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Review

The many roles of IL-7 in B cell development; Mediator of survival, proliferation and differentiation

Steven A. Corfe[a,b,*], Christopher J. Paige[a,b,c]

[a] Ontario Cancer Institute, Princess Margaret Hospital, University Health Network, 610 University Ave., Toronto, Ontario M5G 2M9, Canada
[b] Department of Immunology, University of Toronto, Toronto, Ontario M5S 1A8, Canada
[c] Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 2M9, Canada

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ABSTRACT

Interleukin-7 (IL-7) plays several important roles during B cell development including aiding in; the specification and commitment of cells to the B lineage, the proliferation and survival of B cell progenitors; and maturation during the pro-B to pre-B cell transition. Regulation and modulation of IL-7 receptor (IL-7R) signaling is critical during B lymphopoiesis, because excessive or deficient IL-7R signaling leads to abnormal or inhibited B cell development. IL-7 works together with E2A, EBF, Pax-5 and other transcription factors to regulate B cell commitment, while also functions to regulate Ig rearrangement by modulating FoxO protein activation and Rag enhancer activity. Suppressor of cytokine signaling (SOCS) proteins are inhibitors of cytokine activation and, in B cells, function to fine tune IL-7R signaling; ensuring that appropriate IL-7 signals are transmitted to allow for efficient B cell commitment and development.

1. Introduction

B cells are derived from hematopoietic stem cells (HSCs) in a process by which cells progressively develop B cell traits while repressing traits of other lineages. During development, progenitor B cells undergo rearrangement of their immunoglobulin (Ig) loci in a stepwise and reproducible fashion, whereby recombination of the heavy chain (HC) genes occurs in pro-B cells followed by recombination of the light chain (LC) genes in pre-B cells. Binding of μHC proteins with surrogate LC proteins λ5 and V_{preB} leads to surface expression of the pre-B cell receptor (pre-BCR) on large pre-B cells, which initiates survival, proliferation and maturation signals *via* Igα and Igβ signaling subunits. Small resting pre-B cells exit the cell cycle and once again begin the recombination process, leading to rearrangement of the LC proteins. LC proteins associate with μHC proteins to form the B cell receptor (BCR), which is first expressed on immature B cells. Immature B cells undergo positive and negative selection in the bone marrow (BM) and subsequently exit into the periphery where they undergo additional selection prior to becoming functional mature B cells capable of responding to antigen.

B lymphopoiesis takes place in niches within the fetal liver (FL) or BM, which provide the structure for development to proceed, as well as the necessary chemokines and cytokines that regulate signal transduction pathways in receptive cells. Initiation of signaling pathways in turn activate transcription factors (TFs) that function to induce or repress the expression of various target genes that modulate B cell survival, proliferation and differentiation. Interleukin-7 (IL-7) is a key cytokine during B cell development and is produced by stromal cells in the FL, BM, spleen and thymus. IL-7 receptor (IL-7R) signaling leads to the proliferation and survival of B cell progenitors as well as aids in the commitment of cells to the B lineage. Mice with targeted deletions of IL-7 or the IL-7R display a severe block at the early pro-B cell stage of development [1,2]. The peripheral B cells that exist in these mice appear to have originated during fetal development, a time during which B lymphopoiesis is not absolutely dependent on IL-7. Thymic stromal lymphopoietin (TSLP) is a cytokine that possesses a number of characteristics in common with IL-7 and its receptor is composed of the IL-7Rα chain and TSLPR chain. Fetal derived pro-B and pre-B cells respond to TSLP, while in adult BM, only pre-B cells are TSLP-responsive [3,4]. TSLP was thought to substitute for IL-7 during fetal development, because transgenic expression of TSLP in IL-$7^{-/-}$ mice restored B cell development during fetal and adult life [5]. However, IL-$7^{-/-}$/TSLP$^{-/-}$ mice did not show enhanced defects during fetal B cell development and, instead, it was the absence of IL-7 and fms-related tyrosine kinase-3 ligand (Flt-3L) that completely abolished both fetal and adult development of B cells [6,7]. Flt-3L was also able to recover the development of residual B cells present in IL-7Rα deficient mice [3]. IL-7 transgenic mice displayed increased numbers of immature and mature B cells in the BM, as well as extramedullary B lymphopoiesis whereby pro-B and

* Corresponding author at: Ontario Cancer Institute, 8-105 Princess Margaret Hospital, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada.
Tel.: +1 416 946 4501x3019; fax: +1 416 946 2086.
E-mail address: steve.corfe@utoronto.ca (S.A. Corfe).

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pre-B cells were observed in the spleen, blood, and lymph nodes, and ultimately lead to lymphoproliferative disorders [8].

IL-7 is absolutely essential for murine B cell development, however, in humans it was thought that this was not the case. Genetic mutations in humans that disrupted the IL-7R led to X-linked severe combined immunodeficiency disease (X-SCID), which is characterised by the absence of T cells and natural killer (NK) cells but normal B cell numbers [9]. However, similar to mice, these B cells appear to be the result of fetal or neonatal development. *In vitro*, human B cells can be generated from fetal BM as well as cord blood (CB) in the absence of IL-7 [10,11]. However, human B cell precursors express the IL-7R and displayed increased proliferation and survival in response to IL-7 that was mediated by Signal Transducer and Activator of Transcription-5 (STAT5) [12]. IL-7 also greatly increased the production of B cells in co-cultures containing human BM stroma and either CB or adult BM HSCs, while only neonatal CB was able to give rise to B cell progenitors independent of IL-7 [11]. In humans, TSLP cannot substitute for IL-7, while Flt-3L is able to support IL-7-independent B lymphopoiesis from neonatal CB [11,13].

As important as the signaling pathways initiated by IL-7 and other cytokines, are the mechanisms that exist to regulate and terminate these signals. The suppressor of cytokine signaling (SOCS) family of proteins are key regulators of cytokine signals and are essential for the development and function of a variety of hematopoietic lineages (reviewed in [14]). SOCS proteins contain three functional domains: a central SH2 domain that is necessary for binding to phosphotyrosine residues on target proteins; a C-terminal domain, termed the SOCS box, which is involved in ubiquitin-mediated proteasomal degradation *via* elongins B and C; and an N-terminal domain whose function remains largely undefined. SOCS proteins bind directly to Janus Associated Kinase (JAK) and STAT proteins, as well as cytokine receptor chains, and prevent their interaction as well as target them for destruction. SOCS proteins are often induced by the same signaling pathway that they inhibit, providing a negative feedback loop that functions to limit receptor activation.

