MALIGNANCIES OF THE THYMUS

The Thymus and the Immune System

Layered Levels of Control

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Abstract: Control points of normal thymopoiesis may provide insights into strategies for interrupting cell interactions in thymomas which appear to maintain active T cell production. Thymus production of T cells represents one of two pathways by which peripheral T cell populations are maintained or, if lost, regenerated. The production of T cells by the thymus results from a series of thymus epithelial cell (TEC) - thymocyte interactions from entry of thymocyte precursors into the thymus to release of mature naïve single positive T cells into the periphery. Within this series of interactions, certain control points have been identified, all of which act through TEC to modulate thymopoiesis.

Key Words: Thymopoiesis, Thymic epithelial cells, Thymocytes, IGF-1, KGF, Androgen withdrawal.

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Thymomas are slow-growing primary tumors that arise from thymic epithelial cells (TEC).1 Thymomas are well known for their association with autoimmune disorders, such as myasthenia gravis, suggestive of thymoma-induced distortions in central tolerance induction. This possibility has been raised because the process of thymopoiesis continues in many thymomas, reflecting the complex interdependency between thymocytes and TEC. The neoplastic epithelial cells may continue to express thymic epithelial markers, and thymocytes at all developmental stages can be found in thymoma.2 Indeed, myasthenia gravis is more common in thymoma patients with a greater retention of thymopoiesis, as assessed by a high frequency of CD4+CD8+ thymocytes.2 Understanding the role of the thymus in T-cell generation and homeostasis may contribute in understanding the biologic effects of thymoma and may identify potential points of intervention. Normal thymopoiesis results from a full symbiosis of thymocytes and TEC. The differentiation of cortical and medullary TEC subpopulations is dependent on T-cell lineage commitment, and the maturation of T cells, in turn, is dependent on TEC. Therefore, this review will address factors that regulate normal thymopoiesis by acting through TEC.

T CELL HOMEOSTASIS: THYMIC-INDEPENDENT AND THYMIC-DEPENDENT PATHWAYS

The generation and maintenance of T-cell populations are governed by the interplay of thymic-dependent and thymic-independent pathways (Figure 1). Residual T-cell populations proliferate in severely lympho-depleted hosts, filling the available “space.” The space in this rapid, thymic-independent peripheral expansion is defined by homeostatic cytokines, such as interleukin (IL)-7 and IL-15. These cytokines are constitutively produced at stable levels and consumed by a large population of T cells. At low circulating maintenance levels, these cytokines are critical for survival of their target cells: naive CD4 and CD8 cells are reliant on IL-7 and memory/effector CD8 cells reliant on IL-15. When T cells are severely depleted, however, the relative levels per cell of these cytokines increase. Increased levels of these cytokines not only support survival but also drive expansion of their targeted cells.4 This rapid expansion of residual T cells results in a population composed primarily of memory and effector T cells, but limited by the antigen-response repertoire of the original population.5

The second pathway is the thymic-dependent generation of new naïve T cells from T-cell progenitors. In this pathway, progenitors from the bone marrow mature into T cells in the thymus and are exported into the periphery as naïve T cells. In younger individuals, thymopoiesis plays the principle role in the recovery of T cells, rapidly resulting in a stable population of naïve cells with broad T-cell receptor repertoire diversity. However, the efficiency and time course of the thymic-dependent pathway are highly correlated with the age of the individual.6 In middle-aged individuals, fewer are capable of extensive T-cell recovery, even successful thymopoietic recoveries require 2 to 3 years to complete and may include fewer naïve cells. The delay is caused by the physiologic involution of the thymus with age. To regenerate T-cell populations, the thymus must expand and thymopoietic tissue must increase. Studies of long-term immune reconstitution in adults demonstrate that thymic regeneration and renewal of productive thymopoiesis occurs.8 The question then posed is what factors define the space-limiting thymopoiesis and how this space is regulated.

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ROLE OF THYMOCYTES AND TEC IN THYMOPOIESIS

T-cell progenitors arise in the marrow from hematopoietic stem cells. On leaving the marrow, T-cell progenitors home to the thymus and enter through the vasculature at the cortical medullary junction. The most immature thymocytes are termed double negative cells (DN) due to a lack of expression of CD4 or CD8. In the DN stage, subdivided into DN1 to DN4, thymocytes increase in number, migrate outward through the cortex, and undergo the gene rearrangements needed to form the T-cell receptor (TCR) α and β chains. Signaling through the rearranged TCR-β chain triggers the main proliferative expansion of thymocytes and differentiation into CD4 and CD8 double-positive (DP) cortical thymocytes. On final rearrangement of the TCR-α chain and surface expression of a complete TCR-αβ, the DP cells undergo positive selection, based on affinity for class II or class I major histocompatibility complex molecules, into single positive (SP) CD4+ or CD8+ T cells, respectively. Cells that fail to complete TCR gene rearrangements or to be positively selected die. The SP cells then migrate back through the thymus to enter the medulla, where maturation occurs. Here, SP T cells undergo a stringent negative selection to remove cells that are reactive to self antigens expressed by medullary TEC and dendritic cells. The T cells that survive the selection processes in the cortex and medulla then exit the thymus to join the naive T-cell population in the periphery.

