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Review

Regulation of pre-T-cell development

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Abstract. One important pillar of cellular immune defense in mammals is the T-lymphoid compartment which produces cells that are able to specifically recognize foreign peptide antigens through a membrane-bound receptor. These T-cells can trigger a variety of defense mechanisms upon antigen stimulation ranging from the production of potent cytokines to the direct killing of virus-infected cells. The production of such highly specialized T-cells takes place in the thymus and requires a stringent process of differentiation and selection of precursor cells that are delivered from the bone marrow. In the thymus, several waves of proliferative expansion and selection ensure the production of a large repertoire of antigen-specific T-cells that each bear a unique T-cell receptor (TCR) which is able to recognize foreign antigens but can tolerate the own host-specific peptide structures. Education of precursors to mature T-cells in the thymus requires a dense network of regulatory processes acting at receptor-ligand interactions, signal transduction, genomic rearrangement of TCR gene loci, cell cycle progression, transcriptional control and programmed cell death.

Key words. T-cell development; thymus; β-selection; pre-T-cell receptor; positive/negative selection; lineage decision; CD4; CD8.

Introduction

The development of T-lymphocytes takes place in the thymus but begins with an undifferentiated and uncommitted hematopoietic precursor cell that originates in the bone marrow. These precursor cells migrate into the thymus at a rate of about 50–100 per day [1] where they encounter a three-dimensional structure built by thymic epithelial cells, macrophages and dendritic cells which allows and at the same time controls their maturation [1]. These early T-cell precursors undergo a number of differentiation steps that can each be defined and characterized by the presence of specific surface marker proteins. Similar to antibody-producing B-cells, T-lymphocytes rearrange part of their genome to generate a large repertoire of antigen-specific, heterodimeric T-cell-receptor (TCR) molecules [2, 3]. The presence or absence of the two membrane-bound molecules, CD4 and CD8, which function as costimulatory molecules associated with the mature TCRs is used to distinguish immature pre-T-cells from more mature stages (reviewed in [4]).

The different steps of TCR gene rearrangement are tightly controlled by and conversely drive the process of maturation and selection of pre-T-cells. In contrast to B-cells which produce secretable antibodies that can recognize soluble antigen, the TCR-bearing T-lymphocytes can only respond to processed antigens presented as short peptides by molecules of the major histocompatibility complex (MHC), on antigen-presenting cells [5, 6]. Thus, only those T-cells which are able to recognize MHC molecules will be an efficient part of the adaptive immune system. It is absolutely crucial that

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T-cells be able to distinguish between self and foreign peptide antigen/MHC complexes. Thus, the interaction between TCR and a peptide-loaded MHC must be regulated in such a way that self peptides are tolerated and do not trigger a T-cell activation cascade, whereas foreign peptide/MHC complexes must initiate the activation of a particular T-cell and a subsequent immune response. During pre T-cell development two processes, called positive and negative selection, assure the elimination of self-reactive T-cells and the propagation of self-tolerant T-cells. A large number of pre-T-cells die by apoptosis during positive and negative selection, but the surviving selected cells differentiate to mature T-cells which are ready to migrate into spleen and lymph nodes to exert their protective role at the multiple entry sites of foreign antigens into the organism [7–10].

In this review we will focus on the developmental staged that early T-cell precursors must pass through and on the two critical selection steps that take place in the thymus, namely β-selection and positive/negative selection.

The thymus and its architecture

The thymus is a flat organ which in all mammals consists of two lobes that are situated above the heart and its large vessels. The thymic lobes are built up by smaller lobules that are each separated by strands of connective tissue called trabeculae (fig. 1). Within the lobules, the lymphoid cells of the thymus (the thymocytes) are divided into two compartments: the outer more densely packed cortex and the inner compartment

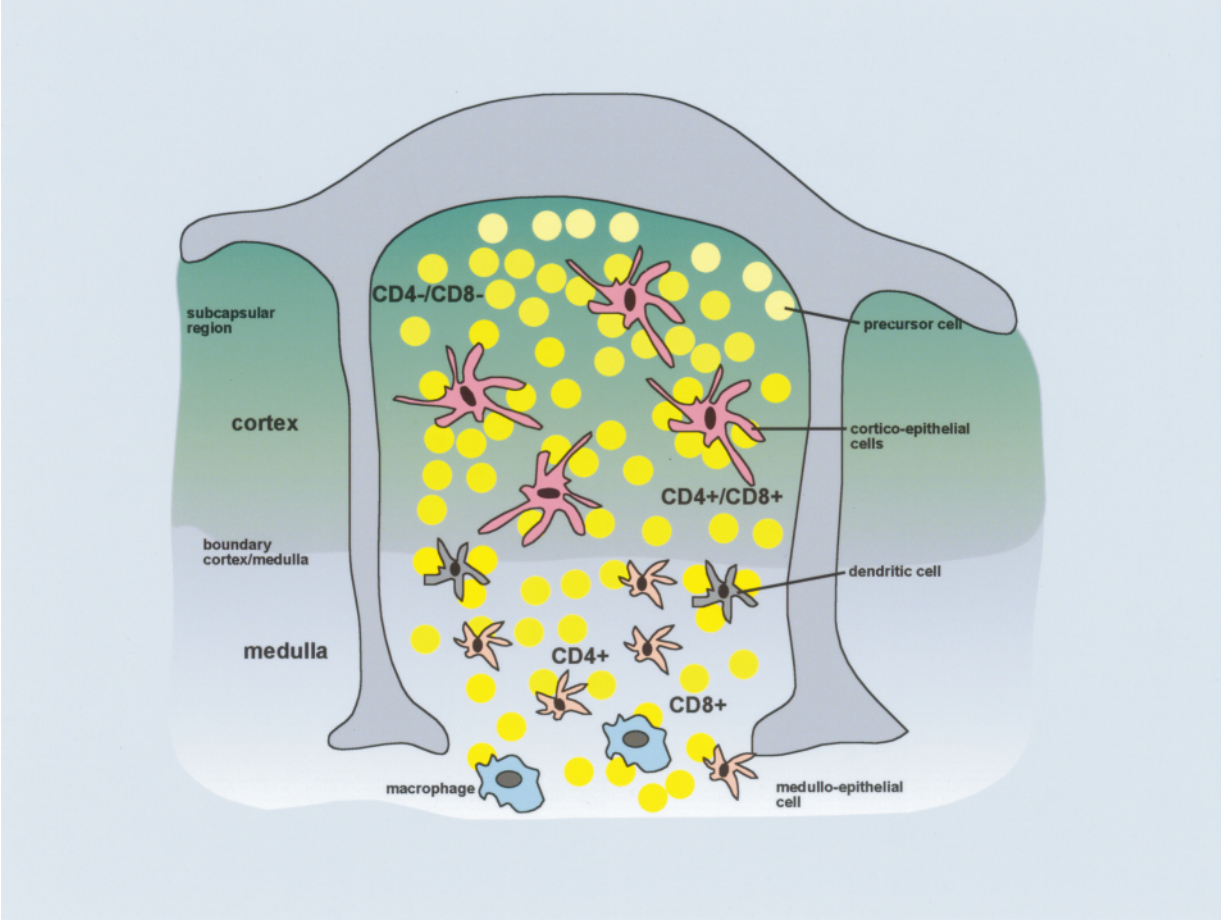

Figure 1. Schematic view of a thymic lobule separated by two trabeculae. The cortical region (dark green) bears the early precursor T-cells which migrate towards the medulla as they differentiate. Cells that survived positive/negative selection enter the medulla and leave the thymus. Precursor T-cells (yellow) encounter a thymic stromal network of nonlymphoid cells as, for instance, the epithelial cells of the cortex and the medulla or dendritic cells. Macrophages are found throughout the thymus and remove apoptotic cells by phagocytosis.

called the medulla (review in [11]). Precursor cells from the bone marrow enter the thymus in the subcapsular region of the cortex situated at the outer area of the lobe (fig. 1). They migrate towards the boundary between cortex and medulla and then into the medulla as they differentiate into more mature cell types. It is generally held that the thymus progenitors proliferate in the cortex and only a few selected cells enter the medulla where they mature and are prepared to leave the thymus towards the peripheral lymphoid organs [12]. A first classification of immature and mature thymocytes has been made according to the expression of the cell surface markers CD4 and CD8. These molecules are coreceptors of the T-cell receptor and allow the distinction of mature T-cells into helper ($CD4^+$) or cytotoxic ($CD8^+$) T-cells (fig. 1) [12, 13].

