The Thymus in Immunity and in Malignancy

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Abstract
The thymus is an essential organ for the generation of the adaptive immune system. By now, the cellular selection events taking place in ongoing life before sexual maturity have been worked out even at the molecular level, and thus thymic lymphocyte development represents one of the best-studied systems in mammalian development. Because thymic lymphocyte development involves ample proliferation and generation of new cells, it is not astonishing that the thymus also represents an organ where malignancy can develop. In this Masters of Immunology primer, the development of lymphocytes and the role of intracellular Notch 1 and cyclins in lymphocytic malignancy are reviewed, offering new therapeutic possibilities.

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No potential conflicts of interest were disclosed.

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The members of the planning committee have no real or apparent conflicts of interest to disclose.

Learning Objectives
The thymus is an essential organ for the generation of the adaptive immune system, and it is one of the best-studied systems in mammalian development. Thymic lymphocyte development involves ample molecular events leading to highly regulated cellular proliferation or malignancy. Upon completion of this activity, the participant should gain a basic knowledge of thymic development and adaptive cellular immune responses.

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Introduction
The thymus is an essential organ for the generation of an adaptive immune system. It is astonishing, therefore, that removal of the thymus in adult life has few consequences. Still, some new T cells develop in adulthood, but they do not seem to be indispensable for the immune system in healthy individuals. The thymus essentially contributes to the immune system before sexual maturity by selecting developing thymocytes in such a way that the T cells that leave the thymus can recognize peptides presented by the body’s major histocompatibility complex (MHC) molecules without causing autoimmunity (as a rule), and functionally different T-cell subsets discriminate class I and class II MHC–presented peptides that are generated either inside the cell or taken up with exogenous proteins.

Early Thymocyte Development
Although the cell type seeding the thymus is unknown, the earliest thymocyte seems to be a cell that can be identified by its high expression of c-Kit [tyrosine-protein kinase Kit, also known as CD117 or mast/stem cell growth factor receptor (SCFR)] and Sca-1 (stem-cell antigen 1), as well as low levels of the interleukin-7 (IL7) receptor (1). Some in vitro studies have indicated that cells of this subset can also generate myeloid cells (2), an observation that could not be confirmed in vivo (3). This so-called DN1a population (CD4/CD8 double-negative population 1a; CD4−CD8−CD25−CD44+CD24lowCD117hi) can extensively proliferate and generate about 50 million CD4+CD8+ thymocytes in the mouse per day, whereas only 1 million medullary thymocytes exit the thymus per day (4), indicating that in a normal thymus most newly generated cells die in situ. It is now a consensus that this DN1a subset, which may also acquire CD4 expression, contains the earliest intrathymic T-cell precursors (5). These proliferating cells acquire consecutively markers of the DN2 (CD4−CD8−CD25+CD44hi) and DN3 (CD4−CD8−CD25−CD44lo) subsets where T-cell receptor (TCR) rearrangement sets in. First rearrangement of the TCRβ locus and the TCRγ and TCRδ takes place within the same cells (6). It is not whether the cell succeeds
in productive rearrangements of $\beta$ and $\gamma$-$\delta$ gene segments that decides whether the cell enters the $\gamma$-$\delta$ or $\alpha$-$\beta$ lineage (even though as a rule the pre-TCR with the TCR $\beta$ chain instructs cells to enter the $\alpha$-$\beta$ lineage) but the intensity of the signaling of the expressed receptor. Taking the differentiation of DN2 (CD4$^-$CD8$^-$CD25$^{hi}$) or DN3 (CD4$^+$CD8$^-$CD25$^{hi}$) cells toward double-positive (DP) CD4$^+$CD8$^+$ cells as being indicative of $\alpha$-$\beta$ lineage choice, it was reported that the intensity of signaling of the TCR rather than the TCR itself instructs cells whether they should enter the $\alpha$-$\beta$ or the $\gamma$-$\delta$ lineage (7–9).

The Pre-TCR Receptor

From here on, only the development of the $\alpha$-$\beta$ lineage of thymocytes will be considered, which starts with the expression of the pre-TCR as a rule (some weak signaling of the $\gamma$-$\delta$ TCR can also make cells enter the $\alpha$-$\beta$ lineage; refs. 7–9), which consists of the invariant pre-TCR $\alpha$ chain (10) and the variant TCR $\beta$ chain. Expression of this receptor by DN3 cells initiates several rounds of cell division that only cease when the cells enter the DP stage of differentiation. This proliferation is mandatory for differentiation (11) and involves intracellular Notch1 (12) that is expressed as a consequence of the binding of the transmembrane Notch receptor to the Notch1 ligand on the thymic epithelium. Ectopic expression of Notch target Myc rescues differentiation in the absence of Notch signaling. This diagram shows that T-cell differentiation in wild-type mice requires TCR$\beta$-selection–induced proliferation, as inhibition of proliferation blocks development of DP cells. Expression of transcription factor E47 (encoded by Tcf3) obligates the progression of T-cell differentiation on proliferation. In the absence of E47, T cells are able to differentiate into DP cells with or without cell division. Adapted from Kreslavsky et al. (11).