This review will focus on the signal transduction pathways activated downstream of the IL-7R, as well as how IL-7R signals are translated into the observed physiological outcomes of survival, proliferation and differentiation. We will also discuss the other surface proteins, signaling molecules and TFs that assist in mediating the effects of IL-7 in developing B cells. Finally, we will describe the mechanisms that exist both extrinsically and intrinsically to modulate and terminate IL-7R signals, ensuring that B cell development proceeds efficiently.

2. IL-7R signaling in B cells

2.1. JAK/STAT and Src activation

The IL-7R is a heterodimer composed of the IL-7Rα chain and the common γ (γc) chain. The γc chain is a shared component of the receptors for IL-2, IL-4, IL-9, IL-15 and IL-21, while the IL-7Rα chain can also dimerize with the TSLPR chain to form the receptor for TSLP. The γc chain is expressed by a variety of hematopoietic cells and is essential for initiating signals downstream of the IL-7R; however, it is the α chain, which is predominantly expressed by lymphoid cells, that confers receptor specificity for binding to IL-7 (reviewed in [15]).

Both α and γc chains lack intrinsic tyrosine kinase activity and thus rely on non-receptor kinases and adaptors to mediate downstream signaling. The JAK family consists of four members (JAK1, 2, 3 and Tyk2), with JAK3 expression being confined predominantly to cells of hematopoietic origin. For the IL-7R, JAK3 is constitutively associated with the γc chain, while JAK1 associates with the α chain. Binding of IL-7 to the IL-7R leads to dimerization of the α and γc chains, which brings JAK1 and JAK3 in close proximity and allows for trans-phosphorylation, resulting in increased kinase activity (Fig. 1). Activated JAK kinases are able to phosphorylate tyrosine residues on the IL-7Rα chain that in turn create docking sites for SH2 containing proteins, which themselves are JAK substrates. Mice deficient for JAK3 or the γc chain exhibit similar phenotypes and closely resemble the defect observed in IL-7 or IL-7R knockout mice; while in humans, mutations in JAK3 or the γc chain result in a $T^-NK^-B^+$ SCID phenotype [9,16–19]. $JAK1^{-/-}$ mice are runted at birth and die perinatally, exhibiting a severe reduction in both B and T cell numbers, while in humans no cases of JAK1 deficiency have been described [20].

Phosphorylation of the IL-7Rα chain is critical in initiating downstream signaling because it leads to the recruitment of STAT proteins. Phosphorylation of STAT proteins allows them to dimerize and translocate to the nucleus where they act as TFs for a variety of target genes by binding to specific promoter elements (reviewed in [21]). A total of seven STAT family members exist (STAT 1, 2, 3, 4, 5a, 5b and STAT6); however, only STAT1, STAT3, STAT5a and STAT5b are activated after stimulation of B cells with IL-7 [22,23]. While STAT1 does not play a direct role in B lymphopoiesis, STAT3 is required. $STAT3^{-/-}$ mice displayed a reduction in pro-B, pre-B, immature and mature B cells, and pro-B cells from these mice exhibited a decreased proliferative response to IL-7 [22]. STAT5 is the predominant STAT protein activated by IL-7 and carries out the majority of STAT-mediated responses in developing B cells, including the activation of ccnd2, ccnd3, Bcl-2, Bcl-xL, and Mcl-1 (Fig. 1) [24,25]. STAT5 is recruited to Tyr449 of the IL-7Rα chain where it is subsequently phosphorylated by JAK and Src kinases. STAT5a and STAT5b have redundant roles during B cell development but are absolutely essential, as B cells in STAT5a/b double deficient mice are arrested at the pre-pro-B cell stage, similar to that observed in $IL\text{-}7R^{-/-}$ mice [26–28]. Additionally, constitutive activation of STAT5b in mice overcame IL-7R deficiency and significantly increased pro-B cell numbers [29]. Recently, the use of Rag1-Cre knock-in mice allowed for the conditional mutagenesis of STAT5. This resulted in the complete inactivation of STAT5 in pro-B cells and caused severe defects in the further development of B cells [30]. The transgenic expression of Bcl-2 restored the development of $STAT5^{-/-}$ B cells in these mice, suggesting that the predominant role of STAT5 during B cell development under these conditions is survival.

Src family kinases are also recruited to the IL-7Rα chain and are activated by IL-7 binding. IL-7 induces the activation of both p59fyn and p53lyn in pre-B cell lines, and Src kinase inhibitors impaired proliferative responses to IL-7 [31,32]. Src family kinases have redundant roles during B cell development and their function in IL-7 signaling has yet to be fully elucidated. One potential role is to help activate STAT proteins, because Src kinases can directly phosphorylate STAT proteins independently or in conjunction with JAK proteins (reviewed in [33]).

2.2. PI3K/Akt and MAPK/Erk activation

Phosphatidylinositol 3-kinase (PI3K) activation is another important consequence of IL-7-induced signaling. PI3K is composed of a smaller regulatory subunit (p50α, p55α or p85α) and a larger catalytic subunit (p110α, p110β or p110δ). IL-7R signaling leads to the recruitment of the p85 subunit to Tyr449 of the IL-7Rα chain, *via* its SH2 domain [34]. Mutation of this site or the p85 subunit, as well as treatment of B cells with PI3K inhibitors, led to a loss in IL-7-induced proliferation and impaired B cell development [35–37]. The p110α and p110δ subunits play essential but redundant roles during B lymphopoiesis, as evidenced by the fact that double deficient mice are arrested at the pre-B cell stage