Progenitor T-cells that migrate into the thymus from the bone marrow do not express readily detectable levels of CD4 or CD8, with the exception of very early uncommitted precursors that have low levels of CD4 which are rapidly downregulated as the cells differentiate [14–16]. Therefore, this population of cells is called the double-negative subpopulation or 'DN cells'. Cells of the DN subset proliferate in the subcapsular region of the cortex. Once these cells have productively rearranged their T-cell receptor genes and express functional TCR α and β chains, expression of both CD4 and CD8 coreceptors is upregulated. As these cells that account for 70–80% of all thymic lymphocytes move deeper into the cortex, they become smaller, cease to proliferate and are subjected to positive and negative selection. At this stage, most of the thymic lymphoid cells ($>95\%$) die by apoptosis and are removed by macrophages; only the very few MCH-restricted and self-tolerant T-cells shut down their CD4 or CD8 expression and become single-positive (SP) T-cells that leave the thymus to populate the secondary lymphoid organs [12].

The lobular spaces of the thymus are not only filled by lymphoid cells but are structured by other nonlymphoid cells which generate the thymic stroma and the entire framework of the organ [11]. This framework is mainly built up by epithelial cells, dendritic cells and macrophages, which all contribute to the maturation and development of pre-T-cells. The importance of this cellular network for the maturation of T-cells and the correct development of the whole organ is illustrated by the nude mouse mutant (*nu*/*nu*) where thymic epithelial cells do not develop and where the entire thymus and functional T-Cells are missing. In this particular mutant, a transcription factor of the winged-helix family, WHN, is absent. Gene-targeting studies have shown that WHN is not required for the formation of the thymic epithelia primordium before any lymphocytes enter the organ but that it is critical for the subsequent differentiation of precursor cells into subcapsular, cortical and medullary epithelial cells of the postnatal thymus [17]. Hence, thymic lymphoid cells need the thymic stroma and the interaction with its components to receive the appropriate signals to develop and mature (fig. 1). The precise receptor ligand interactions of thymic stroma cells and the lymphoid cells are not well understood, but chemokines as well as members of the selectin group of molecules have been proposed to be involved in this non-TCR-mediated selection process.

The DN population: from T-cell progenitors to β-selection

The role of cytokines

The early phases of T-cell development before the appearance of a full TCR are marked by three major events: T-lineage commitment, progenitor T-cell proliferation and pre-TCR selection (fig. 2). These early phases of progenitor T-cell differentiation are subdivided according to the expression of the markers CD44 (phagocyte glycoprotein), CD25 [interleukin (IL)-2 receptor α chain], c-Kit (receptor tyrosine kinase for steel factor) and CD4 [18] (fig. 2). Cells that express CD44, c-Kit and low levels of CD4 are not yet committed and can differentiate into a number of lineages, among them also B-cells, thymic dendritic cells and natural killer (NK) cells [19]. Loss of CD4 expression and upregulation of CD25 marks the first commitment step and defines a population that proliferates in a first wave of pre-T-cell expansion (fig. 2). These cells have now lost their capacity to develop into B cells but can still form thymic dendritic cells [20] and with very low efficiency to natural killer (NK) cells [21]. When these cells shut down their CD44 expression, they enter G1/G0 and start to rearrange their TCR β, γ and δ chains [22, 23]. This population of cells where the rearrangement of the TCR β-chain genes takes place has been termed $CD25^-/CD44^{-,lo}$ DN and has attracted considerable attention as it is subject to the first crucial selection process in T-cell development. $CD25^-/CD44^{-,lo}$ DN cells which directly emerge from this pool express functional TCR β chains and are termed 'β-selected' [24–26]. They quickly upregulate the surface markers CD4 and CD8 to form the major T-cell population of the thymus (see above and [27–29]) (fig. 2).

This early phase of pre-T-cell development from the uncommitted cell to the β-selected T-cell can be divided in a V(D)J recombination and pre-TCR signaling independent part and subsequent events that are triggered by the pre-TCR. Before rearrangement of the TCR β locus, thymic precursor differentiation is regulated by cytokines that are produced by the thymic stroma cells [30–32]. Among these cytokines, IL-7 and c-Kit ligand

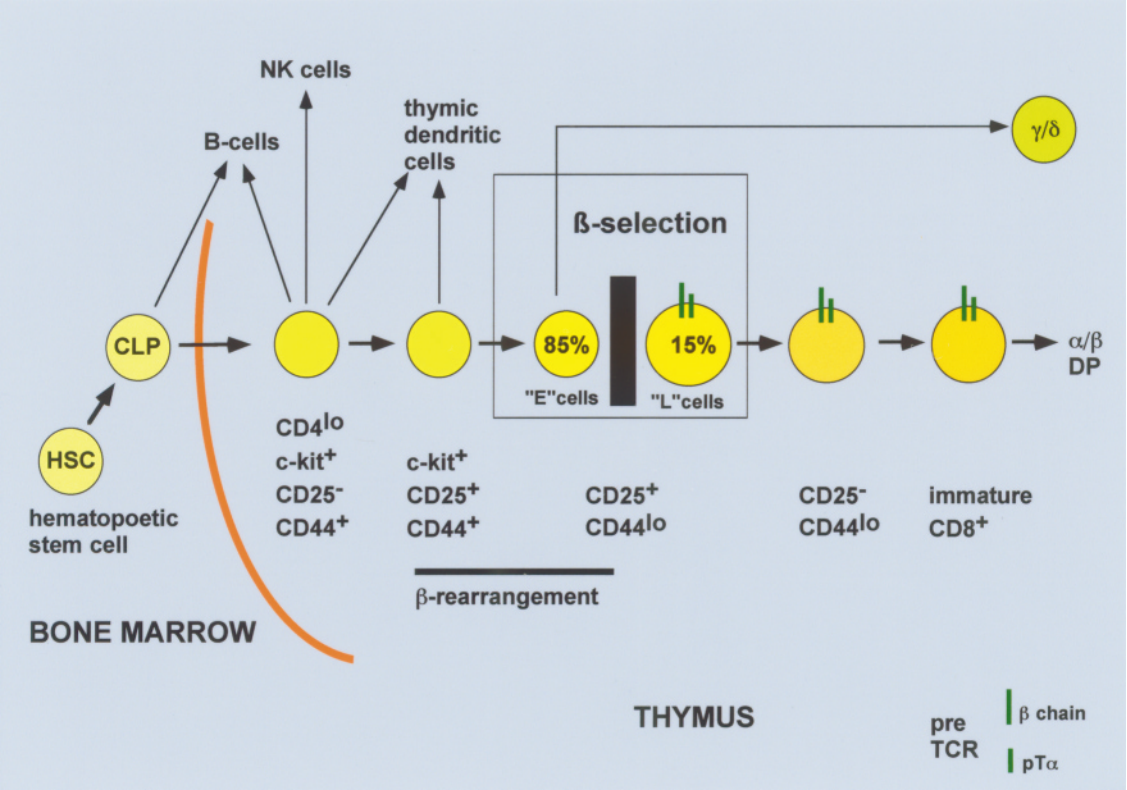

Figure 2. Differentiation of $CD4^-CD8^-$ (DN) thymocytes until the appearance of low levels of CD8. Hematopoietic stem cells (HSC) in the bone marrow develop into common lymphoid precursors cells (CLP) that migrate into the thymus where they undergo a number of differentiation steps. These steps are defined by the presence or absence of specific surface markers, for example CD25 or c-Kit, the receptor for kit ligand. V(D)J recombination and β-selection take place in a particular subset of this DN thymocyte population called E cells. This first critical selection step in the thymus ensures proliferative expansion of pre-T-cells with a functional TCR β chain. In addition, the early DN precursors are a source of γ/δ cells as described in the text. All developmental steps of the DN population take place in the subcapsular region of the cortex.

(steel factor) have attracted the highest attention. For instance, IL-7R-deficient mice show reduced thymic cellularity and a severly disturbed pre-T-cell developmental program characterized, for instance, by the lack of an appreciable number of DP cells and a drop in the number of peripheral T-cells, which underscores the importance of this particular cytokine [31, 32].

