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Modulation of IL-7 Thresholds by SOCS Proteins in Developing B Lineage Cells

Steven A. Corfe,* † Robert Rottapel, †‡‡,§ and Christopher J. Paige∗ †,‡,§

During B lymphopoiesis, IL-7 induces survival, proliferation, and differentiation signals that are important during the pro-B to pre-B cell transition. We showed that murine small pre-B stage cells do not signal or proliferate in response to IL-7, yet they maintain IL-7R surface expression. Loss of proliferative responsiveness to IL-7 is mediated by suppressor of cytokine signaling protein 1 (SOCS-1), the expression of which is regulated during B lymphopoiesis, with the highest levels observed in small pre-B cells. SOCS-1 inhibits IL-7 responses in pre-B cell lines and ex vivo B lineage cells. SOCS-1 expression and, thus, responsiveness to IL-7, can be regulated by IL-7 itself, as well as IFN-γ and IL-21. Additionally, the transcriptional repressor Gfi-1b enhances the proliferative responsiveness of B cells to IL-7. We demonstrated that these molecules act together to form a SOCS-mediated “rheostat” that controls the level of IL-7R signaling in developing murine B lineage cells. The Journal of Immunology, 2011, 187: 3499–3510.

B lymphopoiesis is a regulated process during which B cell progenitors undergo rearrangement of their Ig loci, leading to the eventual expression of a functional BCR and other critical proteins that mediate a response to Ag. During development, committed B cell progenitors (pro-B cells) proliferate in response to IL-7 and begin recombination of their µ H chain proteins (µHCs). µHCs associate with surrogate L chain (LC) proteins VµH and L5 to form the pre-BCR, which is expressed on the surface of newly formed pre-B cells. Successful expression and signaling of the pre-BCR activates survival, proliferation, and differentiation pathways that lead to the selection and expansion of large pre-B cells (1). Large pre-B cells exit the cell cycle, become resting small pre-B cells, and once again begin the recombination process, resulting in rearrangement of the LC proteins. LC proteins associate with µHCs to form the BCR, which is first expressed on immature B cells in the bone marrow (BM).

IL-7 is an essential cytokine during murine B cell development and is produced by cells in the BM, spleen, thymus, and fetal liver (2). Mice with targeted deletions of IL-7 or the IL-7R display a severe block at the early pro-B cell stage of development (3, 4). Increased levels of IL-7R signaling can also alter development, because overexpression of the IL-7R on multipotent progenitor cells leads to a block in B lymphopoiesis prior to CD19 expression (5). Regulation of IL-7R signaling is also critical at later stages of B lymphopoiesis, and a number of recent studies demonstrated the importance of controlling IL-7R signaling in pro-B and small pre-B cells (6–9). During this stage of development, a balance between signals mediated by the IL-7R and the pre-BCR occurs, in which signaling through the IL-7R maintains cells in a proliferative state and inhibits LC recombination, whereas signaling through the pre-BCR brings cells out of cycle and promotes LC rearrangement.

The IL-7R is a heterodimer composed of the IL-7Rα-chain and the common γ-chain. IL-7R binding causes heterodimerization of α- and γ-chains and allows for the trans-phosphorylation of constitutively associated JAK proteins, which leads to IL-7Rα-chain phosphorylation (10). Receptor phosphorylation allows for the activation of Src kinases (Lyn/Fyn/Btk) and creates docking sites for the Src homology 2 (SH2)-containing proteins PI3K and STAT5. As with the majority of ILs, the downstream effects of IL-7R activation depend on the signaling context in responsive cells. In this report, we demonstrate that the suppressor of cytokine signaling (SOCS) family of proteins controls the contextual signaling for IL-7 in developing B cells.

SOCS proteins bind directly to JAK and STAT proteins, as well as cytokine receptor chains, and prevent their interaction, as well as target them for proteasomal degradation (11). In many cases, SOCS expression is induced by the same receptor pathway that it inhibits, providing a negative feedback loop that regulates signaling and limits receptor activation. SOCS-1 and SOCS-3 family members are closely related in structure and have similar, but nonredundant, functions in a variety of immune cells (12). SOCS-1−/− mice die within 3 wk of birth because of monocytic infiltration of multiple organs and fatty liver degeneration (13). SOCS-1−/− mice also have greatly reduced peripheral B cell numbers, which is attributed to the increased apoptosis of pre-B cells unable to initiate negative-feedback mechanisms that limit IFN-γ signaling. IFN-γ was shown to inhibit the response of pre-B cells to IL-7, resulting in reduced proliferation and, ultimately, cell death (14–17). Confirmation that IFN-γ signaling is required for the B cell defect in SOCS-1−/− mice was shown in SOCS-1−/−/IFN-γ−/− double knockout mice, as well as SOCS-1−/− mice injected with IFN-γ-blocking Abs (18, 19). In both of these cases, peripheral B cells recovered to almost normal levels.
It has been established that a B cell’s response to IL-7 is modified by both the amount of IL-7 present and the activation of other signaling pathways. For example, we previously showed that large pre-B cells proliferate in low (picogram) concentrations of IL-7 only in the presence of signals downstream of the pre-BCR (20). Pro-B cells, which lack the pre-BCR and, thus, the signals mediated by this receptor, proliferate only in response to higher (nanogram) concentrations of IL-7. As B cells mature from the pro-B to pre-B stage of development, they reach a point at which they stop proliferating in response to IL-7 (21). Loss of responsiveness to IL-7 is associated with a number of key differentiation events; however, the molecular mechanisms leading to nonresponsiveness remain unknown. Thus, we examined this stage of differentiation using both primary B lineage cells and IL-7–dependent B cell progenitor lines. We found that the response to IL-7 is largely controlled by SOCS-1, and its expression can be influenced by a number of cytokines including IL-7 itself, IL-21, and IFN-γ. We propose a model in which regulated levels of SOCS-1 act as a “thermostat” to control the ability of developing B lineage cells to respond to IL-7.

Materials and Methods

Mice

C57BL/6 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 4–8 wk of age. All mice were maintained at the Ontario Cancer Institute animal facility and used between 6 and 12 wk of age. All animal procedures conformed to institutional animal protocol guidelines.

Isolation of B cells

B cell progenitors were isolated from the BM of 6–12-wk-old mice for all experiments. Single-cell BM suspensions were prepared by crushing and gently grinding leg bones using a mortar and pestle and then flushing with PBS/FCS (PBS + 5% heat-inactivated [HI] FCS) or MACS buffer (PBS + Ca2+/Mg2+ + 1 mM EDTA, 0.25% BSA).