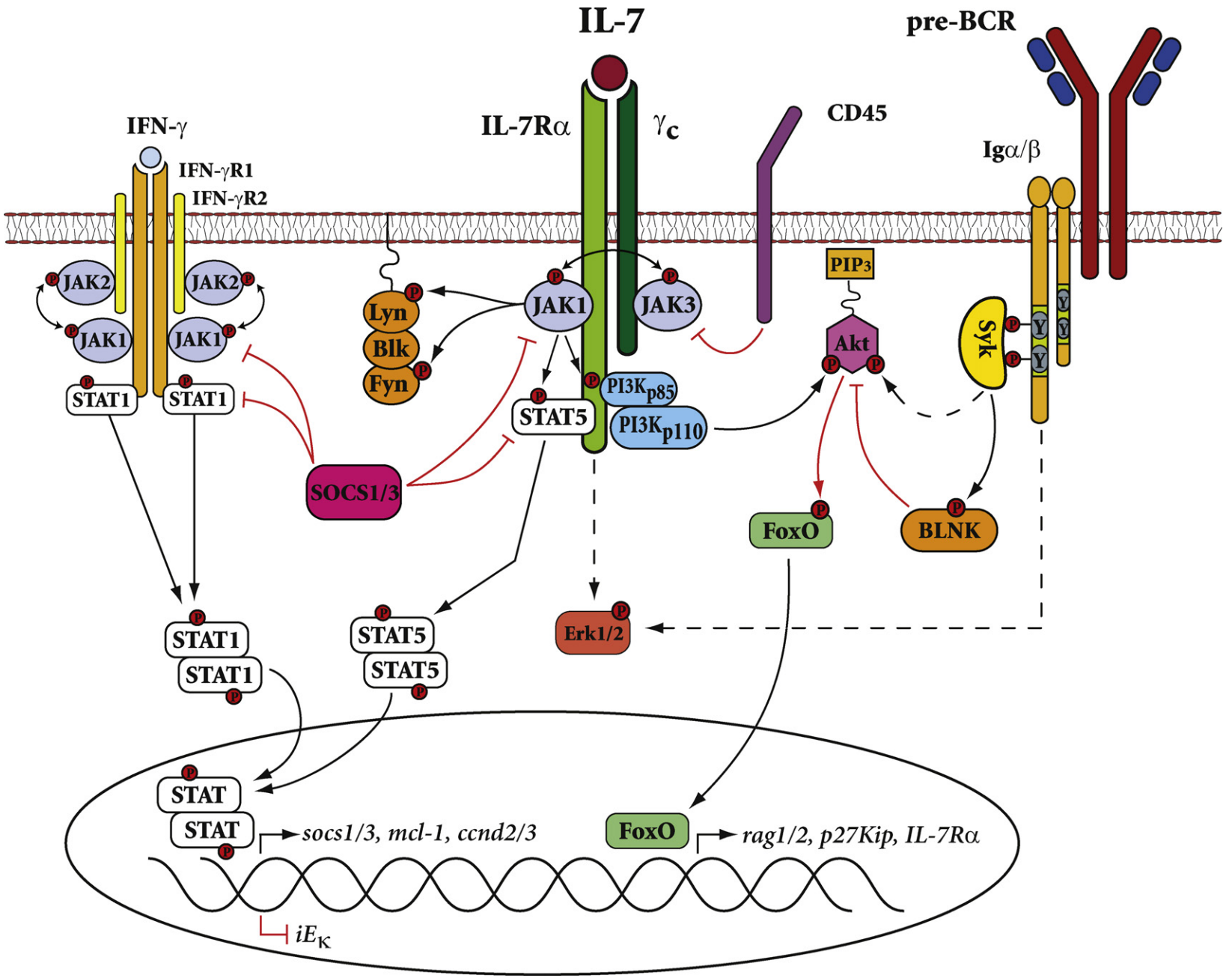

Fig. 1. A schematic diagram of the signaling pathways initiated downstream of the IL-7R as well as other signaling molecules that work with or inhibit IL-7R activation. Red lines denote inhibitory actions and dashed lines denote a multistep pathway. See text for detailed description.

and displayed impaired proliferative responses to IL-7 [38]. One key downstream mediator of PI3K signaling is the serine/threonine kinase Akt (PKB). Akt regulates a variety of pro- and anti-apoptotic factors and also targets the Forkhead box (FoxO) family of TFs (reviewed in [39]). Akt phosphorylation of FoxO proteins causes them to bind 14–3–3 proteins, which retains them in the cytosol where they remain inactive and unable to regulate gene transcription.

IL-7 stimulation of B cells also leads to the activation of the MAPK/Erk pathway. Erk is a critical target of IL-7R and pre-BCR signaling, as demonstrated by the observation that treatment of cells with Erk inhibitors or deletion of Erk1/2 in mice resulted in a block at the pro-B to pre-B cell transition as well as ablated IL-7-induced pro-B/pre-B cell survival and proliferation [36,40]. While the full mechanism of action of the MAPK/Erk pathway has yet to be described in B cells, the adaptor protein Shc may be an important mediator, similar to that observed for T cells [41,42]. In B cells, Shc was phosphorylated after IL-7 stimulation, and pro-B cells containing a defective Shc protein displayed increased apoptosis [43]. Additionally, conditional mutation of Shc proteins led to a defect in development at the pre-pro-B to pro-B cell stage, as well as a significant reduction in pre-B cell numbers.

3. The role of IL-7 during early B cell development

3.1. IL-7 synergy with other early co-factors

Developing B cells rely on a variety of factors that provide signals independently or in conjunction with those from the IL-7R. Stem cell factor (SCF, c-Kit-Ligand) binds c-Kit and is required during the earliest stages of hematopoietic development. SCF acts directly on developing B cells and, *in vitro*, worked synergistically with IL-7 to increase the numbers of pro-B cells in culture [44]. Flt-3L, and its receptor Flt-3, also play important roles in enhancing the survival and proliferation of early progenitors. In Flt-3$^{-/-}$ mice, pre-pro-B and pro-B cell numbers were significantly reduced, while more mature pre-B and immature B cell populations remained relatively normal [45]. Addition of Flt-3 and IL-7 to hematopoietic progenitor cells resulted in the synergistic activation of the MAPK/Erk and PI3K/Akt signaling pathways, which led to increased survival and proliferation of pre-pro-B cells [46]. Experimentation with different cytokine cocktails led to the discovery that IL-11, SCF and IL-7 supported the development of B cells from uncommitted progenitors derived from FL, while the combination of IL-11, Flt-3L and IL-7 was even more potent [47,48]. While IL-11, SCF, and Flt-3L all play important roles in enhancing B lymphopoiesis, only IL-7 is absolutely essential, and once a progenitor cell is capable of responding to IL-7 these other factors are no longer required for cell survival, proliferation or development toward the B lineage.