IL-7 mediates its effects by binding to the high-affinity IL-7 receptor, which is a dimer composed of the IL-7R α chain and the constant γ chain (γc). The constant γ chain is not only an integral part of the IL-7 receptor [33, 34] but also forms the receptors for IL-2, IL-4, IL-9 and IL-15 [35–38]. The constant γ chain is expressed on all thymocytes, but the IL-7R α chain has so far only been detected on DN cells and in TCRs expressing SP but only in a minority of DP cells that contain cells with functional and selectable TCRs [39]. Besides its role in the early phase of DN cell expansion the interaction between IL-7 and IL-7R is also important for the process of positive selection of DP cells that are about to mature into TCR-expressing SP cells [40]. IL-7R signals are transduced by Lck and Fyn, by phosphoinositide 3 (PI3) kinase but also by the Janus kinases Jak1 and Jak3, which trigger the phosphorylation of STAT5, a transcription factor that dimerizes upon phosphorylation and relocates to the nucleus where it initiates transcription of appropriate target genes [41]. The signaling through Lck and Fyn, as well as PI3 kinase signals, has widespread effects that are well described. They elicit the activation and synthesis of a large number of kinases and nuclear transcription factors which are also involved in signaling through pre-TCRs and mature TCRs (see below) [41–44].

What distinguishes signaling through IL-7R from other pathways mentioned above is its ability to activate the transcriptional transregulator STAT5. Consequently, STAT5 target genes have a critical role in this early phase of pre-T-cell development. Among the genes that

are regulated by STAT5, one has recently gained particular attention. The gene encoding the serine threonine kinase Pim-1, known as a protooncogene from previous experiments [45], can be transcriptionally activated by STAT5 [46]. So far the physiological consequences of this remain unsettled, although a role in cell survival and cell proliferation has been discussed [47–50]. Now, recent studies with Eμ pim-1 transgenic mice point to a role for Pim-1 in the proliferative expansion of $CD25^+CD44^{-,lo}$ DN cells [51]; moreover, experiments with combinatorial mutant mice that are IL-7R-deficient or lack expression of the constant γ chain but at the same time express high levels of the Eμ pim-1 transgene show almost a full rescue of thymic cellularity when compared with the single IL-7R-deficient animals [52]. This suggests that Pim-1 kinase can act as a downstream effector of IL-7R signaling and that its activity is responsible through a thus far unknown mechanism for the expansion of the DN thymocyte compartment.

It is still debatable whether IL-7R signaling triggers cell survival or cell proliferation. But the crossing of transgenic mice that express Bcl-2 in pre-T-cells into IL-7R-deficient animals increased total thymocyte numbers significantly and partially reinstalled DN cell numbers and the relative proportions of the DP and SP compartments [53, 54]. This argues for a role of IL-7R in cell survival. However, the rescue by Bcl-2 is only partial, leaving room for other roles of IL-7. Indeed, the ability of Bcl-2 to rescue the IL-7R-deficient phenotype strictly depends on an intact pre-TCR [54], i.e. signaling from the pre-TCR is required for the expansion of early thymocytes and may cooperate with signals from IL-7R. In addition to its role in the expansion of already committed pre-T-cells, expression of the IL-7R α chain in early lymphoid progenitors points to additional tasks for this receptor in cells before the commitment stage that still remain to be discovered.

The other cytokine important for pre-T-cell development is the c-Kit ligand or SCF (for stem cell factor) and its receptor c-Kit. Like IL-7, SCF is made by thymic stroma cells but also by progenitor cells in the bone marrow [55, 56]. $CD25^-CD44^+$ and $CD25^+CD44^+$ DN cells express c-Kit and are also responsive to its ligand, SCF [18, 55, 56]. The interaction of both ligand and receptor and the ensuing signaling is of critical importance for the expansion of early thymocytes before the rise of the pre-TCR in a way that is similar to IL-7/IL-7R interaction [57], although a role of c-Kit-initiated signals in DN cell proliferation has been less clear than for IL-7R. In addition, the consequences of a lack of c-Kit are not as drastic compared with the loss of IL-7R, suggesting the existence of compensatory mechanisms later in thymocyte development that make up for c-Kit deficiency. Interestingly, however, the combination of IL-7R deficiency and the loss of c-Kit has devastating consequences and leaves thymocyte development completely blocked at the DN stage, indicating a synergistic action between IL-7 and SCF [58]. In contrast to IL-7R, c-Kit does not trigger the activation of STAT factors, but as a receptor tyrosine kinase signals through the well-established Ras/Raf/Mapk pathway [41]. Other cytokines such as tumor necrosis factor (TNF) and IL-1 also play a role in early thymocyte development, but their effects are less well documented. Treatment of fetal thymic organ cultures with antibiotics directed against IL-1 α and TNF α showed an arrest of $CD25^+/CD44^{-,lo}$ DN cells. However, both antibodies had to be present, or no effect was detected, indicating that both cytokines have overlapping roles in pre-T-cell development [59].

T-lineage commitment and V(D)J recombination

Among the DN population, $CD25^+CD44^-$ cells begin to rearrange their TCR β, γ and δ genes by a process called V(D)J recombination (reviewed in [60, 61]) at the same time, whereas the rearrangement of the TCR α chain occurs at later stages of pre-T-cell development (see below). V(D)J recombination is a process that depends on two recombination-activating enzymes, Rag-1 and Rag-2, and is initiated at the borders of D and J gene segments within the TCR loci. The joining of D to J elements is followed by the joining of a V (variable) gene segment to the newly formed DJ element. The joining of VD and J segments is not exact. Consequently, V(D)J joining creates diversity of the expressed variable regions of TCR chains, but also causes nonfunctional or nonproductive rearranged gene segments that will not be expressed. This built-in possibility of nonproductive rearrangements makes β-selection absolutely necessary to ensure that only those cells are expanded that bear a functional TCR β chain (see reviews in [60, 61]).

Once such a productive β-gene rearrangement has taken place, the TCR β chain associates on the cell surface together with a molecule called pTα and the CD3 ε, γ, δ and ζ and chains (ffig. 2) [13, 22–25, 62–70]. Although classical costimulatory receptors as CD4 and CD8 are still lacking at this stage, the pre-TCR complex is capable of initiating signal transduction through the CD3 complex into the nucleus and of triggering clonal expansion and proliferation (reviewed in [44], see also [71]). Those cells at the $CD25^+CD44^{-,lo}$ stage that were unable to productively rearrange their TCR β-chain genes either become γ/δ cells or die, i.e. only those cells with a TCR β chain are selected to survive and proliferate and hence are expanded at this stage. This pre-TCR selection process itself is restricted to the $CD25^+/CD44^{-,lo}$ DN cells and appears to be governed by the assembly of the pre-TCR

and its signaling capacity [12, 13, 24, 25, 44]. The signals delivered from the pre-TCR trigger a burst of proliferation and the formation of blastoid-like larger cells (fig. 3). Therefore, $CD25^+CD44^{-,lo}$ DN cells have been divided into two subpopulations: one that contains these blastoid, large, cycling cells (about 15%, termed, 'L' cells) with a high proportion of in-frame β-rearrangements and a second subset that represents the major preselected population. These cells are termed 'E' cells for 'expected' size and are smaller compared with the L cells. They are resting cells without an enrichment of in-frame β-rearrangements and represent about 85% of the whole $CD25^+/CD44^{-,lo}$ DN population [26].

The pre-TCR-triggered expansion of selected cells is driven by the regulation of a few regulators of cell cycle progression. L cells contain higher levels of cyclin A and B and higher CDK2 activity than E cells, but most dramatic is the complete absence of the G1-specfic inhibitor $p27^{KIP1}$ [26, 51] (fig. 3). This inhibitor can negatively regulate all G1 cyclin-dependent kinase (CDK) complexes [72, 73] and is normally expressed at very high levels in almost all thymocyte subsets, including E cells [26, 51]. These findings clearly establish regulation of cell cycle progression at a G1 checkpoint as a significant step in the pre-TCR selection process.

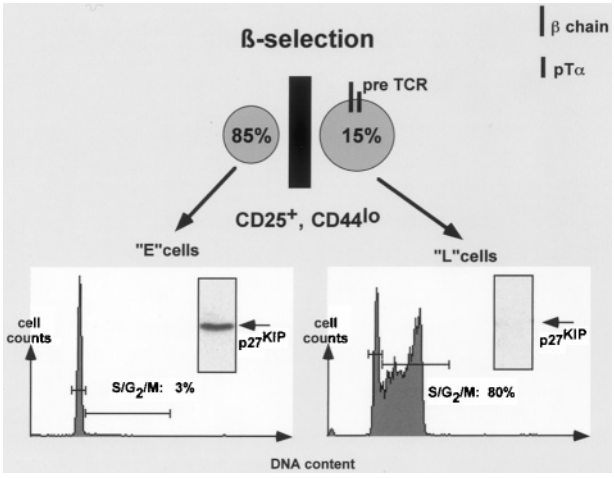

Figure 3. Proliferative burst during β-selection. $CD25^+CD44^{-,lo}$ DN cells that have assembled a functional pre-TCR immediately relay signals into the cell that cause, among other effects, the immediate downregulation of the G1-specific inhibitor $p27^{KIP1}$ (see inserted Western blot). The absence of this inhibitor promotes cell cycle progression from G1/G0 into S phase. At this time about 80% of β-selected $CD25^+CD44^{-,lo}$ DN cells are actively cycling, i.e. are in S, G2 or M phase. This is demonstrated here by a FACS analysis of sorted E and L cells stained with the DNA dye propidium iodide (for details, see text).