MACS

BM cells were selected for expression of either B220 or CD19 by MACS (Miltenyi), as described previously (22). Briefly, cells were isolated from BM using either anti-B220 bead-coupled or anti-CD19 Abs. For CD19 selection, cells were then incubated with a goat anti-rat bead-coupled Ab (Miltenyi). B220+ or CD19+ cells were positively selected in a Vario-MACS magnet with an LS adaptor and eluted with 10 ml OptiMEM (Life Technologies) + 10% non-HI (NIH) FCS. Typically, 10–20 × 10^6 B220+ or CD19+ cells were recovered from BM. Isolation by MACS resulted in >95% purity, as assessed by flow cytometry.

FACS

RBCs were removed from BM cells by resuspending centrifuged cells in 1 ml ACK solution and incubating for 3 min on ice. Incubation was followed by the addition of 9 ml PBS/FCS and centrifugation. Cells were resuspended in 500 μl PBS/FCS, stained with appropriate Abs, and enriched for the desired populations using either MoFlo (Cytofetron) or Aria (BD Biosciences) cell sorters. Sorting strategies and postsort purities for B cell subsets are displayed in Supplemental Fig. 1.

Culturing of primary B cells and B cell lines

Primary B cell cultures were initiated from FACs- or MACS-selected BM by seeding cells in 24-well plates at a density of 5 × 10^5 cells/ml in 2 ml OptiMEM (+ 10% NIH FCS, 50 μM 2-ME, 2.4 g/l NaHCO3, and 100 μg/ml kanamycin or karsamycin). Supernatant from the J558 cell line, which had been stably transfected with the IL-7 gene, was used as a source of IL-7 (supplied by Dr. Ana Cumanos, Institute Pasteur, Paris, France). The concentration of IL-7 used in culture was 5 ng/ml, unless otherwise stated. Cells were cultured in a humidified atmosphere at 37°C and 5% CO2. An aliquot of cells was removed and cultured in fresh media otherwise stated. Cells were cultured in a humidified atmosphere at 37°C in 95% CO2 and 5% HI FCS was used instead of 10% NHI FCS. The B62.1 cell line is a novel cell line that no longer required IL-7 for its growth and survival but was still responsive to IL-7.

Immunofluorescence staining and flow cytometric analysis

Primary BM or cultured cells were washed in PBS/FCS and resuspended in 100 μl PBS/FCS. For surface phenotyping, cells were labeled for 15 min on ice in a total volume of 100 μl. The following Abs were used: anti-B220 (RA3-6B2), anti-CD19 (MB19-1), anti-CD117 (ACK2), anti-CD2 (RM2-7; BD-Pharandm), anti-IgM (33,60; Lab Made), anti-IgD (11-26c), anti-IL-21Rα (ebio4A9), anti-IL-7Rα (7R34), and anti-IL-7Rγc–chain (4G3; BD-Pharandm). All Abs were purchased from eBioscience, unless otherwise stated. For biotin-conjugated Abs, cells were washed twice after primary staining and then incubated with a streptavidin–conjugated PerCP Ab (BD-Pharandm) for 15 min on ice. FACS was performed using a FACSCalibur (BD Biosciences), and FlowJo (version 7.6) software was used for analysis.

Cell stimulation and Western blot analysis

Cell stimulation and Western blot analysis were performed as previously reported (22). Briefly, cells were washed three times in media prior to being incubated for 1 h at 1 × 10^5 cells/ml (cultured) or 5 × 10^5 cells/ml (primary). Cells were stimulated by the addition of 15 μg/ml F(ab’)2 goat anti-mouse μhC (Jackson ImmunoResearch), 25 ng/ml murine IL-7, or both for indicated time periods. Cells were lysed in 200 μl lysis buffer on ice for 1 h. Protein samples were run on a 10% gel, transferred to a nitrocellulose (Invitrogen) and 0.7 M 2-ME and then resolved on a Bis-Tris gradient gel prior to being transferred to a polyvinylidene difluoride membrane in transfer buffer and blocked in TBST with 5% milk for 2 h. Membranes were then washed in TBST and probed for p-ERK1/2, p-STAT5, or p-JAK1. Membranes were washed in TBST and subsequently probed with goat anti-rabbit IgG–HRP (Santa Cruz Biotech) for 1 h. After washing in TBST, membranes were developed using an ECL detection kit (Amersham Biosciences). For loading controls, membranes were stripped using the Re-Blot Plus recycling kit (Chemicon International) and then washed in TBST, blocked in milk, and reprobed for total ERK1/2 or β-actin.

[3H]thymidine-incorporation assay

Cell proliferation was measured by incorporation of [3H]thymidine (Perkin Elmer). Cell lines were plated in triplicate at 2500 cells/well in OptiMEM (+ 5% HI FCS) in 96-well flat-bottom plates (Costar) with varying concentrations of IL-7. Cells were cultured for 3 d; on the final day of culture, 0.5 μCi [3H]thymidine was added prior to incubation for another 6 h. Cells were harvested onto microplate filters, and scintillation fluid was added to each well prior to being read in a scintillation counter (TopCount Systems, Canberra Packard). All cytokines were added on day 0 of culture.

IL-7 limiting-dilution assay

To determine the frequency of IL-7–responding cells, primary BM B cells were harvested, selected by FACs, counted, and plated at limiting dilutions of 1000, 500, 250, and 125 cells/wells or 200, 100, 50, and 25 cells/well in IL-7–supplemented OptiMEM (+ 10% NIH FCS) in 96-well plates. Each cell concentration was repeated in 24 wells. After 4 d, plates were scored visually for colony growth, with wells containing clusters of ≥50 live cells scored as positive. The frequency of IL-7–responsive cells was determined according to Poisson distribution.