3.2. IL-7 regulation of B cell specification and commitment

Prior to embarking down the B cell developmental pathway, hematopoietic progenitors are primed for lymphoid specification by a variety of TFs including Bcl11a, E2A, Gfi-1, Ikaros and PU.1 (reviewed in [49]). Mice deficient for any of these factors display defects in B cell development at the common lymphoid progenitor (CLP) stage, partially due to the failed expression of Flt-3 and/or IL-7Rα, as well as the inappropriate expression of alternative lineage specific genes. Upon expressing the IL-7R, CLPs begin the process of specification and eventual commitment to the B lineage, which is governed by a network of TFs including E2A, EBF1, Pax-5 and FoxO1. IL-7R signaling provides trophic signals during this process, however, constitutive expression of Bcl-2 is unable to compensate for the loss of IL-7 in IL-7Rα$^{-/-}$ or γc$^{-/-}$ mice, suggesting that IL-7 also provides instructive signals [50,51]. Furthermore, CLPs from IL-7$^{-/-}$ mice have a greatly reduced ability to generate B cells, likely due to ineffective activation of EBF1 and Pax-5, both of which are essential for the initiation and maintenance of B lineage commitment [52]. Ectopic expression of EBF or constitutive STAT5 expression in IL-7$^{-/-}$ mice restored B cell development, signifying that these are key downstream mediators of IL-7R signaling [52,53]. A potential mechanism for IL-7R signaling in directing B cell commitment comes from the observation that STAT5 signaling enhances the expression of EBF1 and Pax-5 [53–56]. This activation appears not to be direct though and likely requires priming or assistance from other TFs. The conditional deletion of Pax-5 at various stages of B cell development allowed cells to revert to a non-committed state and develop into alternative lineages [57]. This lineage reversion only occurred in the absence of IL-7 though; when cells were cultured with IL-7 they maintained a pro-B cell phenotype, indicating that IL-7 is able to keep cells committed to the B lineage.

Recently, Miz-1 was identified as an important TF for B cell commitment, as evidenced by the fact that Miz-1$^{-/-}$ mice displayed a block at the pre-pro-B to pro-B cell stage of development [58]. This defect resulted from a lack of IL-7R signaling, due to high expression of SOCS-1, as well as decreased Bcl-2 expression. Interestingly, in Miz-1$^{-/-}$ mice, B cell development could only be recovered by the combined ectopic expression of Bcl-2 and EBF1, demonstrating that in the absence of IL-7R signaling both trophic and instructive signals were required. In contrast to these studies demonstrating an instructive role for IL-7, conditional deletion of STAT5a/b at the onset on Rag expression did not result in decreased expression of EBF or Pax-5 in pro-B cells, suggesting that IL-7R signaling may not be absolutely required for their expression [30]. In addition, the developmental defect observed in STAT5a/b double deficient mice could be partially overcome by Bcl-2 expression, implying that IL-7 plays a permissive role during commitment. Further studies investigating whether transient STAT5 activation prior to its deletion is sufficient to initiate the activation of other factors that then regulate B cell commitment will help address these contradictory findings.

While it has been widely demonstrated that the absolute lack of IL-7R signaling is detrimental to murine B cell development, it has also been shown that excessive IL-7R signaling can be inhibitory. Studies in which the IL-7Rα chain was expressed at high levels in multipotent progenitor cells led to a reduction or block in B cell development prior to the expression of CD19, with cells failing to express EBF1 and Pax5 [59,60]. Therefore, it is not simply the presence or absence of IL-7R signaling that is crucial for B cell commitment, but also the magnitude of these signals. Mechanistically, IL-7R signaling is regulated at the level of IL-7 availability, IL-7R expression, and inhibition of downstream IL-7R signaling pathways. Transcription of the IL-7Rα chain is regulated by PU.1, which works together with signals downstream of Flt-3 to initiate expression of the IL-7Rα chain, while FoxO1 is critical in sustaining receptor expression [61–63]. Flt-3$^{-/-}$ mice display a block in commitment to the B lineage, identical to that observed for mice expressing a dominant negative form of Ras (dN Ras) [64]. This developmental defect was due to impaired Flt-3/Ras dependent proliferation of CLPs and pre-pro-B cells, as well as diminished IL-7Rα chain expression and elevated SOCS-2 and SOCS-3 expression. While PU.1 is necessary for B cell development, elevated levels can be detrimental to lymphopoiesis and PU.1 expression is kept in check, in part, by the transcriptional repressor Gfi-1 [65]. Gfi-1$^{-/-}$ mice contain defective CLPs and exhibit a block in development prior to B cell commitment that is the result of imbalanced IL-7R signaling [66]. Cells from Gfi-1$^{-/-}$ mice expressed elevated levels of the IL-7Rα chain, which may be the result of excessive PU.1 expression or lack of suppression of the IL-7Rα gene, a Gfi-1 target. Decreased STAT5 activation was also observed in Gfi-1$^{-/-}$ progenitor cells stimulated with IL-7, which was due to enhanced activation

of SOCS-3. The abnormal regulation of SOCS proteins that led to defective B cell commitment in Gfi-1$^{-/-}$, Miz-1$^{-/-}$, Flt-3$^{-/-}$ and dN Ras mice highlight the fact that a balance of IL-7R signaling must exist to allow for efficient B lymphopoiesis to proceed.

4. IL-7 regulation of B cell survival, proliferation and maturation

4.1. Cell survival

One of the main functions of IL-7R signaling during B cell development is to promote cell survival by regulating the localization and interaction of anti-apoptotic (Bcl-2, Bcl-xL and Mcl-1) and pro-apoptotic (Bax, Bad and Bim) factors. Both the JAK/STAT and PI3K/Akt pathways play key roles in mediating survival responses. IL-7R signaling enhances pro-B cell survival by increasing the ratio of Bcl-2 to Bax and, correspondingly, mice deficient for the IL-7R or JAK3 displayed increased levels of Bax [67,68]. Mcl-1 is another key mediator of B cell survival and its expression is directly regulated by STAT5 [30]. Mice deficient for Mcl-1 displayed a severe reduction in B lymphocytes as well as a block at the pro-B cell stage of development (reviewed in [69]). Akt regulates cell survival through the induction of Bcl-2 and Bcl-xL, while also inhibiting Bad by phosphorylating it and causing it to be retained in the cytosol where it remains inactive [70]. Finally, Bim has recently been shown to play a role in B cell survival, as Bim deficiency allowed for the partial recovery of B cell development in the absence of IL-7 [71,72].