Accordingly, $p27^{KIP1}$-deficient mice have drastically increased thymic cellularity [74–76] and also show disturbances at the pre-TCR selection steps [H. Karsunky and T. Möröy, unpublished]. The expression of $p27^{KIP1}$ is regulated at a posttranscriptional level in particular by degrading free $p27^{KIP1}$ that is unbound to cyclin/CDK complexes [77].

Signaling through the pre-TCR is very similar to the processes triggered by the mature TCR [78, 79], and the absence of any component of the pre-TCR affects the differentiation of E cells to L cells within the $CD25^+CD44^{-,lo}$, DN population [e.g. 80–83]. The most dramatic example is given by Rag-1- or Rag-2-deficient animals, which are unable to perform V(D)J rearrangements of their TCR loci and accordingly have virtually no L cells 80, 81, 82, 83]. Thus, they lack all other subsequent pre-T-cell stages and have a dramatically reduced thymic cellularity at or below 1% of wild-type cell numbers. Introduction of a rearranged TCR β chain through a transgene can rescue this phenotype and restore the number of DP cells, but it fails to produce SP cells [84]. The importance of the cis-acting TCR-β-enhancer element ($E\beta$) has been nicely demonstrated in animals that lack this control element. These animals cannot rearrange the TCR β locus and completely lack TCR-β-chain expression and have a drastically reduced thymic cellularity to levels at about 20% of wild-type cell numbers [85, 86].

Pre-TCR signaling

Signaling through the pre-TCR is in many aspects similar to the events triggered by the mature TCR (reviewed in [41–44, 79]) (fig. 4). The critical first component after the assembly of the pre-TCR is the activation of the src kinases Lck and Fyn, where Lck seems more important than Fyn as the effect of Lck deficiency in mice is clearly more dramatic at the pre-TCR-selection step than the loss of Fyn expression. In addition, TCR-β deficiency can be rescued by a transgene expressing a constitutive Lck kinase [87–91]. One of the targets of Lck is the molecule ZAP-70, which is associated with the CD3 ζ chain and belongs to the tyrosine kinase family [92–94]. ZAP-70, very much like Lck, is absolutely required for pre-TCR selection and for the maturation of L cells into more mature stages as demonstrated by gene-targeting studies [95, 96]. Through another adapter molecule, called SLP-76, ZAP-70 mediates the further transduction of signals either through the Ras/Raf/Mapk (MAPKIERK) pathway by association of Grb2/Sos with SLP-76 or by recruiting the guanine nucleotide exchange factor Vav to SLP-76. Vav can in turn activate Rac, which functions through the Jun Kinase (JNK/SAPK) pathway and the activation of p38 kinase [97, 98]. Both pathways

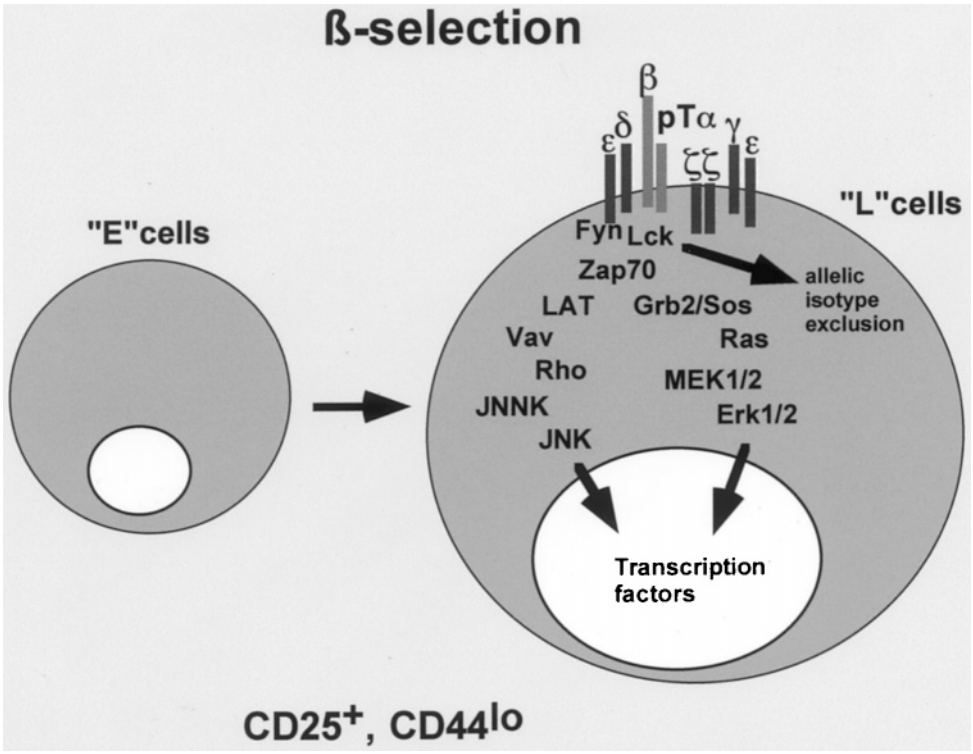

Figure 4. Signaling events triggered by the pre-TCR during β-selection. The pre-TCR consists of a functional TCR β chain, the membrane-bound pTα and CD3 chains ε, δ and ζ. Proliferative expansion of β-selected L cells is mediated by signals from the pre-TCR transduced through either the JNK or the MAPK/ERK pathway or both (grey arrows pointing to the nucleus). The tyrosine kinases Lck and Fyn are responsible for coupling the activated pre-TCR to these signal-transduction pathways. However, allelic and isotype exclusion is not mediated by either one of these pathways. To ensure allelic exclusion and isotype exclusion, Lck has to activate yet another unknown pathway.

lead to the activation of a number of transcription factors that are well established in cellular proliferation (fig. 4).

A number of studies have attempted to establish one or another pathway firmly in pre-TCR selection by gene targeting or transgenesis. Whereas mutations in the SAPK pathway had either no effect on this early stage of pre-T-cell development or produced different results that await to be reconciled [99–101], more progress has been made on the MAPK/ERK pathway, although some experimental results are contradictory or at least did not confirm what was expected. One of the first reports that the MAPK/ERK pathway is of critical importance in the DN-to-DP transition came from a technically very demanding study using retrovirally infected fetal thymic organ cultures (FTOCs) [102]. The experiments showed that the introduction of a constitutively activated Mek-1 kinase promoted the development of DP cells from DN precursors, whereas a dominan-negative form of Mek-1 blocked this transition [102]. However, reports from another group that had expressed dominant-negative forms of Mek-1 did not demonstrate an effect on the generation of DP cells [103]. Nevertheless, other experiments have strongly supported the role of the MAPK pathway in pre-TCR selection. The introduction of a constitutively activated Ha-ras gene into Rag-1-deficient mice, for instance, showed substantial rescue of DP cell production [104–106]. Similar results have been obtained by yet another group that used a constitutively active form of Raf which is an immediate effector of Ha-Ras [107]. Again, rescue of thymic cellularity in V(D)J-recombination-deficient mice was observed. In addition, most recent experiments using a novel technique to introduce genetic material directly into thymic lobes have shown that Erk 1/2 is phosphorylated and activated by signals delivered from the pre-TCR, extending and confirming all previous observations [108, 109]. These experiments establish a role for the most downstream kinases of this pathway, namely Erkl and Erk2, in pre-TCR-mediated signaling. Taken together, all these experiments leave little doubt that the MAPK/ERK pathway is at least

one of the important mechanisms for the transduction of signals from the pre-TCR that ensures the proliferation and expansion of selected β-selected pre-T-cells.

