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Intrathymic IL-7: The where, when, and why of IL-7 signaling during T cell development

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Abstract

The thymus is the birthplace of all T lineage cells. But the thymus is also a cradle as it provides the environment for further maturation and differentiation of immature thymocytes. While many factors contribute to make the thymus a unique place for T cell development, here we review the essential role of intrathymic interleukin-7 (IL-7). In the absence of IL-7 signaling, survival, proliferation and differentiation of immature thymocytes are all severely impaired. Consequently, IL-7 is critical to nurture and guide T precursor cells through the diverse steps of thymic maturation. Interestingly, even as IL-7 signaling is such a critical factor, IL-7 signaling must be also actively suppressed during specific stages of T cell differentiation. These contradictory observations are puzzling but can be satisfactorily explained when understanding the developmental context of IL-7 signaling. In this regard, here we will discuss the spatiotemporal expression of intrathymic IL-7 and address the stage-specific effects of IL-7 signaling in developing thymocytes. Specifically, we will review other facets of intrathymic IL-7 beyond its role as a pro-survival factor and so clarify and reaffirm the unique role of IL-7 as a prime factor in T cell development and differentiation.

Keywords

Thymus; IL-7 receptor; thymocytes; SOCS; positive selection

1. Introduction

All lymphocytes are required to pass through an early developmental stage that is uniquely dependent on IL-7. For developing B lymphocytes, IL-7 signaling is critical at the pre-B cell stage to open up the immunoglobulin heavy (IgH) chain locus and to make this region accessible for the recombination machinery [1]. Similarly, γδ T cell development also requires IL-7 signaling to initiate a STAT5 dependent opening of the TCR γ-chain locus for TCR rearrangement [2, 3], and the same has been also proposed for the TCR β-chain locus during αβ T cell development [4]. Thus, all lymphocytes are born with an innate need for IL-7 signaling that has to be instilled to proceed through the earliest stage of their development. Such a critical role of IL-7 signaling is clearly documented in the developmental block of both B and T cells in the absence of IL-7 and IL-7 receptor α-chain (IL-7Rα) expression, and it is also manifested in severe immunodeficiency and lymphopenia under these conditions [5, 6]. Interestingly, while B cells overcome IL-7

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dependency after successful IgH rearrangement and become IL-7 independent, T cells only start a life-long addiction of IL-7 upon TCRβ rearrangement. In fact, mature peripheral B cells terminate IL-7R expression and survive without IL-7. In T cells, however, IL-7 is a non-redundant cytokine for T cell maturation and differentiation, and it also remains essential for mature T cell survival in peripheral lymphoid tissues [7]. Thus, T cells are generated with an innate requirement for IL-7 signaling throughout their life, and we consider it important to understand where and when, and also when not, IL-7 signaling is necessary for efficient T cell development. Furthermore, the downstream targets of IL-7 signaling can vary depending on the developmental context of IL-7 signaled cells. This leads to another important question of why IL-7 signaling is required at all. Here we will discuss the genetic and cellular basis of IL-7 requirement in thymopoiesis, and we will lay out the distinct effects of IL-7 signaling along a spatiotemporal map of T cell maturation and differentiation in the thymus.

2. An IL-7's-eye view of T cell development in the thymus

While IL-7 signaling intersects with all major events during T cell development in the thymus, T lineage commitment of lymphoid progenitor cells itself is IL-7 signaling independent and rather dependent on Notch [8, 9]. The earliest of such Notch-signaled T lineage cells are characterized by the absence of both CD4 and CD8 coreceptor expression (CD4, CD8 double negative, DN) and also the lack of surface T cell receptors. The first encounter of immature thymocytes with IL-7 happens precisely at this DN stage, and IL-7 signaling is necessary for the survival of DN thymocytes during their progression into their next developmental stage [10]. In fact, recent fate mapping studies of T lineage development using IL-7Rα-Cre mice documented that all T cells are derived from IL-7Rα expressing precursors, suggesting that the ability to receive IL-7 signaling is a critical measure for maturing to competency [11]. The exact timing and the developmental signals leading to the earliest IL-7Rα expression in DN cells, however, are not clearly defined. The DN stage can be further subdivided into four subpopulations, known as DN1 through DN4, determined by the surface expression of CD44 and CD25 [12]. Cell surface staining and IL-7Rα mRNA detection show that IL-7Rα expression starts upon entering the DN2 stage [13], and it is generally understood that IL-7R signaling happens at the DN3 stage, coinciding with TCRβ-selection of DN thymocytes. IL-7 signaling at this particular stage is critical for the survival and the proliferation of β-selected cells as IL-7 signaling deficient cells are normally found developmentally arrested at the DN3 stage [14]. Overexpression of anti-apoptotic molecules such as Bcl-2 or genetic deletion of pro-apoptotic factors such as Bim or Bax can partly compensate for absent IL-7 signaling [15–18], which suggest that the major role of IL-7 at this point is to provide survival signals. Interestingly, IL-7Rα expression does not persist beyond the DN4 stage [19]. Rather, surface IL-7Rα expression is dramatically downregulated in DN4 stage cells and ultimately terminated in immature $CD4^+CD8^+$ double positive thymocytes [13]. In fact, cessation of IL-7 signaling is important since prolonged IL-7 signaling in DN thymocytes seem to impair DP cell differentiation by inhibiting expression of TCF-1 and LEF-1 [13]. Therefore, downregulation of IL-7Rα expression is a cell-intrinsic requirement for DP thymocytes differentiation.