RT-PCR and real-time PCR

RNA was extracted from FACs- or MACS-selected primary B cells, as well as IL-7–expanded B cells and B cell lines, using TRizol reagent (Invi- tropen), as per the manufacturer’s instructions. Five micrograms of RNA were transcribed in the presence of Superscript II (Invitrogen), as per the manufacturer’s instructions. After reverse transcription, soy-c1, soy-c3, fn-v, gfi-1b, and β-actin were amplified by RT-PCR. The following PCR primers were used: sox-c1, 5’-GCCAGCTTGGAAGGAGCTGAGGGA-3’ (sense) and 5’-GCTCCACCTGTGATTACCCGCGG-3’ (antisense); sox-c3, 5’-GCATCGAATTACCTGGAAAC-3’ (sense) and 5’-CAAGTCTTGGATGAACTG-3’ (antisense); fn-v, 5’-ATCTTTGGAGACCCTCTGACTT-3’ (sense) and 5’-TAATGCTGGTCCCAGGATTT-3’ (antisense); gfi-1b, 5’-CGCAGCTATTGCACGCACTTCTCTTGA-3’ (sense) and 5’-AGGTTTGTTTCTTCTAGTCC-3’ (antisense); and β-actin, 5’-TCCCTGGAGAAGGTCACGGA-3’ (sense) and 5’-ATCGGTGCGGAGTGGC-3’ (antisense).
(antisense). PCR reactions were set up using 1 μl cDNA and run for 25–35 cycles. PCR products were separated by electrophoresis on an agarose/Tris-acetate-EDTA gel and visualized by ethidium bromide staining. For real-time PCR, RNA was extracted as described for RT-PCR. After reverse transcription, socs-1, socs-3, and β-actin were amplified by real-time PCR, according to the manufacturer’s instructions (Applied Biosystems). The following PCR primers were used: socs-1, 5′-TGTTGGTGAGGGTG-GAGT3′ (sense) and 5′-CTCCAGACA-AAGTCCTACA-3′ (antisense); socs-3, 5′-TGAAGCTTGAAGGCCACCTCG-3′ (sense) and 5′-GCCAGT-CGAACCGGGAACT-3′ (antisense); and β-actin, 5′-GCAACCCTGTT-GAAAGTACCCAGC-3′ (sense) and 5′-AAGACAGAGGCTACACAGG-ACAG-3′ (antisense). β-actin was used for sample normalization.

Viral infections

Generation of plasmids and GP+E packaging cell lines expressing MIEV retroviral constructs (GFP, SOCS-1, SOCS-1 SH2, and SOCS-1 ACT) were described previously (23, 24). MigR1 retroviral constructs (GFP and Gfi-1b) were obtained from Dr. Barbara Kee (Department of Pathology, University of Chicago, Chicago, IL) and were described previously (25). For infection of B cells, GP+E cells expressing retroviral constructs were irradiated with 2000 rad prior to plating at 4 × 10^5 cells/well in a six-well plate and culturing overnight. The following day, an equivalent number of target cells (MACS-selected BM or IL-7–dependent cell lines) was added to GP+E cultures in a total volume of 5 ml. Cells were cultured in Opti-MEM (+10% FCS) supplemented with IL-7 and 1:4000 polybrene (8 mg/ml stock). An aliquot of cells was taken on subsequent days and checked for infectivity by analyzing for GFP expression. Positive cells were enriched for based on GFP expression and checked for purity prior to being put back in culture.

Results

As B lineage cells mature to the CD22 stage of development, they fail to signal or respond to IL-7

Because of the important role that IL-7 plays during B lymphopoiesis, we tested various B cell subsets for their ability to signal and respond to IL-7. B220+ BM (BM_{B220+}) cells were enriched for pre-B/large pre-B (CD2+IgM+), small pre-B (CD2*IgM+), and immature (CD2*IgM+) B cell populations and plated at varying cell densities with IL-7. Cultures were visually inspected on day 4; we observed that the frequency of IL-7–responsive cells in the CD2*IgM+ population was 1 in 15, whereas the CD2*IgM+ and CD2* IgM+ subsets failed to yield any live cell clusters (≥1 in 1000). Although CD2* IgM+ and CD2* IgM+ cells are unable to respond to IL-7 and die in culture, we were still able to experimentally investigate these populations because they reproducibly arise in cultures containing CD2* IgM+ cells. Flow analysis of B cell fractions from freshly isolated BM and day-4 IL-7–expanded BM_{B220+} revealed that expression of the IL-7Rα is highest on CD2* IgM+ cells and is decreased, but still positive, on CD2* IgM+ and CD2* IgM+ populations (Fig. 1A). The level of expression of the IL-7Rγ-chain was approximately equal in the CD2* IgM+ and CD2* IgM+ populations, whereas higher expression was present on CD2* IgM+ cells.

Freshly isolated BM or BM_{B220+} cells grown in IL-7 for 4 d were fractionated into CD2* IgM+, CD2* IgM+, and CD2* IgM*IgD populations and stimulated with IL-7 for 15 min. The 15-min time point was used based on our previous studies that showed this to be an optimal time to observe JAK and STAT activation after IL-7 stimulation (26). In both freshly isolated BM or IL-7–expanded B cells, the CD2* IgM+ population displayed phosphorylation of both STAT5 and JAK1 after stimulation with IL-7, whereas little to no activation was observed in the CD2* IgM+ or CD2* IgM*IgD populations (Fig. 1B). These observations demonstrated that as developing B lineage cells mature to the CD22 stage pre-B stage of development, they lose the ability to signal or respond to IL-7 but retain IL-7R surface expression.

Enforced expression of SOCS-1 in B cell lines inhibits IL-7R signaling and IL-7–induced proliferation

The role of SOCS proteins was examined in our system because they were previously shown to prevent cytokine signaling in immune cells through the inhibition of JAK and STAT protein activation (12, 27). These experiments were initiated using stromal-independent factor-dependent B lineage cell lines (22). The B62.1 cell line was established by cloning BM_{B220+} cells that had been grown in the presence of IL-7. B62.1 cells express μ on the cell surface in conjunction with surrogate LC proteins or κ LC, have a pre-B/immature B cell phenotype, and have not been experimentally transformed, making this line a good model for investigating signaling pathways representative of cells at this transitional stage of differentiation. B62.1 cells were infected with expression constructs containing either GFP alone or GFP and SOCS-1 separated by an internal ribosome entry site.

Postinfection, we observed visually that B62.1 cells expressing GFP and SOCS-1 died when cultured with or without IL-7 (data not shown). B62.1 cells expressing GFP alone did not die, suggesting
that SOCS-1 expression inhibits signals necessary for the survival of B62.1 cells. We used the B62.1-independent (B62.1 IND) cell line to determine whether the inhibited signals were downstream of the IL-7R. B62.1 IND is a variant of the B62.1 cell line that was created by growing B62.1 cells in gradually reduced concentrations of IL-7 until cells were able to be recovered that no longer depended on IL-7 for growth or survival yet remained responsive to IL-7. B62.1 IND cells have a doubling time of ∼30 h in the absence of IL-7 (baseline) and a doubling time of ∼15 h in the presence of IL-7 (data not shown). Infection of B62.1 IND cells was carried out using the SOCS-1 construct or SOCS-1 mutants that lacked either a functional SH2 domain (SOCS-1 SH2) or the c-terminal SOCS box domain (SOCS-1 ΔCT) (23). The SH2 domain of SOCS-1 is necessary for JAK protein binding, whereas the SOCS box is important in proteasomal degradation via elongins B and C (24, 28, 29).