Transcription factors in pre-T-cell selection

Both the MAPK and the JNK/SAPK pathways end in the nucleus, where a number of transcription factors are activated and begin to regulate the expression of target genes. In contrast to the antigenic activation of peripheral, mature T-cells, the role of downstream effectors of the pre-TCP-initiated pathways is much less clear. It is well established that activation of Erk 1/2 and JNK leads to the formation of the AP-1 transcription factor, which is a heterodimer of Jun and Fos, but also activates other transcription factors such as Atf-2 and Elk-1. Whereas all of these should be prime candidates to transduce Ras/Raf/Mek/Erk signals to the nucleus, gene-targeting experiments have failed to demonstrate unequivocally essential roles for these factors in pre T-cell development. It has been recognized that AP-1 expression is high in DN cells and is downregulated as cells progress to the DP stage [110], but clear evidence that AP-1 or either one of its components is important in pre-TCR selection has been scarce. Transgenic mice expressing a dominant-negative for c-Jun exhibited decreased thymic cellularity due to a proliferative arrest of $CD25^-CD44^-$ DN cells [111]. Earlier experiments with transgenic mice overexpressing the c-fos-related gene FosB demonstrated a role for Fos family members in pre-T-cell development [112]. Both results suggested that AP-1 or related factors really function as downstream effectors of pre-TCR signals and can be essential for DN-to-DP transition. Still, more experiments will show to what extent AP-1 transcription factors govern β-selection and associated processes.

A very large number of other transcription factors have been shown to play a role in pre-TCR selection, and the number of these factors is still growing. The early response gene family member Egr-1, which is upregulated in β-selected cells, is one candidate for an efficient downstream effector of the pre-TCR [113]. In combinatorial mutant mice that lack a pre-TCR but overexpress Egr-1, thymocytes developed past the β-selection stage into $CD25^-CD44^-$ and intermediary single CD8-positive (ISP) cells but not further [113]. Other factors that are important in this early phase of pre-T-cell development are members of the high-mobility group (HMG) box transcription factors T-cell factor 1 (Tcf-1) [114] and lymphoid enhancer factor 1 (Lef-1). Both factors are closely related, and both are expressed during T-cell development in the mouse (reviewed in [115]). Studies with Tcf-l-deficient animals have demonstrated that this factor can play a role in two distinct steps of early thymocyte differentiation. T-cell maturation in $Tcf^{-/-}$ animals deteriorates overtime and completely stops in 6-month-old mice, whereas fetal T-cell development is barely affected [116]. In 4–6-week-old mice the differentiation of DN thymocytes is blocked at the $CD25^+CD44^+$ and $CD25^-CD44^-$ stages. The latter subset emerges directly from β-selected L cells and still shows high numbers of cycling cells. Both transcription factors overlap in their function, as was nicely illustrated in animals that lack either Lef-1 alone or both Tcf-1 and Lef-1 [116]. The deficiency of Lef-1 did not markedly alter DN thymocyte subsets, and Tcf-1 deficiency alone had the described mild effect in younger mice. However, an absence of both factors arrested almost all DN thymocytes in the $CD25^+CD44^-$ stage, suggesting that the downregulation of CD25 is severely affected by these factors [116].

Another factor that is potentially a regulator of pre-TCR-associated processes and may function either as a downstream effector or an upstream regulator of the pre-TCR signaling pathway is the zinc-finger protein Gfi-1. The gene for this potential transcriptional repressor was discovered in retroviral insertional mutagenesis screens performed in cultured cells and transgenic mice [117–122]. In the thymus, Gfi-1 is readily detected in DN and DP cell subsets but in peripheral T-cells only after antigenic stimulation [51, 117]. The analysis of transgenic mice that constitutively express high levels of Gfi-1 in thymocytes showed lower numbers of L cells within the $CD25^+/CD44^{-,lo}$ DN subpopulation [51]. Interestingly, the cytoplasmic serine/threonine kinase Pim-1 that is able to rescue L-cell development in Rag-deficient mice and in animals that lack IL-7R [52, see above], is also able to restore the defect introduced by the Gfi-1 transgene [51]. In addition, transgenic mice that overexpress Pim-1 in pre-T-cells show higher numbers of L cells and higher numbers of cycling $CD25^+/CD44^{-,lo}$ DN cells [51]. As both Gfi-1 and Pim-1 are expressed in the same pre-T-cell subpopulations critical for pre-TCR selection, it was concluded that the level and ratio of both proteins is important for pre-T-cells to correctly pass the E-to-L transition during β-selection [51].

Of particular interest with regard to effectors of pre-TCR signaling might be proteins of the Ikaros gene family. The protagonist Ikaros transcription factor is a zinc-finger-containing protein that is essential for T-cell commitment and homeostasis in general (reviewed in [123]). More recent findings show that pre-TCR-controlled steps are influenced by Ikaros [124]. It is put forward that Ikaros establishes thresholds at different stages of T-cell differentiation or activation. Reduction of Ikaros expression levels in null mutants or in heterozygous animals that retain one intact allele causes disturbances in the DN subset and an increase of the

DN-to-DP transition rate possibly by lowering the pre-TCR signaling thresholds. Ikaros-deficient mice have lower numbers of $CD25^+/CD44^{-,lo}$ DN cells, but a higher percentage of these are in cell cycle, where cell numbers of the emerging $CD25^-/CD44^{-,lo}$ DN subpopulation appear normal [124]. It is concluded that Ikaros may provide a negative regulatory effect for pre-TCR-mediated differentiation steps within the $CD25^+/CD44^{-,lo}$ DN subset [124]. Exactly how Ikaros activity modulates signals delivered from the pre-TCR is not yet known.

Clonal expansion and allelic exclusion in L cells

It is important to note that that besides TCR gene rearrangement and β-selection a third, very important process takes place in the same $CD25^+/CD44^{-,lo}$ DN cells. The successful expression of a functional TCR β chain blocks V(D)J recombination at the remaining TCR β locus [125, 126]. This process is known as allelic exclusion and ensures the future specificity of mature T-cells that each bear a unique α/β T-cell receptor [125–127]. In addition, once the TCR β chain appears as a protein on the surface of L cells, rearrangement at the TCR γ locus is also suppressed. This process is called isotype exclusion and prevents the emergence of T-cells with mixed isotypes different from α/β or γ/δ T-cells. How precisely the shutdown of V(D)J recombination is achieved by signaling through the pre-TCR in conjunction with Lck is still unclear. The most pressing question that remains is how signals delivered from the pre-TCR can on the one hand trigger clonal expansion and proliferation of β-selected cells and on the other hand restrict V(D)J recombination on remaining alleles, after a successful rearrangement of one of the TCR loci. Until recently, it was generally held that pre-TCR/Lck-mediated expansion and differentiation steps are concurrent with allelic exclusion. The model predicted that pre-TCR signaling elicited a strong proliferation accompanied by downregulation of Rag gene expression at the DN stage. Allelic exclusion would then be explained by the halting of further rearrangements during this stage of expansion and limited accessibility of the TCR β locus [24, 26, 128]. However, this model must necessarily include regulation of accessibility of the TCR β locus in all later stages in order to explain how, after reexpression of Rag genes and rearrangement of the TCR α locus, the silencing of the TCR β rearrangement at the remaining intact alleles is assured.

Most recent experiments may have found a first clue as to how this double task is managed by L cells. Experiments with transgenic mice overexpressing active forms of Ras, Raf or Lck showed different behavior with respect to TCR-β-chain expression and rearrangement of the TCR β locus [106, 107]. While transgenes expressing activated forms of Ras and Raf ensured proliferative expansion of DN cells, neither one affected allelic exclusion. However, allelic exclusion clearly occurred in cells from transgenic mice expressing a rearranged TCR β chain or the constitutively active form of Lck [106, 107]. Apparently, at the critical stage of DN-to-DP transition, i.e. where L cells begin to emerge in the $CD25^+CD44^{-,lo}$ subset, the activity of the Ras/Raf/Mek/Erk cascade only mediates those signals from the pre-TCR/Lck complex that are needed for differentiation and proliferative expansion [106, 107]. Termination of rearrangement is not mediated by this cascade and depends solely on Lck signaling. This suggests a bifurcation of signal transduction at the level of Lck, in which one direction leads to Ras/Raf activation and proliferative expansion of β-selected cells and the other direction to allelic exclusion through other yet unknown pathways that are independent of Ras or Raf [106, 170] (fig. 4).

Where is the ligand for the pre-TCR?