Immature DP cells compose the vast majority of thymocytes and they are the first population to express a functional αβ TCR. Curiously enough, DP thymocytes are uniquely small in their cell size, metabolically inactive, and pre-programmed for cell death. These characteristics are due to the absence of *in vivo* IL-7 signaling as shown by their absence of Bcl-2 expression and their failure to express glucose transporter-1 (Glut-1) [19]. In fact, *in vitro* IL-7 stimulation of DP cells expressing transgenic IL-7Rα documented that these characteristic phenotypes could be reversed [20]. Thus, absent IL-7 signaling predisposes DP cells to undergo programmed cell death unless pro-survival signals are delivered. The

transduction of such survival signals, however, is dependent on a thymic selection process known as positive selection. DP thymocytes are developmentally the last population to express the DNA recombination activation genes RAG-1/2 and to be able to rearrange their TCR specificity. The selection of an immunologically meaningful TCR reactivity, which is a self-MHC restricted but not autoreactive TCR specificity, is known as positive selection and happens only in IL-7 signaling refractory DP thymocytes. Positive selecting TCR signals rescues otherwise doomed DP cells by inducing expression of anti-apoptotic Bcl-2. Importantly, it is precisely the positive selecting TCR signal that re-induces IL-7Rα expression on immature thymocytes. The immediate progenitors of TCR-signaled DP thymocytes are known as intermediate cells, and they can be identified by their unique phenotype of being $CD69^{+}CD4^{+}CD8^{lo}$ which results from a selective downregulation of CD8 but not CD4 coreceptor transcription by TCR signaling. Transition of pre-selection DP cells into post-selection intermediate cells is accompanied by IL-7Rα re-expression, thus, making these cells capable of IL-7 signaling for survival. Interestingly, IL-7 signaling at this stage not only provides pro-survival signals but also cues for distinct T cell lineage differentiation [21, 22]. In fact, intrathymic IL-7 signaling turned out to be a critical factor in CD4/CD8 lineage commitment with IL-7 signaling imposing CD8 cytolytic T lineage fate while absent IL-7 signaling is critical for CD4 helper lineage differentiation [22, 23]. Once lineage fate is sealed, both CD8 and CD4 thymocytes embark on a life long dependency for IL-7 signaling that continues with their export into peripheral tissues as mature T cells. Such need is only abandoned with T cell activation or memory cell differentiation when other common γ-chain (γc)-cytokines such as IL-2 or IL-15 replace IL-7s's role as a survival factor [24, 25].

Why IL-7 and no other cytokines play such an indispensable role in T cell development is an important question that remains open. Among other possibilities, both *in vitro* and *in vivo* studies have indicated that IL-7 is the most potent survival factor in T lineage cells [26, 27] and it is also possible that IL-7 is more readily available to thymocytes than any other cytokines in the thymus [28, 29]. Still, it is remarkable that no other γc-cytokine has such a profound effect on thymopoiesis (Table 1). Depletion of *in vivo* IL-7 by injections of anti-IL-7 antibodies into wildtype mice resulted in a dramatic reduction of overall thymocytes numbers (>99%) and a developmental block at the $CD44^{+}CD25^{+}$ DN3 stage [30, 31]. *In vivo* blocking of IL-7 signaling by injecting anti-IL-7 receptor antibodies resulted in the same conclusion [32]. Furthermore, genetic deletion of IL-7, IL-7 receptors, or proximal signaling molecules of IL-7R, all resulted in a severe defect in thymopoiesis and a block at the DN3 stage. These data suggest that IL-7 is essential for the survival of post-β-selection DN thymocytes (Table 1) [5, 6, 14, 31, 32]. Collectively, IL-7 signaling in thymocytes is a developmentally controlled event that leads to the survival and enrichment of thymocytes expressing an immune competent TCR whereas its deliberate absence leads to the removal of cells that have failed to do so.