Figure 1. Development of $\alpha$-$\beta$ lineage thymocytes requires TCR$\beta$-selection–induced proliferation. Thymocyte development to the DP stage requires the expression of a pre-TCR with a productively rearranged TCR$\beta$ chain, which is followed by several rounds of cell division that only cease when cells enter the DP stage. Thymocyte proliferation also requires the expression of intracellular Notch1, which is a consequence of the binding of the transmembrane Notch receptor to the Notch1 ligand on the thymic epithelium. Ectopic expression of Notch target Myc rescues differentiation in the absence of Notch signaling. This diagram shows that T-cell differentiation in wild-type mice requires TCR$\beta$-selection–induced proliferation, as inhibition of proliferation blocks development of DP cells. Expression of transcription factor E47 (encoded by Tcf3) obligates the progression of T-cell differentiation on proliferation. In the absence of E47, T cells are able to differentiate into DP cells with or without cell division. Adapted from Kreslavsky et al. (11).
exclusion at the TCRz locus through feedback inhibition. For this reason, the postulate of one lymphocyte with one receptor (19) is not correct as T cells can express two receptors with two different TCRz chains. This is probably so to facilitate positive selection such that one CD4+CD8+ cell can try more than one receptor during its limited lifetime. It is unlikely that both receptors are specific for the individual’s MHC molecules, i.e., that they interfere at the receptor level during positive selection.

Death from Neglect and Negative Selection

It was clear from the beginning that in the thymus strong selection of cells did occur, as transplantation of many thymi did not result in the accumulation of T cells in peripheral lymphoid organs, supporting an argument that most cells produced in the thymus would also die there (20). It is now believed that the greatest contribution to intrathymic cell death comes from cells that fail to undergo positive selection. "Death from neglect" was shown to occur in TCR transgenic mice that expressed a nonselectable TCR on thymocytes, resulting in a developmental arrest and cell death at the DP (CD4+CD8+) stage of development (21).

This is not the only form of cell death; cells with a TCR that binds with high affinity to self-peptides and MHC molecules will likewise die, and this form of cell death was named "negative selection" (22–24). It was shown later that cells with high-affinity receptors for self-peptides presented by the body’s MHC molecules actually died in situ (25). Another way of avoiding the generation of self-reactive cells is achieved by receptor editing, which apparently is operative in B cells but does not exist in T-cell development (24). Negative selection occurs predominantly in different compartments of the thymus for class I and class II MHC-restricted cells, simply because there are more class I and fewer class II MHC molecules present in the outer cortex. Thus, at least for class I MHC molecules-restricted T cells negative selection can precede positive selection (26).

Positive Selection and Matching Function and T-cell Specificity

Positive selection as defined in TCR transgenic mice represents the appearance of single positive cells in the thymic medulla due to positive selection of DP (CD4+CD8+) cortical thymocytes by thymic MHC molecules plus peptide. It should be stressed here that positively selecting peptides do not bear any recognizable structural resemblance to the peptides that activate the positively selected T cell (27), except when the peptide density is being changed such that the low density of a given peptide mediates positive selection, whereas the high density of the same peptide serves as agonist stimulus for the positively selected T cell (28). Positive selection is more complicated than cell death, and it encompasses multiple stages of development. As postulated by Brugnera and colleagues (29), the encounter with positively selected class I or class II MHC ligands results in the downregulation of CD8, i.e., the generation of CD4+CD8dim thymocytes. If the TCR of these cells is class II MHC-restricted, the coengagement of the TCR and CD4 by class II MHC molecules results in the activation of Th-POK, a transcription factor required for the development of CD4+ T-helper (Th) cells, and further downregulation of CD8, such that CD4+CD8− medullary thymocytes are generated (30). If the TCR is class I MHC-restricted, the weak binding of the TCR and the downregulated CD8 to thymic class I MHC molecules results in what has been named coreceptor reversal (29), i.e., in a cell that now expresses high levels of CD8 and no CD4. Coreceptor reversal seems to depend on IL7 (31). In addition, this signaled cell expresses the transcription factor RUNX3, which helps the downregulation of CD4 and antagonizes Th-POK, thus enabling the development of CD8+, class I MHC-restricted medullary cells (32). It is the mutual antagonism of RUNX3 and Th-POK (Fig. 2) that is responsible for the generation of CD8 and CD4 medullary cells that according to their function are also named T-killer cells and T-helper cells (33, 34). This scenario provides a satisfactory explanation for why a TCR isolated from CD8+ T cells ends up exclusively on CD8+ medullary cells (35). Thus, by this mechanism, the matching of function and specificity is achieved. Here, it must be acknowledged that some CD4+ T cells that have an important role in tumor immunity can actually differentiate into killer cytotoxic T lymphocytes (CTL), i.e., there is some overlap in function of differentiated CD4 and CD8 T cells (36).