Whereas the components and mechanisms of the signal transduction pathways initiated by the pre-TCR are very similar to those triggered by the mature TCR, there are many differences between the receptor types. One of the most intriguing is the missing identity or even existence of a ligand for the pre-TCR. While it is not excluded that the pre-TCR is stimulated by interaction with thymic stromal cells that may be too weak to be detected experimentally, there is compelling evidence that the pre-TCR does not need a ligand. One was provided by experiments with mice that lack MHC class I or II molecules but still showed differentiation of pre-T-cells to the DP stage, thereby excluding a need for any interaction between the pre-TCR and MHC molecules [129–131]. The second most surprising finding was that coexpression of transgenic pTα or TCR β molecules that lack their immunogloblin-like exodomains led to the development of DP thymocytes in Rag-deficient mice and could even restore thymic cellularity [132]. This suggested that a pre-TCR without exodomains was functionally active and could promote DN-to-DP differentiation. It was concluded that the pre-TCR functions without interaction with a ligand.

The question still remains how the pre-TCR is able to transduce signals. One possibility is that the interaction between the CD3 molecules, pTα and the TCR β chain on the cell surface alone or in concert with so-called glycolipid-enriched microdomain membrane rafts is sufficient to activate downstream effectors such as Lck [133–135]. This model has been extended to the view that if an external ligand is not required and the simple association of all components at the membrane is sufficient, signaling should occur already at the endoplasmatic reticulum (ER), where all the components of the pre-TCR are assembled and come together at the ER

membrane (reviewed in [44]). According to this view, signaling could also take place while the pre-TCR is still in the ER membrane but en route to the cell surface. To solve this question, experiments in which a TCR β chain that was modified at the cytoplasmic tail to be retained in the ER were performed [136]. Such a modified TCR β chain could not rescue a TCR-β-deficient phenotype, i.e. reinstall DN-to-DP differentiation, arguing against the ER signaling hypothesis and for a requirement for the pre-TCR to reach the cell surface. However, as the artificial ER retention sequence was appended to the cytoplasmic tail of the TCR β chain, it could formally not be excluded that this may have impaired its signaling capacity in general. Although the ER signaling model is an attractive hypothesis, more experimental evidence that unequivocally establishes this mechanism has still to be provided.

Notwithstanding the above results and the emerging models of pre-TCR function, it is worth noting that activation of Lck through the pre-TCR is still not substantiated by biochemical evidence. Moreover, while clearly implicated in pre-TCR signaling and the generation of L cells as well as allelic exclusion, Lck is known to generate signals through the mature TCR by binding to the CD4 and CD8 costimulatory molecules, which by definition are not expressed in the DN subpopulation (reviewed in [43]). The questions that arise from this conundrum are, does Lck in pre-TCR signaling need other costimulatory molecules or does it function by direct binding to a component of the pre-TCR? If costimulatory molecules are needed, how can they be identified? It is possible that the recently identified pTα isoform of pTα2 or the membrane protein CD2 may have roles as costimulators, but definitive evidence for this is still lacking [78, 137]. The role of CD2 has gained some attention recently as it can, at least in T-cell lines, cause the activation of Jun kinase (JNK) [138]. In addition, two other surface receptor molecules, CD27 and CD81, have been implicated in pre-TCR costimulation. CD27 is a homodimer of 55 kDa and belongs to TNF-receptor superfamily and reacts with the TNF-like ligand CD70 that is present on thymic stromal cells. The expression pattern of CD27 correlates well with the transition from $CD25^+CD44^{-,lo}$, DN to $CD25^-CD44^{-,lo}$ DN cells [139]. CD81 also called TAPA-1 is a 22-kDa polypeptide and more widely expressed than CD27 [140]. The treatment of fetal thymic organ cultures with antibodies against CD81 blocked DN-to-DP transition. In contrast, targeting of CD81 in mice did not reveal any effect on thymocyte differentiation, indicating redundancy of this regulatory mechanism or the absence of a role of CD81 in pre-T-cell differentiation [141]. Largely, the issue of costimulation in pre-TCR signaling is far from being settled and leaves enough room for further experimentation.

The development of γ/δ cells

The primary developmental pathway as well as a unique precursor cell from which γ/δ cells emerge is still unknown. Similar to α/β cells, γ/δ cells have to pass a selection procedure that ensures that only those cells with productive in-frame TCR δ and γ rearrangements are expanded and allowed to survive and differentiate (reviewed in [78]). Clear evidence, however, for a pre-TCR complex similar to the one responsible for establishing α/β cell expansion has not been forthcoming. In addition, the low numbers and the lower proliferative capacity of most γ/δ cells suggest that the development of these cells is managed in a way that is different from the α/β cells. The E-cell subset of the $CD25^+CD44^-$ population can be seen as a source of γ/δ cells [78]. It has been shown that the rearrangement of β-, γ- and δ-TCR loci takes place in E cells. One model suggests that rearrangement on all loci takes place at the same time. A successful rearrangement at the β locus shuts down all other V(D)J recombination events and triggers expansion and differentiation into the α/β lineage by mechanisms that have been developed above. If a productive rearrangement occurs first at the δ locus, silencing of the TCR β locus will ensue and exclude for these cells a differentiation along the α/β pathway. In contrast to β-selected cells, these cells now undergo 'δ selection' [142], which has a different outcome. In the one most probably yielding appreciable numbers of γ/δ cells, δ selection ensures the progress of selected E cells through one cell cycle with subsequent maturation into an arrested state. The difference between a TCR-β-mediated proliferative burst and δ-selection certainly offers a good explanation of the very low numbers of γ/δ cells normally found in the thymus.

Another more direct argument for such a competitive model is, for instance, experiments with γ/δ transgenic mice, where a strong reduction of α/β cell development is found. Moreover, TCR $E\beta^{-/-}$ mice, which were described above, lack a TCR β chain but are still able to engage in V(D)J recombination processes. In these mice, thymic cellularity is depressed, but a significant number of γ/β cells develop that represent a higher percentage than in mice with an intact TCR β locus. Interestingly, $E\beta^{-/-}$ mice still develop DP cells that either bear no TCR at all or express a γ/δ TCR. This suggests that in the absence of a TCR β chain, γ/δ cells can develop along the α/β lineage pathway [85, 86]. It will be of interest whether in this particular situation the TCR δ or γ chain associates with pTα and/or undergoes more rounds of cell cycling than a γ/δ cell that develops under normal conditions.

This competitive model implies that the TCR γ/δ receptor is a molecule able to transduce signals similar to its counterpart, the pre-TCR. The nature of signals delivered by the TCR γ/δ and the relaying molecules in-

volved are not defined yet, but they may be the same as delivered by the pre-TCR with a quantitative rather than qualitative difference. On the other hand, there is evidence that the signal from the TCR γ/δ is qualitatively different from those delivered by the pre-TCR and does not initiate a proliferative response. The difference in the signaling capacity of TCR γ/δ and the pre-TCR is not easily explained. Both TCR γ/δ and the pre-TCR associate with CD3 molecules and, as already pointed out for the pre-TCR, neither one may need a ligand to be able to transduce signals. Until now, the only accountable difference between the pre-TCR and γ/δ TCR would be the pTα molecule. It has been hypothesized that either the presence of pTα as a part of the pre-TCR or a higher susceptibility of TCR γ/δ to IL-7 [143] constitutes the difference in signaling. In addition, signaling by other molecules, for instance Notch, are also being discussed as players in the α/β, γ/δ lineage decision. It has been shown that Notch can affect lineage determination of early T-cells at the stage of α/β, γ/δ decision but also later in development when cells must choose between CD4 and CD8 concurrent with positive/negative selection [144] (see below). As the Notch ligand is expressed on thymic stromal cells, its linking to the membrane-bound Notch on early T-cells may constitute an additional signal that may shift the decision to differentiate in one or the other lineage in a way that depends on its expression level. As so many other aspects of early T-cell development, this matter awaits further clarification.

The DP population: positive and negative selection

The second important selection process that thymocytes must undergo before they emigrate from the thymus is associated with their ability to distinguish self from foreign antigens. It is clear that without this particular education any immune defense system as specific and as sophisticated as T-cells would be fatal and turn its weaponry against the host organisms that it normally should protect. Whereas pre-TCR selection and β-selection ensure the specificity of the T-cell receptor and the generation of a large repertoire of cells able to interact with any antigen, this second selection procedure, which is called negative selection, will sort out those TCR-bearing cells that recognize self antigen with high avidity by inducing apoptosis. In contrast, other cells that can bind with low or intermediate avidity to peptide antigens presented by an MHC molecule will be positively selected for further expansion and differentiation. Both processes of positive/negative selection take place in the deeper part of the cortex than pre-TCR selection but are terminated at the boundary between the cortex and the medulla (fig. 5).