3. IL-7 signaling and IL-7 receptor expression in thymocytes

IL-7 signaling is transduced by the IL-7 receptor, which is a heterodimeric complex of the IL-7Rα and the γc [33, 34]. Neither the IL-7Rα nor the γc has intrinsic kinase activities so that IL-7R signaling is dependent on the receptor-associated kinases Jak1 and Jak3 [35]. Ligand-induced IL-7 receptor heterodimerization is the initial step in IL-7R signaling which leads to the juxtaposition of Jak1 and Jak3 kinases, their trans-phosphorylation and activation, and finally to tyrosine phosphorylation at residue 449 (Tyr449) of the IL-7Rα intracellular domain. The Tyr449 is a critical residue for downstream signaling as it recruits STAT5 and also PI-3K, which are subsequently activated by Jaks to initiate further downstream signaling [35]. The dramatic reduction of thymic cellularity in mice with an engineered mutation of tyrosine 449 to phenylalanine 449 (Y449F) documents the

importance of this single residue as well as the importance of the IL-7Rα chain itself [36]. Nonetheless, the IL-7Rα chain is not unique in promoting thymocyte development as domain-swapping experiments with intracellular regions of IL-4Rα, IL-9Rα, and the prolactin receptor partially replaced IL-7Rα function for αβ T cell development [37]. Interestingly, while all these different intracellular domains induced significant reconstitution of thymic T cell development, none of them were able to quantitatively restore T cell development to the extent of a native IL-7Rα chain. Additionally, none of them could restore γδ T cell generation in an IL-7Rα deficient thymus, suggesting that IL-7Rα specific signals regulate T cell development in both a quantitative and a qualitative manner [37]. Collectively, IL-7Rα plays an important role in IL-7 specific signaling as it recruits downstream signaling molecules through its Tyr 449 residue to induce survival, proliferation and differentiation. Because of such importance, understanding IL-7Rα expression during thymic T cell development is imperative for understanding the role of IL-7.

Furthermore, under steady-state conditions, IL-7 levels *in vivo* are widely considered to be constant. Consequently, it is assumed that IL-7 signaling at a single cell level is controlled by IL-7 receptor expression rather than by expression of IL-7 itself. Also, since γc expression is considered to remain constant during T cell activation and homeostasis [34], modulating IL-7Rα expression alone is potentially sufficient to control IL-7 signaling. Presumably because of such significance, IL-7Rα expression is tightly regulated during T cell development. IL-7Rα is already expressed on the most immature double negative (DN) thymocytes, specifically at the DN2 and DN3 stages, but then is significantly downregulated in DN4 cells. Immature DP thymocytes have completely terminated IL-7Rα expression but they still retain γc expression. It is only after TCR-mediated positive selection that thymocytes re-express IL-7Rα [20, 38, 39]. The molecular machinery that times such stage-specific IL-7Rα expression remains largely unknown. However, a number of transcription factors have been already identified whose interaction and cross-regulatory network might be the basis of such stage-specific IL-7Rα expression.

As such, the Ets family transcription factor, GA-binding protein (GABP), has been identified as necessary for IL-7Rα upregulation in immature DN thymocytes [40]. However, GABP expression alone is not sufficient to upregulate IL-7Rα expression since DP thymocytes, which express high levels of GABP, are transcriptionally silent for IL-7Rα. On the other hand, the zinc finger transcription factor, Growth factor independent-1 (Gfi-1), is a transcriptional repressor whose induction results in downregulation of IL-7R α expression on mature CD8 T cells [41, 42]. Curiously enough, IL-7Rα expression on CD4 cells is Gfi-1 independent and IL-7Rα suppression in DP thymocytes is also not controlled by Gfi-1 as Gfi-1-deficient DP thymocytes still fail to upregulate IL-7Rα expression [41]. Thus, Gfi-1 is necessary for IL-7Rα suppression in CD8 lineage cells but the nuclear factor responsible for this function in CD4 cells and DP thymocytes remains unknown. Furthermore, the runt family transcription factors, Runx1 and Runx3, which have identical DNA binding motifs, were also identified to promote IL-7Rα expression [43]. However, since DP thymocytes express high levels of Runx1 but fail to express IL-7Rα, and since the absence of Runx1/3 did not affect IL-7Rα levels on immature DN thymocytes, Runx's effects seem to be rather restricted to mature T cells. Recently, the forkhead transcription factor, FoxO1, has been additionally identified to upregulate IL-7Rα transcription by activating the IL-7Rα enhancer [44, 45]. Strikingly, FoxO1-deficient mature T cells failed to upregulate IL-7Rα expression, but curiously FoxO1-deficient SP thymocytes still express significant levels of IL-7Rα. More confusingly, the FoxO1 DNA binding site in the IL-7Rα enhancer was recently shown to be also occupied by the repressor protein FoxP1, which now together form an intricate molecular circuitry of IL-7Rα expression by two opposing nuclear factors [46]. In conclusion, a large number of transcriptional regulators have been identified for IL-7Rα

transcription, and it is potentially the interplay between these various factors, and not the expression of a single molecule, that controls the specific and selective expression of IL-7R α during thymocytes differentiation.