Interactions between developing thymocytes and TEC control much of thymopoiesis. Entry of T-cell progenitors depends on interaction between chemokine and adhesion molecules on progenitors, such as CCR9 and P-selectin glycoprotein ligand-1 (PSGL1), and the corresponding ligands (CCL25 and P-selectin) expressed by thymic stroma. T-cell lineage commitment and differentiation are reinforced through the interaction of Notch receptors on DN thymocytes and ligands expressed on stromal cells. Thymocyte migration across the thymus is controlled by chemokine signals in the stroma. Cytokines secreted by TEC, such as IL-7, support thymocyte survival at key checkpoints, such as the transition from DN1 to DN2. Finally, thymic stroma is critical to the positive- and negative-selection processes. Cortical TEC expressing class I and II major histocompatibility complex molecules support positive selection of cortical thymocytes into CD4 and CD8 SP T cells. Medullary TEC and dendritic cells, expressing tissue antigens, support negative selection of autoreactive thymocytes and the development of T-regulatory cells. Thus, all steps in thymopoiesis depend on interaction of thymocytes and TEC.

Yet, this interaction is not unidirectional. After the fetal organogenesis period, the continued maintenance of cortical and medullary TEC in the adult requires the presence of functional thymocytes. Thymuses in which T-cell development is blocked at the earliest DN1 stage show an absence of both cortical and medullary TEC. Conversely, introducing functional T-cell progenitors to a mouse in which thymocyte differentiation is blocked at an early stage can trigger the expansion and growth of TEC. These results demonstrate that interactions between thymocytes and TEC are not limited to the organogenesis period. A key finding is that TEC populations are not static, but rather continuously differentiating from TEC progenitors, expanding, and turning over. Thymocytes are constantly being renewed by an input of T-cell progenitors. The interdependence of thymocytes and TEC may contribute to their mutual decline with aging. Yet, this same interdependence may underlie the ability of thymuses to renew growth and expand.

To further explore these interactions, studies have addressed several known components that influence thymus function. Experiments on insulin-like growth factor (IGF)-1, androgen signaling, and keratinocyte growth factor (KGF) have been carried out to study their effects on thymopoiesis and to determine the cellular and molecular basis of those effects (Figure 2).

IGF-1 IN THYMOPOIESIS

Thymopoiesis can be significantly affected by systemic hormones, such as the growth hormone (GH)/IGF-1 axis. Declines in GH during adult life may contribute to the slow decline in thymopoiesis. Most of the actions of GH are carried out by IGF-1, which is generated in the liver in response to GH. Treatment with either GH or IGF-1 consistently produces an increase in thymic cellularity and peripheral T-cell levels. Identifying the effects of IGF-1 within the thymopoietic process poses a challenge because IGF-1 receptor (IGF-1R) is widely expressed among the subpopulations of interest: T-cell progenitors, thymocytes, TEC, and mature T cells.

Administering exogenous IGF-1 in thymus intact mice elicited an increase in the number of naive T cells and recent thymic emigrant populations in the periphery. Yet, similar treatment in thymectomized mice did not alter peripheral lymphocyte numbers. This supports that IGF-1 has an influence on the thymus-dependent pathway rather than the thymus-independent pathway.
In vitro, IGF-1 enhanced proliferation and inhibits apoptosis of T cells. In vivo, systemic treatment with exogenous IGF-1 increased the numbers of T-cell progenitors (Lin\(^-\) Sca-1\(^+\) c-kit\(^+\)) in both the marrow and the circulation. Increased thymopoiesis could be due to an enlarged precursor population and increased entry into the thymus. Therefore, the effects of IGF-1 on thymic function were tested in PSGL1 knockout mice (PSGL-1KO). Because P-selectin is involved in T-precursor uptake into the thymus, these mice have a severe limitation in thymocyte precursor importation. If IGF-1 mediated its effect of enhancing thymopoiesis through increased entry of precursors, then the effect should be blunted in such mice. However, this was not observed experimentally. Instead, administration of IGF-1 in the PSGL-1KO mice increased thymopoiesis as it did in controls. Therefore, the main target of IGF-1 was not impacted by limiting the T-cell precursor pool.