Cells that have survived pre-TCR selection downregulate CD25 and continue their differentiation pathway by upregulating CD8 (fig. 5). This leads to a recognizable subset ISPs which quickly express both CD4 and CD8 costimulatory receptor molecules [145]. These double-positive cells (DP subset) rearrange the TCR α gene locus in order to express a functional TCR α/β heterodimer. This is an ongoing process during positive/negative selection with the consequence that several different TCR α/β heterodimeric receptors can coexsist on the surface of a particular cell that always bears one specific β-chain isotype but several different TCR α isotypes [146]. The fact that positive/negative selection of these DP cells is mediated by the interaction between TCR and MHC molecules has been verified by several experiments with normal and engineered mutant mice (reviews in [147–149]). For instance, irradiated mice bearing one particular MHC haplotype were injected with bone marrow cells from a donor mouse with another MHC haplotype. After the development of new T-cells, it could be shown that they recognized antigens only when presented by an MHC haplotype from the irradiated host mouse and not by the haplotype of the donor [150–152]. Another experiment used a transgenic mouse that expressed the TCR directed against the male-specific H-Y antigen [153]. Female transgenic mice develop SP cells normally, but male mice failed to produce any $CD8^+$ cells. In these H-Y TCR transgenic mice all T-cells express this particular TCR. As they all encounter the H-Y antigen in male mice, they will all be deleted by negative selection. As this antigen is not present in female mice, cells develop normally. In agreement, mice deficient in either MHC I or MHC II are essentially lacking mature $CD8^+$ or $CD4^+$ T-cells, respectively [131, 154]. From these and numerous other experiments, it was concluded that only T-cells that specifically recognize peptides in conjunction with self MHC molecules are able to differentiate in the thymic cortex. Cells that fail to productively rearrange the TCR α locus do not form a complete TCR and will die because they are not able to interact with MHC/peptide complexes. Similarly, other cells that possess a TCR α/β heterodimer but fail to recognize peptide MHC complexes are also programmed to die by apoptosis, a process that has been called 'death by neglect' (fig. 5).

Expression of CD4 and CD8 during positive selection

The predominant population of the mature thymus is constituted by $CD4^+CD8^+$ thymocytes. Although very homogeneous, they can be subdivided into several subgroups according to variations in cell surface markers such as CD4, CD8 and the TCR [155–157]. There are still some conflicting data in different studies especially about the sequential order of different DP subpopula-

tions during development, but most of the results can be merged into a common model, which is presented below (fig. 6).

The most immature DP thymocytes are large, proliferating blastoid-like cells which become small, nonproliferating DP cells after low-level expression of a TCR α/β complex on their surface. Mice lacking either MHC or TCR α show a developmental block at this stage [158, 159]. Originally, it was believed that these cells were incapable of further differentiation [155], but it became clear that this population represents a crucial step in T-cell development and that early steps in positive selection take place in these cells [160, 161]. These cells can survive for several days, leaving enough time for TCR-α-chain-gene rearrangement to occur. Expression of a functional and appropriate TCR α/β complex allows positive/negative selection, and the small cells enter a population of again enlarged, blastoid DP cells characterized as $CD4^+CD8^+CD69^-TCR^{int}$. Whether this enlargement represents evidence for another round of proliferation has not been investigated. Active signaling from a functional TCR complex is essential for further development and is comparable to pre-TCR signaling during β-selection or activation of mature T-cells (reviewed in detail in [162, 163]). The molecular differences between the prosurvival signal of a positively and the death sign of a negatively selected cell—transduced from the same receptor—is not fully understood. The first clues came from work showing that transgenic expression of a dominant-negative form of MKK-1 selectively inhibits positive selection without influencing negative selection [164]. In agreement are the findings that activation of MKK-1 is sufficient for positive selection, whereas activation of the MKK-6/p38 pathway and/or JNK is critical for negative selection [165, 166].

During development, signaling by the TCR leads to transient downregulation of CD4 and CD8 and upregulation of the TCR and the activation marker CD69, resulting in the $CD4^{lo}CD8^{lo}CD69^+TCR^{int}$ population [167]. It is well established that these cells have undergone positive selection, downregulated Rag-1/2 and therefore shut off V(D)J recombination. In addition,

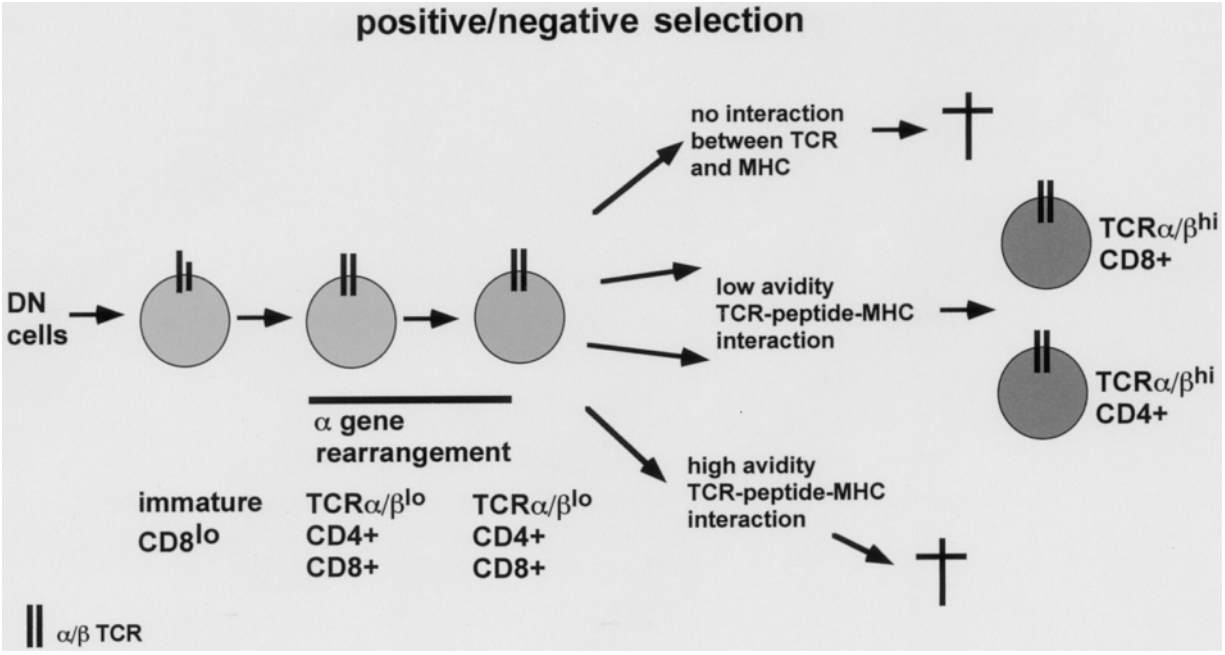

Figure 5. Positive/negative selection of CD4/CD8 DP cells. DN cells that have successfully passed β-selection, have downregulated CD25 and now express both CD4 and CD8 coreceptor molecules undergo positive/negative selection that takes place at the boundary between thymic cortex and medulla. This represents the second critical selection step for thymic lymphocytes after β-selection. Positive/negative selection ensures the maturation and expansion of T-cells that recognize peptide antigens in conjunction with MHC molecules and are able to distinguish foreign from self antigens. This selection step takes place during and after the rearrangement of the TCR-α-chain gene loci and the upregulation of CD4 and CD8, and produces either CD4 or CD8 SP mature T-cells that leave the thymus to colonize peripherial lymphoid organs as spleen and lymph nodes. Positive and negative selection depends on the avidity of the TCR-peptide MHC interaction. A high-avidity binding as well as the complete absence of any interacion of the TCR with MHC-presented peptides leads to cell death (black crosses). Recognition of self-peptide-loaded MHC molecules by the TCR with low to intermediate avidity leads to proliferative expansion and the emergence of positively selected CD4 or CD8 SP mature T-cells.