4.2. Cell proliferation

Cell proliferation is another consequence of IL-7R signaling and is regulated through the induction and inhibition of positive and negative cell cycle regulators. p27Kip is a negative regulator of cell cycle activity and stimulation with IL-7 causes a reduction in its expression [73]. Inhibition of p27Kip function is brought about by distinct but overlapping mechanisms: Akt can directly phosphorylate p27Kip causing it to be retained in the cytoplasm where it is subsequently ubiquitinated and degraded, while Akt phosphorylation and inactivation of FoxO proteins prevent further transcription of p27Kip, a direct FoxO target [74,75]. IL-7 stimulation also regulates cell proliferation by inducing activators of cell cycle. As previously described, Erk activation downstream of IL-7R signaling is important for pro-B and pre-B cell proliferation. Activated Erk proteins are able to translocate to the nucleus where they phosphorylate a variety of TFs including Elk and CREB, which in turn induce the expression of proliferation associated targets Ilf2, Mef2c, Mef2d and myc [40]. Myc proteins are key transcriptional regulators that induce cell cycle progression by stimulating cyclin-dependent kinase (cdk) activity. N-myc and c-myc were both induced by IL-7 stimulation and required E2A activity for full function [76,77]. Additionally, B cell specific deletion of N-myc and/or c-myc led to a block at the pro-B and pre-B cell stages of development, while co-expression of IgH and Eμ-myc transgenes partially restored B cell development in JAK3$^{-/-}$ mice [78–80]. IL-7R signaling or constitutive STAT5 expression also induced the transcription of the ccnd2 and ccnd3 genes, leading to cyclin D2 and cyclin D3 protein expression, which function to promote cell cycle progression by activating cdks [25,29].

4.3. Maturation

In addition to providing survival and proliferation signals, IL-7 has also been implicated in promoting B cell maturation. Injection of IL-7 into IL-7$^{-/-}$ mice, as well as addition of exogenous IL-7 to CLPs *in vitro*, resulted in the generation of B220^{+}CD19^{+} pro-B cells that expressed TdT and cytoplasmic μ (cμ) [81]. In these cases, however, the question remained as to whether IL-7 actually triggered maturation or simply provided proliferative and/or survival signals. In an attempt to address this dilemma, Corcoran et al. inserted mutated IL-7Rα chain molecules into IL-7R$^{-/-}$ progenitor cells and determined what effect they had on cell proliferation and differentiation [35]. They observed that mutation of the Tyr449 site on the IL-7Rα chain resulted in an inhibition of proliferation due to lack of PI3K activation; but the capacity to differentiate to the cμ^{+} cell stage was retained. Conversely, replacement of the entire IL-7Rα chain with the cytoplasmic domain of IL-2Rβ resulted in maintenance of IL-7-induced proliferation, with a lack differentiation. From these studies it appears that signals from the IL-7R can promote B cell maturation and proliferation and these abilities may be mediated by different receptor domains.

The role that IL-7 plays in maturation at the pro-B to pre-B cell stage of development has been highly debated. Much of our understanding regarding the functional mechanisms of B cell development have come from *in vitro* studies, which utilize IL-7 to sustain and propagate B lineage cells in culture. Initial reports investigating the development of B cells in cultures containing IL-7 described a phenomenon in which withdrawal of IL-7 led to increased RAG expression, LC rearrangement, and percentage of IgM^{+} cells in culture [82–85]. These observations led to a hypothesis that IL-7 inhibited the development of pro-B cells into pre-B cells by keeping them in a proliferative state, and that maturation was only induced upon withdrawal of IL-7 from culture. However, theses studies failed to appreciate the highly dynamic and heterogeneous nature of *in vitro* B cell cultures. By carefully monitoring cell maturation in culture and counting absolute cell numbers, our lab determined that the absolute number of IgM^{+} cells that arose in culture was the same in the presence or absence of IL-7 [86]. Therefore, in culture, IL-7 does not prevent the maturation of pro-B/large pre-B cells, but rather selectively enhances their survival and proliferation. Consequently, withdrawal of IL-7 leads to the death of pro-B/large pre-B cells, which results in a relative, but not absolute, increase in small pre-B and immature B cells, suggestive of increased maturation. While IL-7 did not inhibit the maturation of pro-B cells into small pre-B and immature B cells it also did not lead to the survival or proliferation of these more mature populations, demonstrating that after cells reach the small pre-B cell stage they become unresponsive to IL-7 [86,87].

5. IL-7 regulation of V(D)J recombination

5.1. IL-7 regulation of heavy chain recombination

Additional roles for IL-7 in promoting B cell development have been proposed based on studies demonstrating that IL-7R signaling can affect both HC and LC recombination. Several reports have noted that IL-7R activation of STAT5 promoted chromatin accessibility through histone acetylation of the distal V_H genes in pro-B cells [88,89]. In small pre-B cells, which are non-responsive to IL-7, distal V_H sites were hypoacetylated and thus non-accessible, providing a possible IL-7-mediated mechanism for allelic exclusion of the HC locus by restricting recombinase access. Additionally, STAT5$^{-/-}$ mice displayed reduced germline transcription and rearrangement of V_HJ558 genes, while pre-B cells from mice expressing a constitutively active STAT5 displayed increased distal V_H recombination, a failure to "decontract" the IgH locus, and a lack of IgH and IgL association [88,90]. Conversely, rearrangement of the distal V_H genes was found to be normal in Bcl-2 rescued STAT5$^{-/-}$ pro-B cells, suggesting that STAT5 may not be absolutely essential in this process [30]. Clarification regarding these contradictory findings will require further investigation into the possibility that early activity of STAT5 in CLPs, prior to Cre-Rag-mediated deletion, might allow for subsequent V_H rearrangement and development to proceed.