Generation of Regulatory T Cells

Some CD4+ T cells can differentiate in the thymus into forkhead box P3 (FoxP3)-expressing regulatory T cells (Treg). This differentiation is guided by relatively high-affinity ligands for the TCR that can be expressed by thymic stromal cells (37). Such ligands also can lead to the deletion of developing T cells, and it has been suggested that ligands with slightly less affinity than TCR ligands that are responsible for negative selection induce Tregs. Thus, the differentiation of Tregs is not a hard-wired program (38) but depends on the presentation of TCR ligands that induce these cells (37). The close relationship of ligands inducing negative selection and differentiation of Tregs explains the observation that negative selection is often accompanied by the generation of Tregs (37).

Thymocyte Malignancy

Over the years, it has become well known that the transmembrane receptor Notch is crucially involved in proliferation and maturation of immature T cells in the thymus (39). The Notch 1 receptor is expressed on thymocytes and Notch 1 ligands on the thymic epithelium (13). One consequence of the Notch 1 receptor binding to the Notch 1 ligand is the generation of intracellular Notch 1 (ICN1; ref. 12), which has an essential role in thymocyte proliferation. In a murine model of T-acute lymphoblastic leukemia (T-ALL), overexpression of ICN1 by retroviral vector–mediated transfer into hematopoietic cells is sufficient to cause leukemia in the absence of any additional genetic alteration (40). Even though about 50% of human T-ALLs have been reported to exhibit ICN1 overexpression (41), additional genetic alterations are required for the human disease to develop (42). It is
at present unclear whether or not the human disease is different or the additional genetic alterations (42) are simply required as compensation for the lower ICN1 expression in human T-ALL versus the murine model of human T-ALL. It is clear, however, that both human and murine diseases crucially depend on cyclin D (43) for both initiation and maintenance of tumor cells, as the ablation of cyclin D or inhibition of the cyclin D complex (cyclin D associates with cyclin-dependent kinases) leads to regression of tumors or inability to induce them by ICN1 overexpression (43).

It is well established that about 20% of human T-ALLs are resistant to chemotherapy. It is worthwhile to consider whether in such cases inhibition of cyclins (43) by specific inhibitors would have a better chance of managing the disease.

Malignancy can also arise as a consequence of shutting off precursors from the bone marrow that enter the thymus (44, 45). In this scenario, thymopoiesis continues in the absence of new precursors from the bone marrow (44, 45). This can result in tumor formation where the tumor cells again exhibit ICN1 mutations (46), and it has been argued that this represents the mechanism of tumor formation in clinical trials of gene therapy in which the immigration of new T-cell precursors from the bone marrow was also compromised (47), whereas the tumor formation was claimed to be a consequence of the aberrant integration of the vector used for gene therapy by the authors of this study (47, 48).

Summary and Future Directions

The generation of T cells in the thymus is one of the most comprehensively studied events in mammalian development. Uncommitted hematopoietic precursors enter the thymus, proliferate extensively, and eventually commit to the T-cell lineage. The proliferation and differentiation are guided initially by the Notch receptor, later by the pre-TCR and Notch receptor, and finally by the TCR. The pre-TCR ensures allelic exclusion of the TCRβ locus, and the mandatory proliferation following pre-TCR and Notch signaling ensures the generation of a diverse TCR repertoire due to late TCRβ rearrangement and combination of diverse TCRβ chains with amplified TCRβ chains. At the CD4⁺ CD8⁺ DP stage, selection according to TCR specificity sets in; useful cells able to recognize peptides presented by the MHC molecules of the organism are positively selected, whereas useless cells with inappropriate receptors or no receptors die, and the potentially dangerous cells with autospecific TCRs are actively deleted. In addition, function and specificity are matched, such that killer cells recognize...
peptides produced in cells, whereas helper cells recognize peptides ingested by cells. TCR ligands with moderate to high affinity initiate the differentiation of Tregs that modulate immune responses in the periphery. Because the differentiation of T cells in the thymus is accompanied by extensive proliferation, it is not surprising that leukemias originate in the thymus that are often characterized by overexpression of intracellular Notch and are often dependent on D cyclins. Here, it is worthwhile to consider whether conventional chemotherapies of such leukemias can be replaced by inhibitors of cyclin-dependent kinase complexes.

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References


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