4. Spatiotemporal analysis of intrathymic IL-7 expression and signaling

4.1 The “where” of intrathymic IL-7 signaling

One of the greatest mysteries in IL-7 biology has been the source of *in vivo* IL-7. While IL-7 is essential for peripheral T cell survival, IL-7 is undetectable in normal mouse serum, and while IL-7 is critical for T cell development, the source of intrathymic IL-7 has not been mapped until recently [29, 47]. Thus, the identities and locations of IL-7 producing cells have been subjects of intense investigation but also confusion. Furthermore, the fact that IL-7 expression is extremely scarce and that experimental tools to detect IL-7 were not readily available made IL-7 detection even more difficult [48]. It was only recently that a series of four independent *in vivo* studies, which utilized the novel approach of bacterial artificial chromosome (BAC) transgenic reporters, have finally shed light onto these issues [48–51]. These studies confirmed the thymus as a major source of IL-7 and they identified CD45-negative non-hematopoietic epithelial cells as the main producers of intrathymic IL-7. The results of these studies have been recently reviewed and discussed in further detail [29]. In brief, all four studies employed BAC transgenesis to detect IL-7 expression *in vivo* but differed in the reporter proteins they employed. The first IL-7 BAC reporter mouse to be published was designed to express a yellow fluorescence protein (YFP) downstream of the translational start codon of the transgenic IL-7 locus [49]. Curiously, YFP signals were only detected in the thymus and in no other organs. Since peripheral IL-7 expression is a firmly established fact, the failure to detect IL-7 outside of the thymus suggested that either the BAC construct did not contain all the regulatory elements for tissue-specific and proper IL-7 expression or that the level of IL-7 expression in tissues outside of the thymus is too low to be detected by fluorescence microscopy *in situ*. A strong argument against the former possibility was given when an independent study using the same BAC clone successfully detected IL-7 reporter activities in various peripheral organs including lymph node, liver, and intestine [50, 51]. The difference between the two studies lies in the choice of the reporter gene. Fluorescent protein signals were clearly too weak to be detected outside of the thymus [49], and only the use of bioluminescence allowed a strong enough amplification of IL-7 transcriptional activity to identify *in vivo* IL-7 expression [51]. In fact, in all four studies, the only way to detect IL-7 BAC transgene expression outside of the thymus was through the use of indirect detection methods such as the use of BAC transgene-driven luciferase expression or BAC-transgene driven Cre recombinase expression [29]. This is also in agreement with findings of Durum and colleagues [48], who used a cyan fluorescent protein (ECFP) as a reporter in their IL-7 BAC transgene and detected ECFP expression in the thymus but failed to observe any significant ECFP signals in any peripheral organs [48]. In the thymus, however, ECFP expression colocalized with expression of a number of epithelial cell markers such as Ly51, a cortical epithelial cell marker, and also with keratin-5, which is more prominently expressed in the medulla [52]. Additionally, ECFP also colocalized with keratin-8 and keratin-14, which are expressed in both cortical and medullary epithelial cells. However, ECFP expression was not found in endothelial cells, fibroblasts and dendritic cells, each identified by CD31, MTS-15 and CD45/CD11c expression, respectively. Collectively, these results suggested that IL-7 expression is limited to thymic epithelial cells and reaffirms the notion that IL-7 is not produced by thymocytes or T cells themselves. Such highly specific distribution of IL-7 expression was also observed in other studies [50, 53], including the study by Di Santo and colleagues, who visualized a restricted pattern of IL-7 expression concentrated around the cortico-medullary junction (CMJ) in the adult thymus [49]. These results are in agreement with the IL-7 *in situ* hybridization results by Richie and colleagues [53], who were the first to observe the

accumulation of IL-7 expressing cells in the cortico-medullary junction area, and who noted that the thymic cortex is mostly void of IL-7 producing cells.

Altogether, these data suggest that intrathymic IL-7 is not randomly distributed but geographically restricted to areas where the earliest and also the late-stage post-selection thymocytes reside, precisely at the stages where intrathymic IL-7 signaling is required. Thymocyte development starts with the entry of early thymic progenitor cells at the CMJ and progresses with thymocyte migration into the subcapsular zone across the thymic cortex [54]. Upon pre-TCR signaling at the DN3 stage, thymocytes reverse their movement and start migrating towards the CMJ and ultimately enter the medulla only to leave the thymus again at the CMJ. The intrathymic migration of thymocytes is a highly choreographed process, and a combination of chemokine receptor signaling and environmental cues assures that developing T progenitor cells only proceed down the path of migration [54, 55]. Collectively, when assessing the anatomical location of intrathymic IL-7 expression, it is evident that IL-7 producers are strategically located at places where IL-7 signaling is required (Fig. 1). It is also interesting to find that IL-7 producers are rather excluded from the cortex which biological significance becomes clearer when assessing this observation in the context of thymocytes differentiation, as explained below.