5.2. IL-7 regulation of light chain recombination

The role of pre-BCR signaling in LC rearrangement has been substantially supported, however, the role that IL-7 plays in this process has been debated. While it has been demonstrated that LC rearrangement and the production of μ^+ cells occurs in the presence of IL-7, a potential role for IL-7 in regulating the efficiency of this process remains. Decreased concentrations of IL-7 in cultures containing IRF4$^{-/-}$IRF8$^{-/-}$ cells, or IRF4$^{-/-}$IRF8$^{-/-}$ cells in which IRF4 was reintroduced, resulted in an increased percentage of μ^+ cells [91]. Increased recombination was also observed in BLNK$^{-/-}$ cell cultures upon removal of IL-7 or treatment with a PI3K inhibitor [92]. In both of these studies, cell cycle arrest was insufficient to initiate LC recombination, suggesting that activation, or the removal of inhibition, by other signaling pathways was necessary. It should be noted that in these studies the measurement of recombination was determined by percentage and was thus relative. Also, the loss of IL-7-responsive μ^- pro-B/large pre-B cells that normally expand in culture when IL-7 is present must be considered and accounted for when attributing observed changes in development solely to the absence of IL-7. In both situations, low levels of LC rearrangement were observed in the presence of high concentrations of IL-7, demonstrating that IL-7 signaling does not completely inhibit LC recombination. In the case of BLNK$^{-/-}$ cells, sustained PI3K activation led to Akt phosphorylation of FoxO proteins, which decreased the efficiency of recombination [92]. Reintroduction of an inducible BLNK protein, or a mutated FoxO3a protein that was unable to be phosphorylated by Akt, resulted in an increased proportion of κ^+ cells. Correspondingly, mice deficient for FoxO3 display a reduced frequency of pre-B cells in the BM as well as reduced recirculating B cells in the blood and BM [93]. From these studies, a model arises in which activation of the PI3K/Akt pathway downstream of the IL-7R and/or the pre-BCR limits recombination by phosphorylating FoxO proteins; this inhibition is partially alleviated by the subsequent activation of BLNK downstream of the pre-BCR, which prevents Akt activity by a yet to be elucidated mechanism (Fig. 1).

The Igk locus is regulated by two enhancers, the intronic enhancer (iEκ) and the 3′ enhancer (3′Eκ). Deletion of either enhancer resulted in reduced Igκ rearrangement, while deletion of both enhancers completely abolished recombination [94]. Increased recombination in the absence of IL-7 in IRF4$^{-/-}$IRF8$^{-/-}$ cell cultures was shown to be independent of signals from the pre-BCR, and instead, was the result of increased Rag expression and histone H4 hyperacetylation as well as increased binding of E2A at the iEκ enhancer [91]. It should be noted that in these studies the IL-7 concentration was merely reduced and not fully removed. In other studies in which IL-7 was completely removed from IRF4$^{-/-}$IRF8$^{-/-}$ cell cultures, no increase in κ expressing cells was observed [95]. Our lab has previously shown that even at picogram concentrations, IL-7 is still able to exert physiological effects on receptive cells and work in conjunction with signals from the pre-BCR to affect downstream targets [36]. Further evidence that IL-7R signaling normally regulates LC recombination was provided when it was shown that Bcl-2 rescued STAT5 deficient pro-B cells displayed a six-fold induction of Vκ–Jκ recombination as well as increased κ^0 germline transcripts [30]. A potential mechanism for this inhibition came from studies that showed that STAT5 bound directly to the iEκ enhancer in an IL-7-dependent manner and limited E2A accessibility [25,30].

5.3. IL-7 regulation of Rag protein expression

Rag1 and Rag2 are expressed at distinct stages during B cell development and are absolutely essential for antigen receptor rearrangement. A number of TFs regulate the expression of Rag genes, including Pax5, and mice deficient for E2A, Ikaros or FoxO1 all display a deficiency in Rag expression (reviewed in [96]). FoxO1 is a key factor in this system, as it directly regulates Rag1 and Rag2 expression and conditional deletion of FoxO1 resulted in impaired B cell development at the early pro-B and small pre-B cell stages, precisely when Rag activity is necessary [61,97]. Furthermore, deletion of the mTORC2 subunit Sin-1, which is required for Akt phosphorylation of FoxO1, resulted in increased IL-7R and Rag expression [98]. Therefore, IL-7R and pre-BCR regulation of FoxO1 activity is of critical importance during B cell development. Activation of the MAPK/Erk pathway downstream of BCR activation has also been shown to reduce Rag transcription by phosphorylating E47, which inhibited its binding to the Rag enhancer regions [99]. While it has yet to be shown if activation of Erk downstream of the pre-BCR or IL-7R utilize this mechanism, it could provide and additional level of regulation of Rag expression that may work in synergy between these receptors. Turnover of Rag proteins is also important, because Rag2 stability is cell cycle regulated and accumulates in the G0 and G1 phases, but is degraded in the S phase [100,101]. Therefore, IL-7R signals that keep cells in a proliferative state by inducing cell cycle progression would indirectly reduce Rag2 levels. These observations suggest a model in which IL-7R signaling must be kept under tight control during the pre-pro-B cell stage of development to limit proliferation and allow for FoxO activation of Rag proteins, both of which are necessary for HC rearrangement. Increased IL-7R signaling in pro-B cells would result in cell proliferation and survival, while also inhibiting FoxO transcription of Rag and p27Kip genes, and pre-BCR signaling in large pre-B cells would enhance this activity *via* the PI3K/Akt and MAPK/Erk pathways. Inhibition of FoxO activity is alleviated in small pre-B cells by the termination or alteration of IL-7R and pre-BCR signals, thus allowing for re-expression of Rag and p27Kip leading to LC rearrangement (Fig. 1).

6. Regulation, modification and termination of IL-7R signaling

6.1. Modulation of IL-7R signaling by CD45 and the pre-BCR

A variety of signaling pathways are initiated in developing B cells. The factors activated by these pathways can interact with signaling molecules downstream of the IL-7R, modifying the magnitude, duration or quality of the response. B220 is the 220-kDa isoform of the surface membrane phosphatase CD45. CD45 is present on all mouse B cells, except terminally differentiated plasma blasts, as well as in varying isoforms on dendritic cells, T cells, macrophages and NK cells. CD45 dephosphorylates Src kinases leading to their activation or inhibition depending on the tyrosine residue targeted, and in its absence, Lyn is constitutively phosphorylated (reviewed in [102]). CD45$^{-/-}$ mice exhibit reduced proliferation of mature B cells in response to foreign antigens but not to mitogens such as lipopolysaccharide (LPS) [103]. Increased numbers of early pro-B cells were also observed in CD45$^{-/-}$ mice and this population was elevated in *in vitro* cultures initiated with CD45$^{-/-}$ precursors [104]. This result was due to the increased survival of pro-B cells, which displayed prolonged JAK and STAT phosphorylation in response to IL-7. These observations highlight that IL-7R signaling is normally kept in check and that one function of CD45 in developing B cells is to limit IL-7R signaling.