B62.1 IND control cells expressing GFP alone proliferated to a similar extent as did uninfected cells when exposed to IL-7, whereas those expressing SOCS-1 completely lost the ability to proliferate in response to IL-7 (Fig. 2A). However, SOCS-1 expression did not alter IL-7–independent cell proliferation or result in cell death, as demonstrated by the baseline level of [3H]thymidine incorporation observed for SOCS-1–expressing B62.1 IND cells at all concentrations of IL-7. Cells expressing the SOCS-1 ΔCT construct also lost the ability to proliferate in response to IL-7 across most of the titrated range, whereas cells infected with the SOCS-1 SH2 construct did not show this proliferative defect.

Expression of SOCS-1 in B62.1 IND cells prevented the phosphorylation of JAK1 and STAT5 after IL-7 stimulation, as well as downstream ERK phosphorylation (Fig. 2B). This inhibition was also observed in the SOCS-1 ΔCT-expressing cells but not in cells expressing the SOCS-1 SH2 mutant. Flow analysis of the surface expression of the IL-7Rα– and γ-chains confirmed that SOCS-1 expression did not downregulate the IL-7R (Fig. 2C). Enforced gene-expression experiments can lead to unnatural expression levels that result in observations that may not be physiological. To account for this, we used RT-PCR to examine the levels of SOCS-1 mRNA in B62.1 IND GFP control cells and compared the results with those observed in B62.1 IND cells with enforced expression of SOCS-1, as well as with those observed in B62.1 IND GFP cells stimulated with IL-7, IFN-γ, or IL-21. Band-density analysis revealed that B62.1 IND cells with enforced expression of SOCS-1 expressed elevated levels of SOCS-1 compared with B62.1 IND GFP cells (Fig. 2D). However, SOCS-1 levels were similar to those observed in B62.1 IND GFP cells stimulated with IFN-γ, suggesting that SOCS-1 expression was within a range found in induced cells. Lack of proliferation and JAK/STAT phosphorylation in response to IL-7 demonstrated that enforced expression of SOCS-1 in B cell lines inhibits IL-7R signaling, mimicking events observed in CD2+ small pre-B cells.

Expression of SOCS-1 and SOCS-3 in developing B lineage cells

We next examined the expression of SOCS-1 and SOCS-3 in ex vivo BM B lineage cells. RT-PCR analysis revealed that mRNA transcripts for SOCS-1 and SOCS-3 are expressed in whole BM, as well as in CD19+ B cells (Fig. 3A). Using c-Kit as an additional maturation marker, we further separated developing B cell
populations into four distinct fractions: CD19-c-Kit"CD2" IgM" early pro-B cells, CD19"c-Kit"CD2" IgM" late pro-B/large pre-B cells, CD19"c-Kit"CD2" IgM" small pre-B cells, and CD19"c-Kit"CD2" IgM" immature B cells. By real-time PCR, we observed that SOCS-1 mRNA expression did not change between the CD19"c-Kit"CD2" IgM" and CD19"c-Kit"CD2" IgM" populations, but it was greatly increased (∼12-fold) in the CD19"c-Kit"CD2" IgM" population and slightly increased (∼3-fold) in the CD19"c-Kit"CD2" IgM" population (Fig. 3b). SOCS-3 mRNA expression remained unchanged in all B cell populations. These results demonstrated that expression of SOCS-1, but not SOCS-3, is regulated as B lineage cells mature, with highest levels observed in small pre-B cells.

IL-7 induces SOCS-1 and SOCS-3 expression in B cell lines

B6.2.1 IND cells were stimulated with IL-7 to determine whether IL-7R signaling regulated the expression of SOCS-1 and SOCS-3 in our B cell lines. SOCS-1 mRNA levels were ∼6-fold higher in IL-7-stimulated cells than in unstimulated cells, whereas SOCS-3 mRNA levels were increased ∼3-fold (Fig. 3c). We also observed that induction of SOCS-1 and SOCS-3 is concentration dependent, because reducing the amount of IL-7 used to stimulate cells resulted in a stepwise reduction in SOCS-1 and SOCS-3 expression (Fig. 3d).

In vitro maturation of primary B lineage cells expressing SOCS-1

Knowing that SOCS-1 can inhibit IL-7–induced proliferation and signaling in pre-B cell lines, we questioned what effect it would have on the maturation of ex vivo B lineage cells. BM220+ cells cocultured for 2 d with GPlaE virus-producing cells were enriched for CD19"c-Kit"CD2" IgM" cells that did or did not express GFP and were placed back in culture with IL-7. Four days later, GFP+ and GFP− cells from control cultures looked virtually identical based on c-Kit/CD2 and CD2/IgM surface phenotyping (Fig. 4). For these experiments, GFP− cells were used as internal controls, because they represent the same sorted and cultured population, only without GFP or SOCS-1 expression. GFP+ cells from cultures of cells expressing SOCS-1 showed a sharp reduction in the percentage of cells in the c-Kit"CD2" and c-Kit"CD2" populations, with a corresponding increase in the percentage of cells in the c-Kit"CD2" and CD2"IgM" populations. The absolute number of cells in SOCS-1 GFP+ versus SOCS-1 GFP− cultures was reduced ∼10-fold, whereas c-Kit"CD2", c-Kit"CD2", and CD2"IgM" populations were reduced by ∼85%, 20%, and 10-fold, respectively. In contrast, CD2"IgM" cells were reduced by <1.5-fold (Table 1). This alteration in B cell development showed that expression of SOCS-1 in ex vivo B lineage cells greatly reduced the proliferation and/or survival of IL-7–responsive B cells (CD2"IgM"), as well as the survival, proliferation, or replenishment of newly formed small pre-B (CD2"IgM") cells, yet it did not greatly prevent the further development of these cells into mature B (CD2"IgM") cells.

IFN-γ is expressed in BM cells and inhibits IL-7–induced proliferation in B cell lines

It was reported that pre-B cells are sensitive to IFN-γ; however, the mechanism of inhibition and cause of pre-B cell death have not been fully elucidated (14–17). Thus, we wanted to determine whether IFN-γ was playing an active role during B cell development in our system and whether that role was using SOCS activation. RT-PCR analysis of MACS-selected BM revealed that IFN-γ transcripts can be detected in whole BM, as well as in B220+ cells, but not in CD19+ B cells (Fig. 5a).