4.2 The “when” of intrathymic IL-7 signaling

IL-7 signaling is required from the earliest stage of T cell development in DN thymocytes. Generally, IL-7 signaling is thought to sustain the survival and proliferation of DN2/3 cells, but a role for IL-7 signaling as early as DN1 cells has been suggested by mice deficient for the zinc finger protein Miz-1 [56]. Because *in vivo* IL-7 seems to be limited and since DN thymocytes are critically dependent on IL-7, there is fierce competition for limiting amounts of *in vivo* IL-7. This has been visualized in mixed bone marrow chimera experiments of two different strains of mice expressing distinct levels of IL-7Rα, and where DN thymocytes with an endogenously higher level of IL-7Rα outcompeted other cells in survival and reconstitution [57]. Along this line of thinking, it has also been suggested that DP thymocytes have downregulated IL-7Rα expression for altruistic reasons so that they do not compete with DN thymocytes for the limited IL-7 and ensure their survival [33, 58]. The significantly reduced thymus size in IL-7Rα transgenic mice, where transgenic IL-7Rα was expressed on all T lineage cells including DP thymocytes, is in support of this idea [20, 58]. Collectively, the first encounter of αβ T lineage cells with intrathymic IL-7 is at the DN thymocytes stage and IL-7 signaling in these cells determines the size of the pre-selection thymocytes pool.

While IL-7 is a pro-survival factor and important for maintaining thymocyte survival, there are two distinct stages during T cell development in which IL-7 signaling must be disabled. The first stage is at the pre-selection immature DP compartment before thymocytes undergo positive selection [20]. The second stage is at the CD4/CD8 lineage decision point and specifically during the process of CD4 lineage commitment [22]. A series of *in vitro* and *in vivo* experiments have suggested and confirmed that thymocytes at pre-selection DP and during CD4 lineage commitment are uniquely refractory to IL-7 signaling [20, 21, 38]. In fact, it turns out that the immune system employs multiple layers of mechanisms to prevent IL-7 signaling at these two stages, which re-emphasizes the importance of absent IL-7 signaling in these cells. Why IL-7 signaling has to be avoided specifically at these stages is a critical question that touches the fundamental mechanism of T cell development. Thanks to recent advances on the downstream effects of cytokine signaling in immature thymocytes, we are currently much closer to understanding the molecular basis for such requirements.

As such, permissive IL-7 signaling in pre-selection DP thymocytes could potentially lead to the indiscriminate survival of immature DP thymocytes, including those which failed to

rearrange a functional and/or useful TCR. Accordingly, *in vitro* experiments have shown that DP thymocytes, which were genetically manipulated to be responsive to IL-7, induced Bcl-2, upregulated metabolic activities, and increased in cell size upon IL-7 treatment [20]. Since the major purpose of positive selection is to form a random but self-MHC-specific TCR repertoire, the survival of DP thymocytes with a non-self-MHC restricted or even non-functional TCR would result in the rescue of "useless" TCR specificities and nullify the effect of positive selection. Thus, non-discriminatory IL-7 signaling in pre-selection DP thymocytes must be prevented to ensure effective positive selection of a functional self-MHC restricted repertoire. As aforementioned, the thymus utilizes a series of redundant mechanisms to prevent IL-7 signaling in pre-selection DP cells. Firstly, DP thymocytes are unique among thymocytes because they have transcriptionally terminated IL-7Rα gene expression [20, 22]. Consequently, DP thymocytes are unable to bind IL-7. Additionally, DP thymocytes transcribe and express very high levels of the Suppressor Of Cytokine Signaling-1 (SOCS1), which is a cytosolic molecule that binds to cytokine receptor-associated Janus kinases (JAK) through its kinase-inhibitory region and suppresses the downstream JAK-STAT pathway in IL-7 signaling [59]. Finally, immature DP thymocytes reside in the thymic cortex, which is mostly devoid of IL-7 producing cells, and they do not become exposed to IL-7 until positive selection induces expression of the chemokine receptor CCR7 and directs their migration towards the cortico-meduallary junction where IL-7 is available in larger amounts [22, 54]. Thus, multiple overlapping pathways in thymocytes-intrinsic and -extrinsic manners ensure the complete insulation of pre-selection DP thymocytes from intrathymic IL-7 signaling.

$CD4^+CD8^{lo}$ intermediate thymocytes are the immediate progenies of post-selection DP cells. Intermediate cells have regained their potential to respond to IL-7 as they have upregulated IL-7Rα while downregulated SOCS1 expression [20–22]. Importantly, while intermediate cells are not the first thymocyte population that can respond to IL-7 – this would be the immature DN cells-, they are the first αβ TCR expressing population in the thymus that can respond to IL-7. However, whether intermediate cells get IL-7 signaled *in vivo* and whether such IL-7 signaling would have any developmental consequences is still under intense debate. In fact, only recently, Hogquist and colleagues reassessed this question by short term *in vivo* injection of neutralizing anti-IL-7Rα antibodies into wildtype mice [60]. They came to the conclusion that two major post-selection events, namely 1) expression of the transcription factor Kruppel-like factor 2 (KLF2), which controls thymic egress, and 2) CD8 lineage specification in positively selected cells, which has been suggested to be downstream of IL-7 signaling, were both independent of IL-7 signaling [60]. While thymocyte egress was never seriously considered to be IL-7 dependent, IL-7's role in CD8 lineage specification has been repeatedly documented in both *in vitro* studies and *in vivo* models [20–23]. In this regard, dismissing IL-7's role in CD4/CD8 lineage choice [23] would be in striking contrast to the report where *in vivo* deletion of STAT5/6 in immature DP thymocytes resulted in a dramatic and specific impairment of CD8 lineage differentiation [22]. It is also incompatible with the observations that forced IL-7 signaling in pre-selection DP thymocytes imposed CD8 cell fate and induced functional maturation into cytolytic CD8 cells both *in vitro* and *in vivo* [19, 22]. One potential explanation for such contradictory results could be the use of a mild regimen of anti-IL-7Rα treatment in that particular study, which consisted of only three doses during six days [60]. Total thymocytes numbers are indicative of *in vivo* IL-7 availability, and the complete blockade of IL-7 signaling or genetic deletion of IL-7Rα usually results in 100~1,000 fold reduction of thymocyte numbers rather than the 2-fold reduction as observed by Hogquist and colleagues [5, 14, 60]. Thus, an incomplete block of IL-7 signaling *in vivo* could possibly account for intact CD8SP lineage generation, despite anti-IL-7Rα injections. Furthermore, the existence of a small number of CD8SP thymocytes generated independently of IL-7 signaling had been well documented in the original reports of IL-7 or IL-7 receptor deficient mice [5, 14],

