not appear to be required for the initial specification of endoderm to form a thymic epithelial cell but subsequent steps in thymus development are (at least transiently) dependent on this cell type. For example, the particular importance of neural crest cells and their progeny for the formation of the thymus has clearly been demonstrated in chick embryos where the physical ablation of these cells resulted in a small thymus barely competent to support the development of a limited number of thymocytes [15]. Thus, neural crest-derived mesenchyme is critical to sustain the development of pharyngeal epithelium leading to the formation of a regularly composed and structured thymic primordium that attracts and supports lymphoid precursor cells.

The mesenchymal component of the thymic primordium provides the epithelial cells with a yet to be defined inductive signal, which is obviously essential for the organ's correct morphogenesis. This mesenchymal-epithelial interaction may be effected by several molecules including the epidermal growth factor, insulin-like growth factors-I and -II and the fibroblast growth factors (Fgf-7 and -10) [11–14, 16–18]. As a result, it is likely that mesenchymal cells regulate both the proliferation and differentiation of immature thymic epithelial cells. Importantly, despite the absence of some of these factors, the homing of lymphoid precursor cells to the thymus primordium is maintained and the lymphoid precursor cells mature normally to T cells (see below). Once development of the thymus has progressed to a stage able to support immature thymocytes, the further differentiation of thymic epithelial cells is largely rendered independent of ongoing mesenchymal support [18].

The colonization of the thymus by haematopoietic precursor cells

In the thymus primordium of mouse embryos, lymphoid progenitors are detected as early as day 11.5 of gestation, well before the establishment of a blood supply [19–21]. It had, therefore, been postulated early on that the foetal thymus rudiment must produce diffusible factors that act as chemoattractants for haematopoietic progenitor cells and that these cells access the thymus primordium via transmigration through the surrounding mesenchymal layers. Indeed, experiments in trans-filter migration assays [20] and in time-lapse visualization assays [22] are compatible with a role for chemotactic factors secreted by foetal thymus epithelial cells. Further experiments have, moreover, established that this chemoattraction is mediated by pertussis toxin-sensitive G-protein-coupled receptor signals, suggesting an involvement of chemokine signals for the pre-vascular colonization of the foetal thymus [21, 22].

Transcripts for different chemokines including CCL25 (TECK) and CCL21 (SLC) can be detected in the foetal thymus as early as E12.5 [23]. In contrast, mice deficient for the expression of the receptor for CCL25, CCR9, exhibit a three-fold decrease in total thymocyte cellularity until the establishment of the thymus vasculature by E14.5 to E15.5 when compared to wild-type animals. These results reveal a partial, non-redundant involvement of CCL25 in the events leading to foetal thymus colonization by haematopoietic precursor cells [24]. Mice either deficient for CCL21 or its receptor, CCR7, also display a decreased thymus cellularity during the pre-vascular phase of thymus colonization [22]. However, it remains to be defined whether other chemokines also contribute to the colonization and if so, which of these factors are alone or in combination critical for this process to occur.

In addition, and possibly positioned upstream of chemokines, other molecules have also been identified as being necessary for the colonization of the thymus by haematopoietic precursor cells. The transcription factor Foxn1 is expressed in thymic epithelial cells and its function is required for the entry of haematopoietic precursor cells to the thymus primordium. A deficiency in Foxn1 affects not only the initial formation of a thymus but also prevents the homing of haematopoietic precursor cells to the thymic microenvironment, thus precluding the generation of T cells. Known as nude mice due to their concomitant lack of a regular coat, Foxn1-deficient mice are not only unable to recruit haematopoietic precursor cells to the thymus primordium but are also wrongly accumulating these cells in the parathyroid anlage which is also derived from the 3rd pharyngeal

pouch [18, 23, 25]. It has, therefore, been suggested that the colonization of the non-vascularized thymus occurs in two independent but sequential steps. The first step concerns the recruitment of haematopoietic precursor cells to the vicinity of the thymus primordium. The molecular mechanism(s) responsible for this initial step appears to be independent of Foxn1. The second step controls in a Foxn1-dependent fashion the accumulation of these precursor cells amidst the thymic epithelium. Interestingly, the chemokines CCL25 and CXCL12 are not expressed in Foxn1-deficient thymic epithelial cells placing these molecules in the signalling cascade downstream of Foxn1 [23].

Blood vessels begin to sprout into the thymus anlage between days 14.5 and 15.5 of mouse gestation, which then allows haematopoietic progenitor cells to access the thymus via the vasculature. It has been suggested that molecular mechanisms may be operative in the colonization of a vascularized thymus that are analogous to those controlling the homing of mature T cells into secondary lymphoid organs. Indeed, integrins and CD44 have been suggested by several experimental studies to play a role in the process of cell entry into a vascularized foetal thymus [26]. In addition, functional evidence has also been provided that the interaction between the P-selectin ligand PSGL-1 (expressed on circulating lymphoid progenitor cells) and its receptor P-selectin (expressed on thymic epithelium) is of paramount importance for lymphoid progenitor cells to enter the adult thymus [27], further underscoring a role for adhesion molecules in the colonization of the postvascular thymus. However, a precise role for chemotactic signals in this process remains to be defined.