Signals downstream of the pre-BCR have also been shown to converge with and regulate those initiated by the IL-7R. As described earlier, Erk is phosphorylated downstream of both the IL-7R and the pre-BCR, and allows for cell proliferation and survival in picogram concentrations of IL-7 [36]. Further evidence demonstrating the importance of signals emanating from these receptors was provided when it was observed that mice deficient for both the IL-7R and pre-BCR displayed defects greater than observed with the

loss of either alone [105]. Signals emanating from the pre-BCR have also been proposed to subsequently limit pre-BCR and IL-7 proliferation. Ikaros and Aiolos were shown to bind directly to the c-myc promoter and repress its expression, which resulted in increased expression of p27Kip and repression of ccnd3 [106]. As previously discussed, large pre-B cells must come out of cell cycle prior to rearranging their LC genes, and pre-BCR activation of Ikaros and Aiolos appears to be one way by which this outcome is accomplished.

6.2. Modulation of IL-7R signaling by IFN-γ

Signaling pathways downstream of a variety of cytokine receptors have been shown to intersect and both positively and negatively regulate B cell survival, proliferation and maturation. IFN-γ, a type II interferon produced mainly by T cells and NK cells, possesses antiviral activity that is mediated through the regulation of cell growth, differentiation, apoptosis and antigen presentation (reviewed in [107]). The IFN-γ receptor is composed of two IFN-γR1 chains and two IFN-γR2 chains, which dimerize after binding to IFN-γ, leading to transphosphorylation of constitutively associated JAK proteins and subsequent recruitment and activation of STAT1

Treatment of B cells with IFN-γ suppressed IL-7-mediated pre-B cell colony formation and abrogated IL-7-induced proliferation of *ex vivo* pre-B cells and pre-B cell lines resulting in their apoptotic death [108,109]. However, IFN-γ did not exert an affect on small resting pre-B cells, nor did IFN-γ lead to increased apoptosis of Eμ-Bcl-2 transgenic pre-B cells; even though IL-7 proliferative signals were inhibited. Elevated expression of IFN-γ *in vivo* yielded similar results, whereby IFN-γ transgenic mice displayed significantly reduced B cell numbers in the periphery and BM, with cells arrested at the pro-B cell stage [110]. Administration of exogenous IL-7 was unable to correct this defect, suggesting that these cells were unable to respond to IL-7. Mice deficient for T cell protein tyrosine phosphatase (TC-PTP) also possessed a decreased number of pre-B cells, which was due to increased IFN-γ production by BM stromal cells [111]. TC-PTP$^{-/-}$ pre-B cells displayed constitutive phosphorylation of STAT1 but decreased JAK1 and STAT5 activation after IL-7 stimulation. Regulation of mitochondrial apoptotic factors was shown to be important for IFN-γ-mediated apoptosis of pre-B cells, as evidenced by the fact that IL-7-dependent pre-B cells treated with IFN-γ displayed decreased Bcl-2 and Bcl-xL expression, as well as increased expression of the pro-apoptotic molecule Diablo [112]. These findings demonstrate that IFN-γ can inhibit the proliferation and survival of B cell progenitors, but do not fully address the mechanism by which inhibition occurs.

We have recently published a study that sheds some light on the mechanism of action of IFN-γ-mediated B cell death. These studies were initiated utilizing stromal cell-independent IL-7-dependent pro-B and pre-B cell lines that were established by cloning B220^{+} BM cells that had been cultured in IL-7 [113]. One particularly useful line was a pre-B cell line that was selected for its ability to survive independent of IL-7, but retained the capacity to signal and proliferate in response to IL-7. Treatment of this line with IFN-γ alone did not lead to reduced proliferation or cell death; however, IFN-γ did inhibit IL-7-induced cell proliferation [87]. These results are in line with those utilizing Eμ-Bcl-2 cells and highlight the fact that IFN-γ does not inherently cause pro-B/large pre-B cell death. Instead, initiation of apoptosis in IFN-γ treated cells is likely due to inhibition of IL-7 survival and proliferation signals. We have shown that stimulation of pre-B cells with IFN-γ led to a robust expression of SOCS-1 and SOCS-3 [87]. Previous studies have described that induction of SOCS proteins after treatment with IFN-γ inhibited STAT activation downstream of IL-4 or TNF-α stimulation [114–116]. In light of these findings, we suggest a novel mechanism of action for IFN-γ induced inhibition of pro/large pre-B cell proliferation and survival, in which IFN-γ signaling activates SOCS proteins that function not only to limit IFN-γ signaling, but also inhibit signals mediated by the IL-7R.

While these studies demonstrate the potential of IFN-γ to regulate B lymphopoiesis, the physiological role of IFN-γ-mediated inhibition of IL-7 signaling has yet to be elucidated. Interestingly, infection and inflammation responses in the mouse lead to preferential myelopoiesis and extramedullary B lymphopoiesis, which is mediated in part by inhibited production of CXCL12 and SCF by BM stromal cells (reviewed in [117]). IFN-γ is a key factor produced during infectious responses and its activation may regulate B cell development in these situations by inhibiting IL-7R signals. In line with this hypothesis, IL-15, a cytokine important for NK cell development under naïve and inflammatory conditions, selectively expanded Mac-1^{+}B220^{+} NK cells in the BM, which were then capable of inhibiting pre-B cell proliferation and survival in an IFN-γ-dependent manner [118]. Finally, recently emigrated IgD^{-}CD21^{-} immature B cells in the periphery have been shown to be able to produce low levels of IL-15 and IFN-γ [119,120]. If one or both of these factors are produced by immature B cells in the BM they could potentially play a role in regulating the development of earlier precursors, either directly or through the development of NK cells.

6.3. Regulation and termination of IL-7R signaling

Having highlighted the many functions that IL-7 plays during B cell development, one thing remains clear; regulation of IL-7R signaling is critical. Local availability of IL-7 is key, and removal of IL-7 is the most direct manner by which IL-7R signaling can be abrogated. As previously described, IL-7 is produced by stromal cells in the BM, which are a class of large adherent cells that include fibroblasts, reticular cells, preadipocytes, endothelial cells, and macrophages. The distribution of B cells in the BM is dependent on their interaction with stromal cells, which utilize chemokines, such as CXCL12, to attract cells to distinct cellular niches [121].