and a redundancy of intrathymic cytokine signaling in the absence of IL-7 can not be ruled out. Collectively, IL-7 signaling in $CD4^{+}CD8^{lo}$ intermediate thymocytes is proposed to impose CD8 lineage fate, and if so, the distinct modes of IL-7 signaling could mediate CD4/CD8 cell fate decision in the thymus.

If IL-7 signaling is sufficient to drive CD8 cell differentiation, it is imperative that IL-7R signaling should be desensitized during CD4 lineage commitment. And in fact, *ex vivo* analysis of CD4SP thymocytes showed that, in contrast to CD8SP cells, CD4SP cells failed to express cell surface Glut-1, which is indicative of absent *in vivo* IL-7 signaling [19]. How CD4 lineage committed post-selection thymocytes avoids *in vivo* IL-7 signaling, however, is not known. But persistent TCR signaling induced by MHCII and TCR/CD4 coreceptor engagement is clearly a necessary component, as shown by the failure to maintain stringent CD4 lineage choice when TCR signals are interrupted [61]. Altogether, preventing IL-7 signaling in pre-selection DP thymocytes and also during CD4 lineage commitment are two important steps during T cell development that are required to ensure a functional and mature T cell pool.

4.3 The “why” of intrathymic IL-7 signaling

IL-7 is a multifaceted protein that plays a diverse array of functions within the immune system. In addition to its role in immature lymphocytes where it is involved in opening the antigen receptor gene loci for recombination and drives further maturation of precursor cells, IL-7 signaling is also essential for naïve T cells survival and contributes to memory T cell homeostasis [62–64]. Specifically in naïve T cells, IL-7 also controls the TCR activation threshold by tuning CD8 coreceptor expression levels to the self-specificity of their TCR [65]. In this way, IL-7 signaling presumably maintains a self-reactive but not autoimmune peripheral T cell pool. IL-7 signaling also results in downregulation of its own receptor and thus marks IL-7 signaled cells with reduced IL-7Rα levels, allowing IL-7 unsignaled cells to outcompete IL-7 signaled T cells in the next round of IL-7. Maintaining this negative feedback loop was shown to be necessary for maximizing the size of the naïve T cell pool [41]. Furthermore, in dendritic cells, increased IL-7 signaling has been reported to downregulate MHCII expression resulting in contraction of the CD4 T cell pool [47], whereas in liver cells, IL-7 downregulated SOCS3 but induced expression of the cytoprotective cytokine IL-22 to limit organ damage by viral infection [66]. Additionally, IL-7 signaling is also implicated in the development of secondary lymphoid tissues as shown by defect lymph node organogenesis in IL-7Rα deficient mice [6, 67, 68]. In particular, IL-7 seems to impact the size of the lymphoid tissue inducer (LTi) cell pool that interacts with stromal organizer cells to form secondary lymphoid tissues [69]. Collectively, IL-7 is a critical cytokine that governs the development, differentiation, and maintenance of different immune cells in a non-redundant manner.

However, during T cell development, the foremost reason of IL-7’s requirement is its role as a pro-survival factor. A number of anti-apoptotic molecules have been identified downstream of IL-7, which include among others Bcl-2 and Mcl-1 [70–72]. IL-7 also promotes cell survival by redistributing the pro-apoptotic proteins Bax and Bad, but interestingly not Bim, at least not during thymocyte development [16, 18, 73–75]. Additionally, IL-7 signaling induces activation of PI-3 kinase and phosphorylation of Akt which upregulates cell metabolism by inducing expression of the glucose transporter, Glut-1 [76]. Altogether, IL-7 is a potent anti-apoptotic factor that also provides trophic signals to developing thymocytes. Consequently, neither the transgenic expression of anti-apoptotic proteins alone nor constitutively activated Akt by itself could substitute IL-7 signaling in its entirety [77]. Importantly, IL-7 signaling in “moderation” is important to ensure normal T cell development since excessive IL-7 signaling in form of non-physiologically strong

overexpression have resulted in overt B cell development in the thymus and paradoxically in a smaller thymus size as well as lymphoproliferative disorders [78, 79].