The direct contact of haematopoietic progenitor cells with epithelial cells within the thymic microenvironment allows for the initiation of distinct signalling events relevant to the development of either cell type. For example, thymic epithelial cells express the Notch-ligand Delta and the cytokine interleukin (IL)-7 that promote the commitment of the T cell lineage and the survival of lymphoid progenitor cells, respectively [28, 29]. Importantly, early T-lymphoid precursor cells and/or their immediate progeny provide the signals that promote the development of thymic epithelial cells and their differentiation into a distinct cortical compartment. Later in ontogeny, the differentiation of thymic epithelial cells to a medullary phenotype and the subsequent formation of a medulla proper are clearly dependent on the presence of positively selected thymocytes [30]. However, the precise molecular nature of the signals that are provided by developing thymocytes and that shape the thymic stromal compartment are still incompletely defined. Taken together, bi-directional "cross-talk" between lymphoid cells and thymic stromal cells is initiated following the colonization of the thymus anlage that promotes the differentiation of both cell types.

Homeostasis of peripheral T cell numbers

The thymus of a pre-adolescent mouse exports approx. $1–2 \times 10^6$ newly generated and fully functional T cells every single day. This respectable output decreases physiologically over time by up to a 100-fold as a consequence of thymic involution, which starts in puberty [31, 32]. However, the number of peripheral T cells remains surprisingly constant despite this limitation in the generation and export of newly generated T cells. The complex mechanism by which the peripheral lymphoid pool is maintained at a constant size is referred to as homeostatic regulation [33]. However, several conditions and medical interventions have been identified that lead to a reduction of the pool of peripheral T cells, including specific genetic mutations (such as severe combined immunodeficiencies, SCID), infections such as HIV, radiochemotherapy and the therapeutic provision of immunosuppressive drugs. Not surprisingly, reduced T cell numbers have been associated with a higher risk for infections, a reduced response to vaccinations, a dysregulation in the immune response and a higher frequency in cancer development [33, 34].

Enhancing thymic function and T cell output are, therefore, likely measures by which a contracted peripheral T cell pool could be restored to its regular size [34]. One of the strategies to achieve such a goal is the use of thymic epithelial stem cells as building blocks to recreate the functional microenvironment needed for T cell development and tolerance induction. Importantly, the thymic epithelial stromal compartment constitutes a dynamic structure that is continuously remodelled by cells derived from epithelial precursors. This process can be modulated by the provision of exogenous growth and differentiation factors, such as keratinocyte growth factor and by physical or chemical castration as either treatment brings about an absolute increase in thymic stromal cells and, consequently, a higher number of thymocytes [31, 35]. Boosting thymic T-lymphopoiesis will also result in a higher output of newly generated and functionally competent T cells, thus improving the peripheral T cell compartment.

Characterisation of a thymic epithelial precursor cell

Studies in mouse embryos have identified a thymic epithelial cell precursor population that gives rise to fully differentiated thymic epithelial cells with either a cortical or a medullary phenotype. Upon isolation, ex vivo reaggregation and a subsequent engraftment of such precursor cells into mice, a functionally competent three-dimensional stromal network is newly formed and now competent to support regular thymopoiesis [36, 37]. As a population, these thymic epithelial precursor cells express in the mouse the surface marker MTS24, a so far unidentified glycoprotein with ubiquitous tissue expression (fig. 1). The percentage of MTS24 expressing thymic epithelial cells is highest early in development and by far exceeds the frequencies normally observed for stem cells in other tissues.

The potential therapeutic applications of these thymic epithelial cells to rebuild thymic function will ideally advocate their use in an autologous fashion, thus necessitating their successful isolation from adult thymus tissue. However, adult thymic epithelial stem cells with a capacity to regenerate the entire epithelial stromal compartment have yet to be documented, as numerous attempts to employ these cells in reaggregate assays have uniformly failed to produce a thymic microenvironment able to support regular thymopoiesis (fig. 1). We, therefore, wanted (i) to improve the culture conditions to achieve the necessary complexity of the three-dimensional epithelial structures required for regular T cell development, (ii) to characterize and compare the phenotype of MTS24-positive cells in both embryonic and adult mice so as to identify in adult tissue the functional equivalent to the population of foetal thymic epithelial stem cells; and (iii) to define by extensive gene expression profiling the transcriptome of MTS24-positive epithelial cells taken from either foetal or adult thymus tissue. As part of the national research program 46 of the Swiss National Science Foundation (NFP46), this research was specifically undertaken to understand the molecular circuits operative in thymic cells that are able to reconstitute the entire epithelial compartment. Insights gained from these studies are expected to enhance the in vitro conditions to expand thymic epithelial precursor cells and to direct their differentiation to fully functional cortical and medullary epithelial cells, respectively.