CXCR4, the receptor for CXCL12, is expressed on subsets of developing B cells and both CXCR4 and CXCL12 are essential for B lymphopoiesis at the earliest stages of development [122]. Different classes of stromal cells produce varying levels of IL-7 and CXCL12, and expression of these factors has been shown to vary under certain conditions, such as cytokine stimulation, pregnancy and ageing [123–125]. *In vivo*, pre-pro-B cells were found to be associated with CXCL12^{Hi} expressing cells, while pro-B cells associated with IL-7 producing stromal cells that expressed low levels of CXCL12 [126]. Small pre-B cells have been shown to up-regulate CXCR4, and this expression has been proposed to initiate cell migration away from IL-7 producing stromal cells [91,127]. Immature B cells on the other hand, were found not to be associated with CXCL12 or IL-7 producing cells, thus allowing for their emigration out of the BM and into the periphery [126]. Interestingly, the ATPase ATP11c has recently been identified as an essential molecule in BM mediated development of B lineage cells, and functions to control the structure of cellular membranes. Mice deficient for ATP11C display a significant reduction in pro-B, pre-B and immature B cells in the bone marrow, as well as a diminished response to IL-7, yet exhibit normal FL lymphopoiesis and marginal zone B cells [128,129]. While it has yet to be determined exactly how this molecule mediates its action, it highlights the complex and fundamental importance of stromal cell–B cell interactions.

It is also possible that the local concentration of IL-7 is dependent on B cell consumption rather than stromal cell production. The greater the number of IL-7R molecules present on the cell surface, as well as the number of cells expressing the IL-7R that exist in any given environment, the more IL-7 that may be consumed and the less that would remain for other cells to utilize. This effect is

amplified by the fact that stimulation with IL-7 leads to cell survival and proliferation, resulting in the generation of daughter cells that continue to consume IL-7 until they either move away from the IL-7 source, terminate IL-7R signaling, or decrease IL-7R surface expression. This model has been proposed for regulating T cell development in the thymus (reviewed in [130]). During B cell development, pro-B and large pre-B cells proliferate in response to IL-7 and/or pre-BCR expression, and in doing so may effectively bring about their death by diluting out local IL-7 concentrations. However, expression of a functionally rearranged pre-BCR on the cell surface allows large pre-B cells to proliferate in low concentrations of IL-7, giving them a survival advantage [36]. Our lab has suggested that as B cells develop they are exposed to gradient concentrations of IL-7 that are either created by stromal cell niches or local consumption of available IL-7, which provides a mechanism to positively select for functionally rearranged cells [36,131]. While it has been shown that different B cell subsets associate with different types of stromal cells, it is not possible at this time to determine the direct effect that consumption has on the availability of IL-7 *in vivo*, because conventional immunohistochemistry is not sensitive enough to visualize the IL-7 protein in tissues.

While consumption and migration away from a source of IL-7 are two ways in which cells can limit their exposure to IL-7, other mechanisms also exist. This is highlighted by the fact that both IL-7-responsive and IL-7-non-responsive populations exist in B cell cultures matured *in vitro* under conditions in which cells are provided with excess amounts of IL-7 and are unable to migrate away from the IL-7 source [86]. We and others have demonstrated that the IL-7R is expressed on IL-7-responsive pro-B and large pre-B cells, while more mature small pre-B and immature B cells maintain IL-7R expression, but are unable to respond to IL-7 [81,87,104,132,133]. We have recently shown that SOCS proteins play a role in regulating IL-7R signaling in pro-B and pre-B cells. IL-7-induced the expression of SOCS-1 and SOCS-3 in developing B cells and the magnitude of this induction was dependent on the concentration of IL-7 [87]. Thus, as B cells respond to IL-7 they also initiate negative feedback mechanisms that regulate the extent of IL-7R signaling. When this balance is disrupted, such as in $CD45^{-/-}$ mice or by enforced expression of SOCS-1, B cell development is altered [87,104]. IFN-γ, together with IL-7, synergistically increased the expression of SOCS-1 and led to inhibition of IL-7-induced proliferation; a similar phenomenon was observed with IL-21 and IL-7 [87]. Our model system allowed us to alter the levels of SOCS proteins expressed in developing B cells and demonstrated that once a threshold of SOCS expression is reached, further IL-7R signaling is abrogated. Investigation into the expression levels of SOCS-1 and SOCS-3 in developing B cell populations revealed that the small pre-B cell population, which is non-responsive to IL-7, expressed the highest levels of SOCS-1 [87]. We therefore propose that it is the elevated level of SOCS proteins in small pre-B cells that contribute to their IL-7-non-responsive nature. While we have demonstrated that IL-7, IFN-γ and IL-21 can all alter SOCS expression, it has yet to be shown if any or all of these factors directly contribute to the elevated levels of SOCS expression observed in small pre-B cells. What can be concluded is that a variety of known and possibly yet to be identified factors work together to regulate a SOCS-mediated "rheostat" that controls the magnitude of response to IL-7 in developing B lineage cells.

7. Conclusion

IL-7 is a key factor that regulates B lymphopoiesis and plays multiple roles in the commitment, survival, proliferation and differentiation of B cell progenitors. During development, B cells receive both activation and inhibition signals from IL-7, which are modified by the amount of IL-7 present and the activation of other signaling pathways (Fig. 1). Certain aspects of the IL-7R signaling pathway have been well characterized, however, others have yet to be fully elucidated, including; the degree to which IL-7 activation regulates FoxO protein activity at various stages of development, the role of Src kinase phosphorylation downstream of IL-7R activation, and the identification of the signaling intermediates that lead to Erk activation. Additionally, while several studies have pointed to an instructive role for IL-7 in the commitment of cells to the B lineage as well as HC recombination, recent evidence suggests that these functions may not be as conclusive as originally thought. However, the inhibitory role that IL-7 plays in the efficiency of LC recombination is becoming more evident and further studies will help to determine the detailed mechanisms by which IL-7 exerts this effect.

While factors such as c-Kit, Flt-3L, and IL-11 work with IL-7 to enhance cell survival and proliferation, signals downstream of IFN-γ and CD45 limit IL-7R activation; and the pre-BCR appears to both positively and negatively modulate IL-7R signals. It is becoming increasingly clear that expression and signaling downstream of the IL-7R is not a on or off situation, but one that is highly regulated and kept in balance by a dynamic network of TFs and signaling molecules. SOCS proteins are emerging as important members of this network that function not only as a feedback inhibitors, but also play key developmental roles during B cell commitment as well as during the pro-B to pre-B cell transition, by modulating a B cell's ability to respond to IL-7. Regulation of IL-7R expression along with SOCS protein levels allows B cells to control their ability to respond to IL-7-independently of external IL-7. This internal control in conjunction with external regulation of IL-7 production and availability provides a model in which cells can be positively selected and expanded during various stages of development, ensuring that functional HC rearrangement has taken place while not inhibiting further LC rearrangement and maturation.

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