In addition to its survival effects, there is an emerging role of IL-7 as a lineage differentiation factor. Cytokines are powerful mediators of lineage fate decision as in case of IL-2 for regulatory T (Treg) cell development in the thymus [80, 81] and for a series of other specific cytokines during Th1, Th2, and Th17 differentiation in peripheral T cells [82, 83]. A role of IL-7 in "CD4/CD8 lineage choice" during T cell development has been proposed with the "kinetic signaling" model of lineage commitment [21–23]. DP thymocytes are the precursors of both $CD4^+$ helper and $CD8^+$ cytolytic cells but it is only after positive selection that DP thymocytes make a lineage decision and differentiate into one or the other subset. This process is generally known as "CD4/CD8 lineage choice" and delineating its mechanism has been debated for the last couple of decades. Because the specificity of the positively selecting TCR also determines the CD4/CD8 lineage outcome, traditionally it had been assumed that quantitative or qualitative differences in TCR signaling would dictate cell fate decisions. As potential operational mechanisms, either distinct strengths of TCR signals, explained by different binding affinities of the $p56^{lck}$ kinase to CD4 or CD8 coreceptors, or distinct lengths of TCR signals, for which no plausible mechanistic explanation was given, were proposed. These classical models are known as "strength of signal" model or as "duration of signal" model. In contrast, the kinetic signaling model is a non-classical model of cell fate decision which proposes that the kinetics of TCR signaling, which is usually dependent on the kinetics of coreceptor expression, determines CD4/CD8 lineage choice by regulating cytokine responsiveness [23]. Importantly, the kinetic signaling model proposes that persistent TCR signals as mediated by MHCII and CD4/TCR interactions is necessary to desensitize IL-7R signaling and that cessation of TCR signaling, which happens during MHCI and CD8/TCR signaling, will permit IL-7 signaling. And, it is IL-7 signaling that induces the CD8 lineage specification factor Runx3 which imposes CD8 lineage fate [84]. Altogether, intrathymic IL-7 signaling mediates CD4/CD8 lineage choice as it controls expression of the CD8 lineage specifying nuclear factor Runx3 [22]. Therefore, IL-7's requirement in T cell development clearly goes beyond just providing survival signals. In conclusion, IL-7's effect in CD8 cell differentiation reveals that IL-7 joins the list of other γc-cytokines in directing T lineage differentiation such as IL-21 in follicular T helper cell [85], IL-4 in Th2 cell [82], and IL-2 in natural Treg cell differentiation [86, 87].

5. Perspectives

The absolute requirement for IL-7 in thymocyte development and differentiation is intriguing. IL-7 is required for the survival of immature thymocytes and also for lineage differentiation upon positive selection. In moderate amounts, increased IL-7 signaling promotes thymopoiesis but excessive IL-7 signaling can reverse such effects. These results indicate that IL-7 signaling has to be carefully titrated and timed during T cell development. *In vivo*, this is achieved by tight regulation of IL-7Rα expression in a cell intrinsic manner and by the strategic distribution of IL-7 producing cells in a thymocyte extrinsic fashion. It is likely that other pathways are also involved in fine tuning IL-7 signaling during development, such as during CD4 cell lineage differentiation, but these mechanisms remain still veiled. While new roles for intrathymic IL-7 continue to be discovered, unresolved questions like these keep this field an exciting venue for further scientific endeavors.

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Highlights

- Immature thymocytes require IL-7 signaling for survival, proliferation, and differentiation.
- IL-7 is produced by thymic stromal cells but not by thymocytes themselves.
- However, desensitization to IL-7 is also critical for thymocyte development.
- Positive selection and CD4 lineage commitment are proposed to be induced in IL-7 refractory cells.
- Correct spatiotemporal regulation of IL-7 signaling is important for T cell development.