Upon exposure to a matrix scaffold, single-cell suspensions of foetal thymic epithelial cells spontaneously reaggregate into a three-dimensional structure in a period of time as little as 12–24 hours. Such a three-dimensional structure is indispensable for the successful induction of T cell development from haematopoietic precursor cells [38]. In analogy, adult thymic epithelial cell grafts with a correct architectural composition and transplanted under the kidney capsule of syngeneic recipient mice are, therefore, expected to promote a correct microenvironment that supports and maintains T-lymphopoiesis. To achieve the formation of reaggregates using adult thymic epithelial precursor cells, two related issues needed to be first addressed: (i) adult MTS24-positive cells are unable to reaggregate and (ii) an extended ex vivo culture of (adult and foetal) thymic epithelial cells alters their gene expression profile [39]. Despite a greatly increased frequency of viable cells at the time of engraftment and an exceedingly shortened ex vivo culture time to form reaggregates, adult MTS24-positive cells continued to fail to generate functional thymic tissue in vivo. Nevertheless, these cells can persist in the cellular context of foetal thymic lobes but their expansion is difficult to determine under the experimental conditions chosen. Taken together, these observations underscore the possibility that there may be a difference between foetal and adult thymic tissue concerning the actual stem cell frequency among MTS24-positive cells.

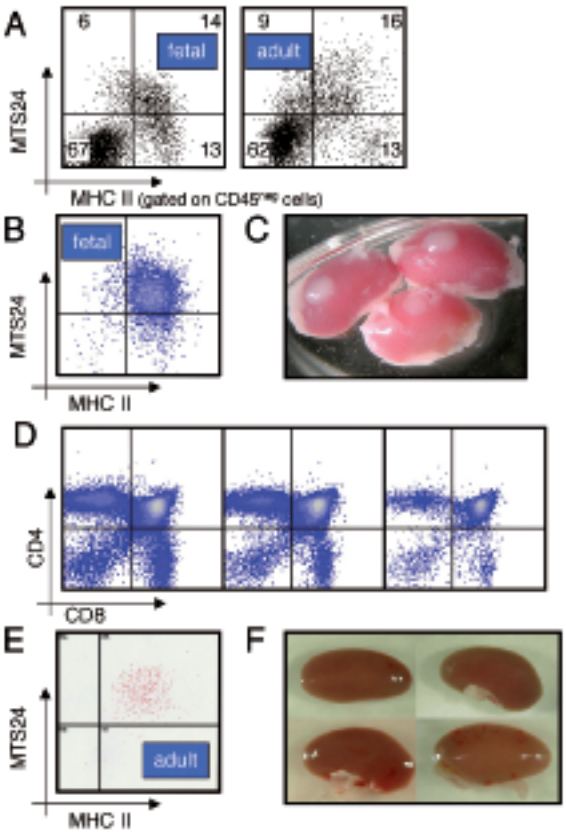

Figure 1

Thymus regeneration potential of fetal (E15.5) and adult (wk6) MTS24^{pos} thymic epithelial cells. (A) MTS24^{pos} epithelial cells are present in the fetal and adult thymus. (B) Purity of sorted E15.5 MTS24^{pos} cells. (C) Sorted MTS24^{pos} cells were reaggregated for 24h in gelfoam and grafted under the kidney capsule of host mice. Mice were analysed 10 weeks after grafting. (D) CD4 and CD8 FACS profiles of newly generated thymi indicate normal T cell development. (E) Sort purity of wk6 MTS24^{pos} cells. (F) Sorted cells were reaggregated, grafted and analysed as their fetal counterparts.

Concluding remarks

Although the potential thymic epithelial stem cell in the adult mouse remains elusive, the observed response of the thymic epithelial cell compartment to growth and differentiation stimuli strongly suggests that such a cell is likely to exist. Novel approaches to identify the phenotype of such a precursor cell are currently being evaluated in our laboratory and show very promising preliminary results. The knowledge gained on thymic epithelial stem cell biology coupled with a detailed understanding of the molecular and cellular events during their differentiation should take us closer to a potential therapeutic application.

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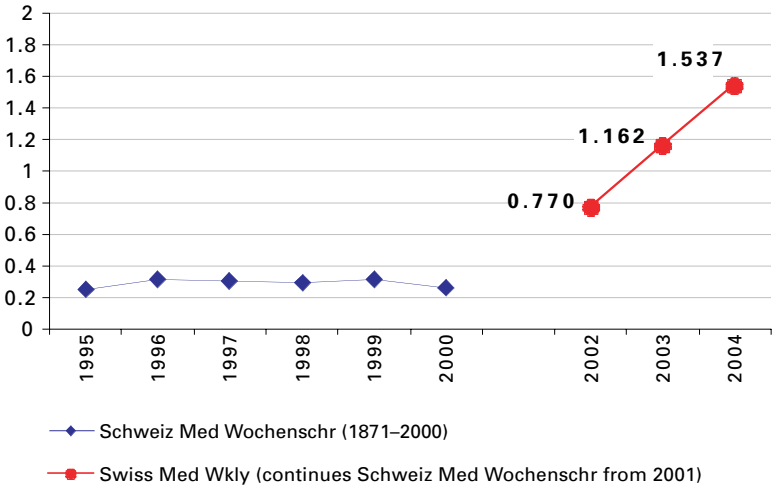

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