FIGURE 3. Endogenous or induced expression of SOCS-1 and SOCS-3 in B lineage cells and B cell lines. A, RNA was extracted from whole BM or CD19+ MACS-selected BM cells and analyzed for the expression of SOCS-1, SOCS-3, and β-actin. B, B220+ B lineage cells isolated from BM were cultured for 4 d in IL-7–supplemented media. Cells were stained and gated on CD19+ live cells and then enriched for c-Kit"CD2" IgM", c-Kit"CD2" IgM", c-Kit"CD2"IgM", and c-Kit"CD2"IgM" cell populations prior to extraction of mRNA for analysis of SOCS-1 and SOCS-3 expression by real-time PCR. Displayed values are normalized to β-actin and presented as fold change compared with unstimulated cells (C) or cells stimulated with IL-7 (D). Figures show fold change for normalized values from three independent experiments. C and D, B6.2.1 IND cells were starved for 1 h prior to stimulation with either IL-7 (25 ng) (C) or varying concentrations of IL-7 (D) for 90 min. RNA was extracted from stimulated cells and analyzed for SOCS-1 and SOCS-3 mRNA expression by real-time PCR. Displayed values are normalized to β-actin and represented as fold change compared with unstimulated cells (C) or cells stimulated with IL-7 (D). Figures show normalized values from three replicate wells from a single experiment. All data presented are representative of at least two independent experiments.
prior to incubating with \(^{3}H\)thymidine, we observed that no live proliferation (Fig. 5).

Concentration of IFN-\( \gamma \) led to enhanced reduction in proliferation (Fig. 5). When the cultures were visually inspected prior to incubating with \([^{3}H]\)thymidine, we observed that no live cells could be detected in wells containing IFN-\( \gamma \) and IL-7Hi. When using either constant high or low concentrations of IL-7, increasing the amount of IFN-\( \gamma \) led to decreased proliferation of B62.1 IND cells (Fig. 5C). However, the degree of IFN-\( \gamma \)-induced inhibition observed for cells grown with IL-7Hi exceeded that for cells cultured with IL-7Lo. In fact, cells grown in IL-7Lo continued to proliferate above baseline levels (no IL-7) at all concentrations of IFN-\( \gamma \) tested, whereas cells grown in IL-7Hi decreased below baseline (no IL-7) proliferative levels. These observations demonstrated that the inhibitory effect that IFN-\( \gamma \) has on B cell proliferation is dependent on the presence of IL-7 and is amplified by increasing concentrations of IL-7.

**IFN-\( \gamma \) and IL-7 induce SOCS-1 and SOCS-3 expression in B cell lines**

B62.1 IND cells were treated with IL-7 and/or IFN-\( \gamma \) to determine whether SOCS-1 or SOCS-3 expression is induced. Treatment of B62.1 IND cells with IFN-\( \gamma \) for 90 min led to an increase in both SOCS-1 (~7-fold) and SOCS-3 (~3-fold) mRNA compared with IL-7 alone (Fig. 5D). This induction was concentration dependent, because reducing the amount of IFN-\( \gamma \) used resulted in lower levels of SOCS-1 induction. Stimulation of B62.1 IND cells with both IFN-\( \gamma \) and IL-7 for 90 min led to a minor increase (~5-fold) in SOCS-3 and a dramatic increase (~40-fold) in the expression of SOCS-1 mRNA (Fig. 5E). The increase in SOCS-1 was greater than that observed for either IL-7 or IFN-\( \gamma \) alone and demonstrated that IL-7 and IFN-\( \gamma \) can cooperate to increase the expression of SOCS-1.

**In vitro maturation of primary B lineage cells treated with IFN-\( \gamma \)**

After observing that IFN-\( \gamma \) and IL-7 can work together to induce SOCS-1 expression and inhibit the proliferation and/or survival of B cell lines, we questioned what effect IFN-\( \gamma \) would have on the maturation of ex vivo B lineage cells. BM\(_{B220} \) cells cultured for 4 d in the presence of IL-7 and low (picogram) concentrations of IL-7 (IFN-\( \gamma \)Lo) were reduced in cell number compared with cells grown in IL-7 alone, but they showed little difference in the distribution of B cell populations (Fig. 6, Table II). Cells cultured in IL-7 and high (nanogram) concentrations of IFN-\( \gamma \) (IFN-\( \gamma \)Hi) were greatly reduced in absolute cell number and displayed a decrease in the percentage of CD2- IgM- cells, with a relative increase in CD2+IgM+ and CD2+IgM+ cells (Fig. 6, Table II). Comparison of cell size in the live cell gate showed that with IL-7 and IFN-\( \gamma \)Hi, the majority of cells surviving on day 4 were small noncycling cells. The observed shift in development toward more mature CD2+ cells suggested that the CD2+ IgM+ cells, with a relative increase in CD2+IgM+ and CD2+IgM+ cells (Fig. 6, Table II), were the most affected cells in cultures containing IL-7 and IFN-\( \gamma \)Hi.

### Table I. Absolute cell numbers for the in vitro maturation of infected B lineage cells

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<th>cKit+CD2+</th>
<th>cKit-CD2-</th>
<th>cKit+CD2-</th>
<th>CD2- IgM+</th>
<th>CD2+IgM-</th>
<th>CD2+IgM+</th>
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<td>0.84</td>
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<td>0.80</td>
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</tr>
<tr>
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<td>6.79 × 10^4</td>
<td>1.93 × 10^5</td>
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<tr>
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<td>0.01</td>
<td>0.05</td>
<td>0.15</td>
<td>0.04</td>
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IL-21 inhibits IL-7–induced proliferation and induces SOCS expression in B cell lines at high concentrations of IL-7

IL-21 is a known regulator of peripheral B cell maturation, and we recently showed that it is expressed by CD4+ T cells in the BM and that B cell progenitors respond to IL-21 (30). B62.1 IND cells express the IL-21Rα–chain and IL-21Rγ–chain on their surface, and expression of the IL-21R was not changed upon culturing cells with IL-7 (Fig. 7 A). B62.1 IND cells displayed little to no growth inhibition when treated with IL-21 alone or IL-21 in the presence of IL-7Lo (Fig. 7 B). Treatment of B62.1 IND cells with IL-21 and IL-7Hi resulted in a significant reduction in proliferation. Titration of IL-21 in the presence of IL-7Hi resulted in a dose-dependent inhibition of IL-7–induced proliferation, whereas cells cultured in IL-7Lo were not inhibited at any concentration of IL-21 (Fig. 7C). These results suggested that a threshold of IL-7 signaling must be reached before IL-21 has an inhibitory effect on IL-7–induced proliferation.