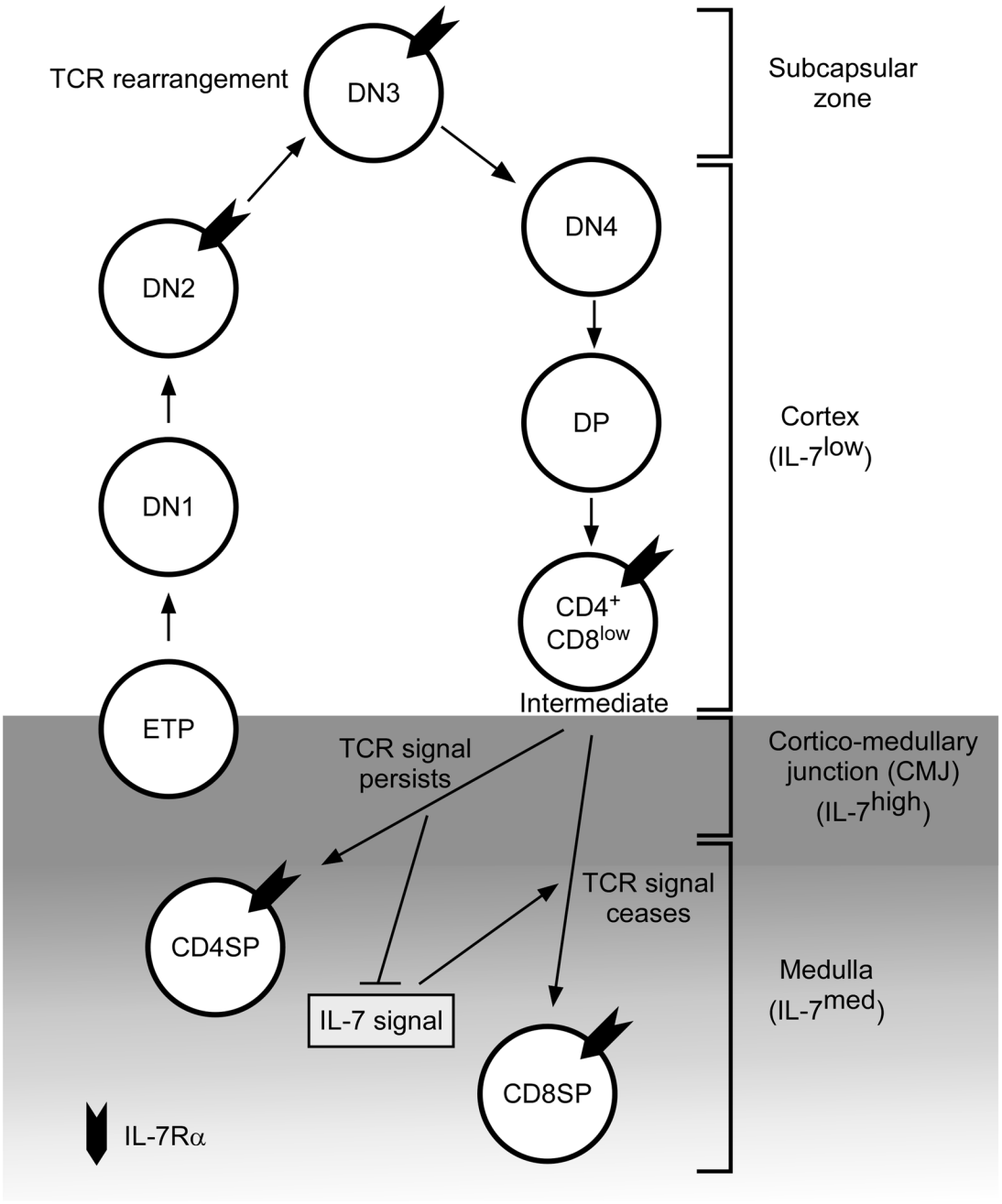

Figure 1. IL-7 receptor expression and IL-7 signaling during T cell development in the thymus

T cell development starts with the entry of early thymic progenitor cells (ETP) through the cortico-medullary junction (CMJ) followed by their migration towards the subcapsular zone. IL-7Rα expression is initiated during this migratory phase, specifically in DN2 stage cells, which is critical for IL-7 dependent survival and proliferation of TCRβ-selected thymocytes. Upon further differentiation into DN4 and DP cells, IL-7Rα expression is terminated. DP thymocytes then reside in an IL-7 poor environment refractory to IL-7 signaling and pre-programmed to cell death. It is only after TCR-mediated positive selection that IL-7Rα expression is re-induced and that these cells become IL-7 signaling competent. Persistent TCR signaling desensitizes IL-7 signaling and permits CD4 lineage differentiation.

Cessation of TCR signaling, however, allows IL-7R signaling in intermediate cells, and it is intrathymic IL-7 signaling that imposes CD8 lineage specification during CD4/CD8 lineage choice in the thymus.

Table 1

Phenotype of cytokine signaling deficient mice

	Genetic deficiency	Cellularity		Reference
		Thymus	LN	
Cytokine	IL-2	N	N	[88]
	IL-4	N	N	[89]
	IL-5	N	N	[90]
	IL-6	PI	PI	[91]
	IL-7	I	I	[6]
	IL-9	N	N	[92]
	IL-10	N	N	[93]
	IL-15	N	N	[94]
	IFNγ	N	N	[95]
Cytokine receptor	IL-2Rα	N	N	[96]
	IL-2Rβ	N	N	[97]
	IL-2Rγ	I	I	[67, 98]
	IL-4Rα	N	N	[99]
	IL-7Rα	I	I	[5]
	IL-15Rα	N	N	[100]
	IL-21R	N	N	[101]
	TSLPR	N	N	[102]
Cytokine signaling molecule	JAK1	I	I	[103]
	JAK3	I	I	[68, 104, 105]
	STAT5	I	I	[106]

(N) Normal; (PI) Partially impaired; (I) Impaired.