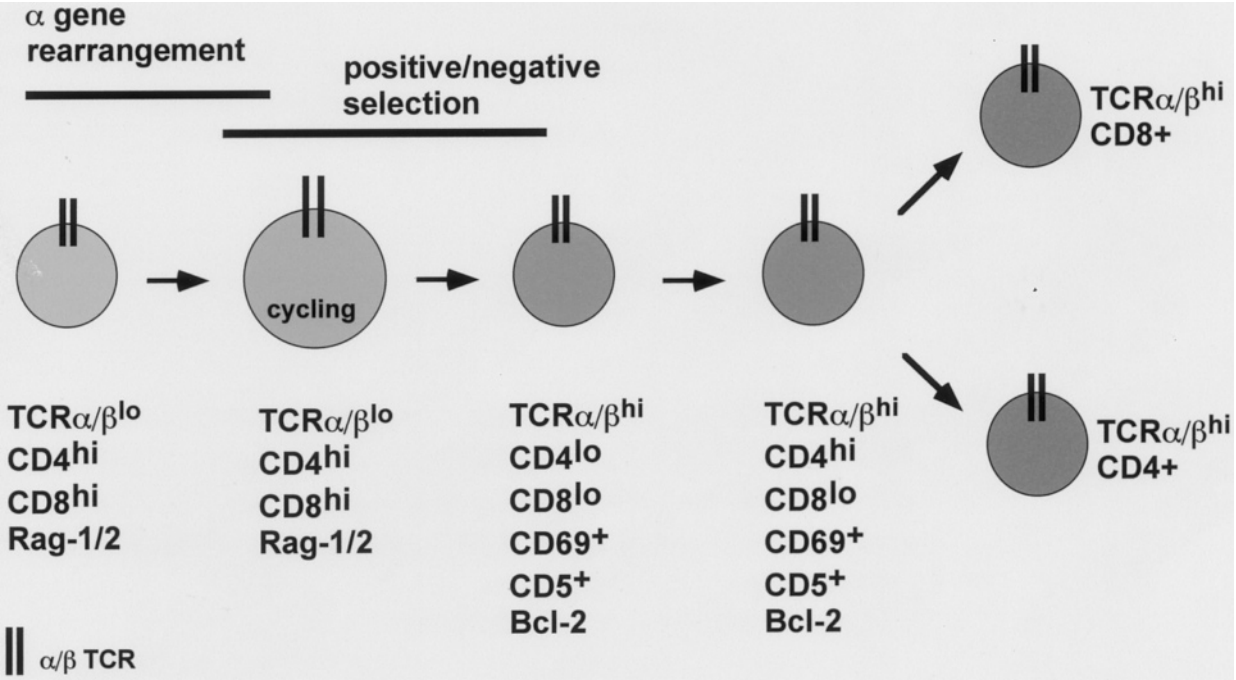

Figure 6. Scheme showing sequential order of thymocyte subpopulations at DP and SP stage. Shown is one possible model for the development sequence of TCR-$\alpha\beta$-bearing thymocytes during positive/negative selection. Single steps are defined by different expression levels of the surface markers TCR $\alpha\beta$, CD4, CD8, CD69 and CD5. Bars on the top delineate general developmental processes and in conjunction with different subpopulations. The most immature populations shown are $DP^{hi}CD69^{-}CD5^{lo}$, expressing Rag-1/2 during ongoing α-gene rearrangement. Upon expression of a functional $\alpha\beta$ TCR, thymocytes stop recombination by downregulating Rag-1/2 and upregulate Bcl-2 if they are positively selected. Phenotypically this step is related to a decreased expression of CD4 and CD8 and an upregulation of TCR $\alpha\beta$ chains as well as CD69 and CD5. It appears that the following thymocyte populations are already committed to be either MHC class I- or II-restricted, leading finally to mature CD4 or CD8 SP T-cells which have downregulated CD69 again and leave the thymus to settle the periphery.

they have upregulated the antiapoptotic protein Bcl-2 [168], whereas DP cells that fail to express an appropriate TCR have no Bcl-2 and are destined to die. In the following maturation step it appears that first CD4 is reexpressed, leading to a small population of $CD4^{+}CD^{lo}CD69^{+}TCR^{int/hi}$ cells [167, 169]. Recent data suggest that these cells are already lineage-committed but are still a mixture of both CD4- and CD8-committed cells. Independent studies confirmed that this population gives rise to either $CD4^{+}$ SP thymocytes or to $CD4^{lo}CD8^{+}CD69^{+}TCR^{int}$ cells which contain exclusively MHC I-restricted thymocytes [170–172] and will further develop into the population of $CD8^{+}$ SP thymocytes (fig. 6). Further insight into the process of positive selection came from studies phenotypically analyzing the expression of CD5 [168, 173]. DP thymocytes expressing low levels of CD5 show V(D)J recombination and express TCR α chains (fig. 6). If thymocytes bearing an α/β TCR successfully engage intrathymic ligands, they become uncommitted $CD5^{hi}$ DP thymocytes which downregulate Rag-1/2 expression. Subsequent signals of the TCR induce lineage commitment or, alternatively, clonal deletion. The resulting $CD5^{hi}$ short-lived and committed cells need an additional rescue signal by the TCR to mature into long-lived SP T-cells. In this context, CD5 is not only of interest as a differentiation marker but also functions as a negative regulator of TCR signaling [174].

A more complex and in part contrary picture comes from a detailed flow cytometric analysis, showing the expression of c-Kit (CD117) and IL-7R in subsets of DP thymocytes [53, 175]. The data described suggest a model where $DP^{lo}TCR^{-/lo}$ thymocytes develop further into $DP^{int}TCR^{lo}$ cells which undergo positive selection, and recognition of self MHC drives them through a c-Kit-dependent maturation pathway towards the generation of SP cells. $DP^{int}TCR^{lo}$ $c\text{-}Kit^{+}$ cells that fail positive selection will upregulated CD4 and CD8 and downregulate c-Kit and IL-7R. This population of $DP^{hi}TCR^{lo}c\text{-}Kit^{-}$ cells is given the chance for an alternative α-chain rearrangement and to mature mainly to CD4 SP thymocytes, but the majority of such cells fail

positive selection and die by neglect. These results would implicate the existence of two distinct pathways of positive selection for thymocytes: one c-Kit-dependent, and a second, less efficient c-Kit-independent pathway. Furthermore, in this model the major part of small DP^{hi} cells would enter a dead-end street of T-cell development and would be sentenced to death by neglect.

Lineage commitment: helper or killer?

Positive/negative selection is closely connected to the process of lineage decision and ensures the exclusive expression of either CD4 on MHC class II or CD8 on MHC class I-restricted T-cells, respectively. The preceding section has already described thymocyte populations within T-cell development in which the lineage decision to become either a CD4 or CD8 SP cell occurs. But which signals lead from indistinguishable DP thymocytes to mature SP T-cells with totally different functions during an immune response? Traditionally, lineage commitment is explained using two different models: the instructive model [176], and the stochastic/selective [177] model. In the former model, a matching α/β TCR and coreceptor would generate a different signal when they engage the adequate MHC/peptide complex, terminate expression of the inappropriate coreceptor and force the cell to the accordant lineage. That would implicate a significant difference between signals generated from the CD4 and CD8 molecules, respectively. In the stochastic/selective model, downregulation of either CD4 or CD8 occurs stochastically, and a T-cell with an appropriate TCR coreceptor combination will be selected and will survive (reviewed in [79, 177]).

As in both models the coreceptor plays a dominant role for the decision to become either a helper or a killer T-cell, the question remains to which extent its signal influences the lineage decision. Several studies have raised the question if and to what degree the intracellular signals differ [178]. On the molecular level, both coreceptors augment TCR signaling at least by recruiting the tyrosine kinase Lck to the TCR complex [179] and probably also by additional mechanisms. Furthermore, they participate in the overall avidity of the TCR for the MHC/peptide complex [180–183] and thereby directly affect the developmental fate of the T-cell. Several transgenic or gene-deficient mouse models have documented the influence of CD4 and CD8 on lineage commitment [176, 182–186]. Another method of regulation may be the expression of alternatively spliced forms. Recent data indicate that the alternative CD8α' product cannot interact with Lck and favors the development of CD8 T-cells, perhaps by reducing Lck activity upon MHC/peptide recognition by the TCR [187].

Besides the signals delivered from the TCR and its coreceptor, members of the Notch family of transmembrane receptors seem to affect thymocyte commitment. Notch also influences the α/β versus γ/δ T-cell lineage decision (see above and [188]), perhaps acting in parallel or downstream of the pre-TCR, promoting the development of γ/δ cells. Constitutive expression of an activated form of Notch in developing thymocytes causes thymocytes normally destined for the CD4 lineage to adopt the CD8 lineage instead [189]. Furthermore, development of CD8 SP cells is favored even in the absence of MHC I, indicating again that Notch may act downstream of TCR-mediated signals. This finding would suggest a normal function for Notch to direct DP thymocytes into the CD8 lineage (reviewed in [144]).

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