Treatment of B62.1 IND cells with IL-21 for 90 min did not lead to a significant change in SOCS-1 or SOCS-3 mRNA expression (Fig. 7D). As observed previously, IL-7 stimulation resulted in a ~6-fold increase in SOCS-1 expression. Addition of both IL-7 and IL-21 resulted in an ~9-fold increase in SOCS-1 mRNA, greater than observed with either condition alone, whereas the levels of SOCS-3 did not change. Thus, although IL-21 does not seem to alter SOCS expression on its own, it can work together with IL-7 to induce SOCS-1.

Expression and function of Gfi-1b in developing B lineage cells and B cell lines

In addition to examining cytokines that regulate IL-7R signaling, we investigated transcriptional factors that might be exerting control over IL-7–induced proliferation in developing B cells. Gfi-1b is a zinc finger transcriptional repressor that has a variety of targets, including SOCS proteins (31). Gfi-1b mRNA expression was highest in the CD19+c-Kit+CD2−IgM− population and decreased as cells matured toward becoming CD19+c-Kit−CD2+IgM+ cells (Fig. 8A). Next, we retrovirally infected B62.1 IND cells with a Gfi-1b expression vector and tested for changes in the proliferative response to IL-7. The level of expression of Gfi-1b in BM, B62.1 IND GFP cells, and B62.1 IND cells infected with Gfi-1b were compared by RT-PCR. B62.1 IND cells expressed a higher level of Gfi-1b than that observed in BM, whereas B62.1 IND Gfi-1b cells expressed a greater, but not excessive, amount of Gfi-1b (Fig. 8B). The proliferation of Gfi-1b–expressing cells was equal to that of uninfected cells in the absence of IL-7; however, enhanced proliferation was observed in response to IL-7 (Fig. 8C). These results demonstrated that Gfi-1b expression is regulated during B cell development and can amplify IL-7–proliferative signals in our B cell lines.
expression acts as a “rheostat” to control IL-7R signaling during the pro-B to immature B cell transition.

SOCS proteins are important negative regulators of cytokine signals and are essential for the development of a variety of hematopoietic lineages (12). In mature T cells, IL-7R signaling was shown to be able to both increase and decrease SOCS-1 and/or SOCS-3 expression, depending on the signaling context of the responsive cells (32, 33). Furthermore, during T cell development, SOCS-1 expression is transiently suppressed after pre-TCR signaling (24). In B cells, a potential role for SOCS proteins was described during the very early stages of B cell lymphopoiesis (34). In this article, we provided evidence that SOCS proteins have a novel and yet-to-be-appreciated role during the pro-B to immature B cell transition. In our system, SOCS-1 acts as a negative-feedback inhibitor and plays a key developmental role as part of a dynamic signaling network that modulates the ability of cells to respond to IL-7.

We showed that, as B cells mature to the small pre-B stage of development, they no longer respond to IL-7. Expression of the IL-7Rα varies between B cell subsets, but is positive on all populations tested, with high receptor levels persisting on IL-7 nonresponsive CD2⁺IgM⁻ small pre-B cells. CD2⁺IgM⁺ cells express the lowest level of the IL-7Rα–chain and highest level of the IL-7Rγ–chain. We believe that increased surface expression of the common γ-chain observed on CD2⁺IgM⁺ cells is likely due to its pairing with one of the other receptors that use the common γ-chain. The IL-21R is a likely candidate, because we recently published that this receptor is expressed at elevated levels on CD2⁺IgM⁺ cells (30). Additionally, CD2⁺IgM⁺ cells isolated and analyzed directly from BM also include mature and recirculating IgD⁻ B cells. Comparison of the IL-7Rα–chain and IL-7Rγ–chain expression on CD2⁺IgM⁺IgD⁻ and CD2⁺IgM⁺IgD⁺ populations revealed that CD2⁺IgM⁺IgD⁻ immature B cells express higher levels of the IL-7Rα–chain and lower levels of the IL-7Rγ–chain compared with more mature CD2⁺IgM⁺IgD⁺ cells (data not shown).

The intensity of IL-7Rα–chain expression also differed slightly between fresh and cultured cells, with freshly isolated CD2⁺IgM⁻ and CD2⁺IgM⁺ populations expressing slightly higher levels of IL-7Rα and slightly lower levels of the IL-7Rγ–chain than observed for cultured cells. IL-7R expression was more uniform and homogeneous on cultured populations compared with freshly isolated cells, highlighting the more heterogeneous nature of freshly isolated populations. We concluded that although receptor expression is not identical between freshly isolated and cultured cells, the same pattern is observed, and lack of signaling or proliferation in response to IL-7 is not due to the absence of receptor expression.

B cells cultured with IL-7 reach an upper proliferative limit, at which point increasing the concentration of IL-7 no longer leads to more proliferation. This finding suggests that a mechanism exists that limits IL-7 signaling, allowing for a certain level of response to IL-7 but nothing further. An alternative explanation for the proliferative limit is receptor saturation. We do not favor this interpretation because we demonstrated that the upper limit can be surpassed in cells with enforced expression of Gfi-1b. Our finding that SOCS-1 and SOCS-3 can be induced in B cell lines after IL-7

**Table II.** Absolute cell numbers for the in vitro maturation of IFN-γ-treated B lineage cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cell No.</th>
<th>CD2⁺IgM⁻</th>
<th>CD2⁺IgM⁺</th>
<th>CD2⁺IgM⁺</th>
</tr>
</thead>
<tbody>
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<td>$7.82 \times 10^5$</td>
<td>$6.12 \times 10^5$</td>
<td>$3.06 \times 10^5$</td>
</tr>
<tr>
<td>IL-7 + IFN-γ₇₀₀</td>
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<td>$3.15 \times 10^5$</td>
<td>$2.01 \times 10^5$</td>
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<tr>
<td>IL-7 + IFN-γ₇₀₀₀</td>
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<td>$4.75 \times 10^4$</td>
<td>$1.13 \times 10^5$</td>
<td>$8.75 \times 10^4$</td>
</tr>
</tbody>
</table>

**FIGURE 6.** In vitro maturation of B lineage cells treated with IFN-γ. B220⁺ B lineage cells isolated from BM were cultured for 3 d in IL-7 (5 ng/ml), IL-7 and low IFN-γ (0.01 ng/ml), or IL-7 and high IFN-γ (1 ng/ml). On day 4, cells were analyzed for in vitro maturation by the surface expression of B220, CD2, and IgM. Population percentages are displayed within the FACS plots and are representative of at least two independent experiments.