The final determination was whether IGF-1 acted primarily on thymocytes or TEC. Both populations increased after systemic IGF-1 treatment and both showed increases in the frequency of proliferating cells. Therefore, a transgenic mouse model was developed with a T-lineage-specific knockout of the IGF-1 receptor (T-IGF-1R\(^-\)) on thymocytes and mature T cells; IGF-1 receptor expression was retained on TECs and T-cell progenitors. Treatment with IGF-1 produced comparable increases in thymocytes in the mice lacking the IGF-1R on thymocytes, as in those with the receptor present. Therefore, the key factor was the expression of IGF-1R on TEC. IGF-1 acted primarily by stimulating proliferative expansion of TEC. This increase in cortical and medullary TEC then, in turn, supported an increase in thymocyte populations. Thymopoietic space was regulated by stimulating TEC with IGF-1.

**ANDROGEN SIGNALING IN THYMOPOIESIS**

In contrast to IGF-1, androgens are negative regulators of thymopoiesis. Castration or androgen withdrawal results in an enlargement of the thymus and an increase in thymic T-cell generation. Furthermore, transplant studies have demonstrated enhanced thymic size and cellularity, and increased numbers of peripheral recent thymic emigrants, after androgen withdrawal. As in the case of IGF-1, both TEC and thymocytes express androgen receptors, but the TEC have again been implicated as the controlling element in androgen regulation of thymopoiesis. When androgen receptors on TEC were dysfunctional, the effects of androgens on thymocytes were abrogated.

At issue then was which of the multiple thymopoiesis-supporting functions of TEC was critical to the increase in thymic productivity. Androgen withdrawal increased the medullary and cortical epithelial cell areas, which, as noted, play many roles in thymopoiesis. One of the earliest effects was an increase in early T-lineage progenitors (ETP), the earliest thymocyte precursor to enter the thymus. This increase was found to be due to improved ETP immigration by comparing uptake of adoptively transferred congenic T progenitors in intact and castrated hosts. Finally, this increased uptake was associated with an increase in TEC production of CCL25, a chemokine critical for T progenitor entry and migration in the thymus. Blockade of CCL25-ETP interactions with antibody prevented the increase in thymopoiesis. Hence, androgen withdrawal triggered increases both in TEC numbers and in TEC production of CCL25. These changes in TEC then both enhanced the influx of ETP and supported increased thymopoietic space.

**KERATINOCYTE GROWTH FACTOR IN THYMOPOIESIS**

KGF is another factor that enhances thymic productivity and peripheral naive T-cell populations in intact mice, but does not affect peripheral T cells in thymectomized mice. KGF belongs to a family of structurally related fibroblast growth factors and acts as an epithelial cell mitogen. KGF

![FIGURE 2. Thymus recovery is regulated at different points by factors such as IGF-1, androgen signaling, and KGF. KGF, keratinocyte growth factor; IGF, insulin-like growth factor.](image)
binds to the fibroblast growth factor receptor-2 of the IIIb variant (FgfR2IIIb). In the thymus, FgfR2IIIb is exclusively expressed on thymic epithelial stromal cells. In murine studies, the addition of exogenous KGF rapidly triggered proliferative expansion of TEC and TEC progenitors. KGF treatment increased the uptake of labeled T progenitors, that is the number of engraftment niches, although whether this was the result of the same induction of TEC chemokines as androgen withdrawal has not been tested. Furthermore, the increase in ETP and TEC numbers resulted shortly afterward in a wave of proliferative expansion in thymocytes and subsequent export of naive T cells. Even in aged mice, KGF treatment increased thymopoietic capacity and reversed involutional changes. Repeated monthly KGF treatments prolonged these effects and reversed involution in aged murine thymic structure, returning the thymuses to the size of those in young adults. Therefore, KGF seems to play a major role in regulating the niche size defined by TECs in the thymic-dependent pathway.

An interesting aspect of KGF is that thymocytes produce KGF at specific stages of thymus development. Therefore, KGF is an intrinsic factor to the thymus, rather than a systemic one. KGF is not detectable in DN, but the level of KGF expression proportionally increases with thymocyte maturation. These findings also point out the interconnections between thymocytes and TEC. Recombination activating gene knockout (RAG\( ^{-/-} \)) thymocytes, perhaps because they are arrested before the DP stage, do not produce KGF. The RAG\( ^{-/-} \) medullary region is rudimentary and disorganized. This region can be induced to develop normal structure either by transplant of normal hematopoietic stem cells, or by treatment with KGF. These linkages between thymocytes and TEC illustrate the interdependence of these populations.

**SUMMARY**

The finding that IGF-1, cessation of androgen signaling, and KGF all act at the level of TECs identifies these cells as playing a central role in the regulation of thymopoiesis. The direct linkages between expansion of TEC populations and increases in thymocytes suggest that these interactions underlie the maintenance of thymopoiesis in thymomas. Considering that the mechanism common to these three model systems is regulation of TEC proliferation, the identification of genes that may be generally expressed in cells or specific to TECs and are involved in regulation of cell division may be of special interest in understanding the biology of thymomas. Further, the association of autoimmunity with the apparent continued production of T cells and their export to the periphery poses questions concerning alterations in TEC/T-cell interactions in thymomas.

**REFERENCES**