**Discussion**

Signaling through the IL-7R is of critical importance during the pro-B to pre-B cell transition. In this report, we showed that abrogation of IL-7R signaling in B lineage cells is not due to the disappearance of the IL-7R, but rather is mediated downstream by the modulation of SOCS-1, which, in turn, controls the levels of JAK/STAT phosphorylation. The highest level of SOCS-1 expression was in the c-Kit⁻CD2⁺IgM⁻ small pre-B population, coinciding with the stage of development at which cells no longer respond to IL-7. We further showed that SOCS-1 expression can be regulated by a number of factors, including IL-7 itself, IL-21, and IFN-γ. Additionally, Gfi-1b can enhance the IL-7–proliferative response of B cell lines. We propose that the dynamic regulation of SOCS-1 and SOCS-3 can be induced in B cell lines after IL-7
stimulation provides a potential pathway by which IL-7R signaling could regulate its own signal-transduction pathway. In this model, low (picogram) concentrations of IL-7 induce minimal levels of SOCS, which is inadequate to inhibit IL-7R signaling. Increasing the concentration of IL-7 leads to an increased level of proliferative signals, as well as an increased level of SOCS expression. However, at this point, the balance is still in favor of activation over inhibition; thus, increased proliferation is observed. At high (nanogram) concentrations of IL-7, a critical threshold of SOCS expression is reached, and the scale tips, wherein IL-7R–mediated signals are dampened, resulting in a balance between IL-7R activation and inhibition.

Using both primary B lineage cell cultures and established B cell progenitor cell lines, we showed that SOCS expression in B cells regulates IL-7R signaling, thus limiting or completely inhibiting responses to IL-7. The SH2 domain of SOCS-1 mediates the majority of the inhibitory action by binding to JAK/STAT proteins and preventing their phosphorylation. Proteasomal degradation of JAK/STAT proteins, or other signaling molecules, by the C-terminal SOCS box is not a major component of SOCS-1–mediated inhibition of IL-7 signaling in our system.

Elevated expression of SOCS-1 in primary B lineage cells, either enforced or induced by IFN-γ, inhibits the expansion of IL-7–responsive CD2⁻ pro-B/large pre-B cells, whereas more mature CD2⁺ small pre-B and IgM⁺ immature B populations are relatively unaffected. Reductions in the cell number observed in the CD2⁺ populations is likely due to fewer CD2⁻ input cells feeding the mature compartments. These observations support our model, in which elevation of SOCS expression beyond a certain threshold results in inhibition of IL-7–induced proliferation, although IL-7 is present, and the IL-7R is expressed.

Various factors may be part of this dynamic signaling network designed to regulate SOCS expression. Our studies with IL-7 and IFN-γ highlight a case in which two separate signaling pathways work together to modulate SOCS expression and, ultimately, responsiveness to IL-7. On its own, IFN-γ treatment did not alter cell proliferation. However, depending on the concentration of IL-7 used, IFN-γ–treated cells either displayed proliferation levels equal to those observed for cells grown without IL-7 or exhibited enhanced reductions in proliferation and ultimately cell death when cultured at high concentrations of IL-7. IL-7 and IFN-γ can act independently and together to induce SOCS-1 protein expression, and the balance of signals from these cytokines can regulate the SOCS "rheostat." Induction of SOCS-1 by IFN-γ stimulation alone may be sufficient to inhibit IL-7–induced proliferation or it may be that the combined effects of IFN-γ and IL-7 are required to surpass the threshold necessary to cause this effect. However, we do not believe that the reduction of proliferation below baseline (no IL-7) levels or the cell death observed with high concentrations of IL-7 is due solely to SOCS inhibition of JAK/STAT activation downstream of the IL-7R, because we showed that B62.1 IND cells can survive and proliferate IL-7-independent of IL-7. Therefore, although IL-7–induced proliferation is inhibited in the presence of IFN-γ, other IL-7R signals may still persist and work together with IFN-γ signals, leading to a further reduction in cell proliferation and, ultimately, cell death. One

FIGURE 7. Proliferative responses and induced expression of SOCS-1 and SOCS-3 in B cell lines after treatment with IL-7 and/or IL-21. A. B62.1 IND cells were cultured with or without IL-7 for 4 d and then stained for the surface expression of the IL-21Rα and IL-21Rγ–chain. B62.1 IND cells were plated in media with either varying concentrations of IL-7 with or without IL-21 (30 ng/ml) (B) or varying concentrations of IL-21 and IL-7 (C). Cells were cultured for 3 d prior to the addition of [³H]thymidine for 6 h and subsequent measurement of [³H]thymidine uptake. Data are presented as the mean of triplicate wells; error bars represent SD. D. B62.1 IND cells were starved for 1 h prior to stimulation with IL-7 (25 ng/ml), IL-21 (30 ng/ml), or both for 90 min. RNA was extracted from stimulated cells, and samples were analyzed for SOCS-1 and SOCS-3 expression by real-time PCR. Displayed values are normalized to β-actin and presented as fold change compared with unstimulated cells. Figures show normalized values from three replicate wells from a single experiment. All data presented are representative of at least two independent experiments.
of IL-7 and cultured for 3 d prior to the addition of [3H]thymidine for 6 h. SOCS-1–infected cells were plated in media with varying concentrations of IL-7 and cultured for 4 d in IL-7–supplemented media. Cells were stained and gated on CD19+ live cells and then enriched for c-Kit+CD2+IgM−, c-Kit−CD2−IgM−, c-Kit−CD2+IgM−, and c-Kit+CD2+IgM+ cell populations prior to extraction of mRNA and analysis for Gfi-1b expression by real-time PCR. Displayed values are normalized to β-actin and represented as fold change compared with the c-Kit−CD2−IgM− population. Figure shows fold change for normalized values from three independent experiments. B and C, B62.1 IND cells were retrovirally infected with expression vectors for SOCS-1 or Gfi-1b protein. B, RNA was extracted from whole BM, B62.1 IND cells, and B62.1 IND Gfi-1b cells and analyzed for Gfi-1b and β-actin expression by RT-PCR. Displayed arbitrary units (A.U.) were calculated by measuring the band intensity for Gfi-1b and normalizing to β-actin. C, B62.1 IND GFP, B62.1 IND Gfi-1b, or B62.1 IND SOCS-1–infected cells were plated in media with varying concentrations of IL-7 and cultured for 3 d prior to the addition of [3H]thymidine for 6 h and subsequent measurement of [3H]thymidine uptake. Data are presented as the mean of triplicate wells; error bars represent SD. All data presented are representative of at least two independent experiments.

The possibility is that the high levels of SOCS induced in the presence of these two factors inhibits other essential signaling pathways in these cells.

Whether IFN-γ actually plays a role in B cell maturation in vivo or only mimics SOCS-modulating potential that is normally mediated by different signaling molecules remains an unanswered question. It was recently identified that IFN-γ production by Mac-1+B220+ NK cells from the BM can inhibit IL-7–induced proliferation of pre-B cells (16). This may be the same B220+ BM population that we observed producing IFN-γ transcripts and suggests that IFN-γ remains a possible in vivo mediator. During infection and inflammation, preferential myelopoiesis occurs, and extramedullary B lymphopoiesis is induced, which is thought to be brought about by the inhibition of CXCL12 and stem cell factor production by BM stromal cells (35). Increased IFN-γ production during an immune response, either by IL-15–dependent expansion of the Mac-1+B220+ population or other mechanisms, may be inhibiting further B cell proliferation within the BM, allowing for preferential myelopoiesis. Our results provide a novel SOCS-mediated mechanism of action for IFN-γ–induced inhibition of IL-7R signaling in pro/large pre-B cells.

Our studies with IL-21 demonstrated another example of how independent signaling pathways can work together to alter SOCS expression and regulate responsiveness to IL-7. Similar to what we found for IFN-γ, we noted that the concentration of IL-7 is critically important in yielding different results with the same concentration of a secondary factor. In this case, only at high concentrations of IL-7 does IL-21 affect cell proliferation. This was also true for induction of SOCS-1 expression, because IL-21 stimulation alone induced little to no expression of SOCS-1, whereas the combination of IL-7 and IL-21 led to greater levels of SOCS-1 than did either alone. We recently published a study detailing that IL-21 promotes the differentiation of developing B cells and induces the expression of aid and blimp1 (30). The CD4+ T cell population in the BM expresses IL-21, and this population accounts for ∼3% of the naive lymphocyte population but significantly increases after immunization (30, 36). In light of these studies and the data presented in this article, we suggest that it would be of interest to further characterize the regulation of the CD4+ IL-21–producing T cell population present normally in the BM, as well as how that population is altered during inflammation, to better understand the role that IL-21 plays in regulating B cell development.

Using IFN-γ and IL-21, we demonstrated that we can dampen, as well as shut off, signals emanating from the IL-7R, whereas with Gfi-1b we showed that we can also enhance responses to IL-7. Gfi-1b expression did not alter normal cell proliferation, but it did enhance the proliferative responses to IL-7. In this case, the previously observed upper proliferative limit is surpassed, highlighting the fact that a B cell’s response to IL-7 is normally controlled and kept in check. However, when negative regulators are removed, cells are released from this inhibition and exhibit enhanced proliferation. Gfi-1b is a transcriptional repressor of a variety of genes, including SOCS proteins, and in our system it is reasonable to believe that Gfi-1b is repressing SOCS-1 in infected cells, thus allowing for increased JAK/STAT activation after IL-7R signaling. Our laboratory previously described a similar phenomenon for CD45−/− pro-B cells, which proliferate normally in low concentrations of IL-7 but display increased proliferation in high concentrations of IL-7, as well as exhibit prolonged JAK/STAT signaling after IL-7 stimulation (26).

The role of IL-7R signaling during the pro-B to pre-B cell transition has been the subject of intense investigation by a number of laboratories. Both instructive and permissive roles have been proposed for IL-7 during various stages of B cell development. Recent work showed that a primary role of IL-7R signaling during pro-B cell development is one of survival, via Mcl1, as well as suppression of premature Igk gene rearrangement, via binding of the intronic iEk enhancer (6). While IL-7R signaling inhibits LC

![Figure 8](http://www.jimmunol.org/content/journals/10.4049/jimmunol.1700127/Figure8.png)
rearrangement in pro-B/large pre-B cells, pre-BCR activation of Irf-4 initiates LC rearrangement, which is then enhanced by attenuation of IL-7R signaling (7). This balance between pre-BCR and IL-7R signaling was further characterized to show that signals emanating from the pre-BCR coordinate exit from the cell cycle, as well as enhance Igκ transcription by activating E2A and inhibiting ID3, whereas IL-7R signaling countered this effect by increasing Cnd3 expression and preventing E2A binding of the Igκ intronic enhancer (9). It was also demonstrated that signals from the pre-BCR terminate expansion of pre-B cells by suppressing c-Myc, leading to a reduction of cyclin D3 and, thus, limiting pre-BCR and IL-7–proliferative signals (8). Collectively, these reports demonstrated that signals emanating from the IL-7R diminish but do not prevent LC rearrangement and that IL-7R signals need to be regulated or attenuated for optimal LC rearrangement to proceed. They also provide evidence that signals emanating from the pre-BCR may help to reduce exposure to, or dampen IL-7R signals, but do not describe a mechanism by which abrogation of IL-7R signaling arises.

Our results demonstrated that regulation of signaling molecules downstream of the IL-7R occurs in developing B lineage cells and is critical during B lymphopoiesis. Previous work published by our laboratory showed that pro-B cells mature into pre-B cells at equal frequencies, regardless of whether IL-7 is present in culture (21). We now propose that during development, pro-B cells encounter IL-7 and receive both positive and negative signals that induce proliferation and survival, while also activating SOCS proteins, ensuring that excessive signaling does not occur. As cells reach the large pre-B cell stage, they remain responsive to IL-7 and now also receive signals from the pre-BCR that allow for proliferation in low concentrations of IL-7. As cells mature further to the small pre-B stage of development, SOCS levels are elevated past a threshold required to fully inhibit IL-7R signals. This allows for LC rearrangement to proceed more efficiently as proliferative signals and LC rearrangement inhibition are removed.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Figure 1. Sorting strategy for B cell subsets

Bone marrow was isolated and treated with ACK prior to staining for CD19, CD2, IgM, and IgD. Samples were gated on CD19+ live cells and then sorted for CD2−IgM−, CD2+IgM−, or CD2+IgM+IgD− B cell subsets. Sort parameters (A) and post sort purities (B) are displayed.
Supplemental Figure 1