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Suppressor of Cytokine Signaling 1 Stringently Regulates Distinct Functions of IL-7 and IL-15 In Vivo during T Lymphocyte Development and Homeostasis

Sheela Ramanathan,* Julien Gagnon,* Chantal Leblanc,* Robert Rottapel,* and Subburaj Ilangumaran2*

SOCS1−/− mice accumulate within the thymus and periphery CD8+ lymphocytes that express memory cell markers and display heightened in vitro responses to common γ-chain cytokines. To investigate whether dysregulated homeostasis of T lymphocytes and acquisition of memory phenotype by CD8+ cells in SOCS1−/− mice were mediated by IL-7 and/or IL-15 in vivo, we have generated SOCS1−/−IL-7−/−, SOCS1−/−IL-15−/− and SOCS1−/−IL-7−/−IL-15−/− mice. We observed that in mice lacking SOCS1, either IL-7 or IL-15 skewed thymocyte development toward CD8 lineage, whereas IL-15 is the principal mediator of dysregulated homeostasis in the periphery. Homeostatic proliferation of SOCS1−/− CD8+ lymphocytes in Rag1−/−, Rag1−/−IL-7−/−, Rag1−/−IL-15−/−, and Rag1−/−IL-7−/−IL-15−/− mice showed that SOCS1 deficiency did not overcome the requirement for IL-7 and IL-15 to sustain homeostatic expansion. Differential expression of memory phenotype markers CD44, CD122, and Ly6C by SOCS1−/−IL-15−/− CD8+ lymphocytes suggest that multiple signals contributed to the memory cell differentiation program. To address whether increased IL-15 responsiveness of SOCS1−/− CD8+ lymphocytes required prior TCR sensitization, we generated SOCS1−/− H-Y TCR transgenic (Tg) mice. Using female SOCS1−/− H-Y TCRtg mice in Rag1−/+ and Rag1−/− backgrounds, we show that acquisition of the memory phenotype by SOCS1-deficient CD8+ lymphocytes did not require prior antigenic stimulation, but required the presence of activated T cells. SOCS1 deficiency accelerated the maturation of CD8 single-positive thymocytes expressing Tg TCR, but did not compromise negative selection in HY-TCRtg males. Our findings illustrate distinct functions for IL-7 and IL-15 in T lymphocyte development and homeostasis, and stringent regulation of these processes by SOCS1.


 interleukins 7 and 15 are important regulators of T lymphocyte development and homeostasis (1, 2). Within the thymus, IL-7 is a survival and growth factor for immature CD4−CD8− double-negative (DN) thymocytes (3). Mice lacking IL-7 or IL-7Rα exhibit impaired T cell development resulting in severe thymic atrophy and 10- to 100-fold reduction in the number of mature T cells in secondary lymphoid organs (4, 5). IL-7 is also implicated in the differentiation of CD4+CD8+ double-positive (DP) cells into CD8 single-positive (SP) thymocytes (6, 7). In the periphery, IL-7 promotes survival of naive T cells, and down-regulation of IL-7Rα expression by IL-7 induces a potential homeostatic regulatory mechanism (8, 9). IL-7 is required for the generation of CD4+ and CD8+ memory T cells as well as to maintain their survival via up-regulation of Bcl-2 (10–12). A subset of activated CD8+ T cells that have re-expressed IL-7Rα has been shown to possess the developmental potential to differentiate into memory cells (13). IL-15 is not essential for thymocyte development or differentiation, however, IL-15 is critical for the survival and proliferative renewal of memory CD8+ T cells in the periphery. Mice lacking IL-15 or IL-15Rα harbor fewer CD8+CD44high memory phenotype T cells (14, 15). Both IL-7 and IL-15 transgenic (Tg) mice display an increased frequency of CD44high memory phenotype CD8+ T cells, whereas IL-7 Tg mice also exhibited a lymphoproliferative disorder (16–18).

IL-7 and IL-15 are members of the IL-2 family of cytokines that use distinct α receptor subunits to bind their respective ligands, and the common γ-chain (γc) to transduce signals by activating JAK3 and STAT5 (19–21). In addition, the IL-7Rα chain and the IL-2Rβ chain (CD122) of the IL-15R complex transduces signals via JAK1 and STAT3 (19). The JAK-STAT pathway of cytokine receptor signaling is regulated by multiple signal attenuation mechanisms that regulate the magnitude and/or the duration of signaling (22). In recent years, members of the suppressor of cytokine signaling (SOCS) family have emerged as critical negative regulators of the JAK-STAT pathway (23). SOCS proteins attenuate the JAK-STAT pathway either by binding to JAKs and blocking their activation, and/or competitively blocking the recruitment of STAT molecules to the phosphorylated receptor chains to prevent downstream signaling events. Of the SOCS family proteins, SOCS1 exerts a profound regulatory role on cells of the immune system including T lymphocytes, macrophages, and dendritic cells (24, 25).

SOCS1 is a critical regulator of T lymphocyte development and maturation within the thymus. In mice lacking SOCS1, the thymus...
undergoes rapid atrophy, which is accompanied by a decrease in thymic cellularity and accelerated thymocyte maturation (26, 27). We and others have shown that SOCS1 deficiency causes a decrease in the frequency of DP thymocytes and an increase in the proportion of CD8 SP cells with a concomitant decrease in CD4:CD8 ratio (28, 29). At least part of the effects of SOCS1 deficiency on thymocyte maturation is mediated by IFN-γ, as IFN-γ deficiency restores thymic cellularity and the frequency of DP cells in SOCS1-deficient mice (28). However, the skewed CD4:CD8 ratio arising from the increased frequency of CD8+ thymocytes remained unchanged in SOCS1−/−IFN-γ−/− mice (28, 29). The peripheral T lymphocyte compartment of SOCS1−/−IFN-γ−/− mice displays defective homeostasis of CD8+ cells due to their accumulation, which causes a decrease in CD4:CD8 ratio (29, 30). In vitro studies using thymocytes and peripheral T cells isolated from SOCS1−/−IFN-γ−/− and SOCS1−/−IFN-γ−/− mice have implicated IL-7 and IL-15 in the defective intrathymic development of CD8+ T cells and their accumulation in the periphery, respectively (28, 30–32). IL-15-stimulated phospho-STAT5 persists for a longer duration in SOCS1-deficient CD8+ T cells than in control cells whereas the IL-7-induced STAT5 phosphorylation was comparable both in magnitude and kinetics (28, 30). These observations suggested that the most likely cause for the accumulation of CD8+ T cells in SOCS1-deficient mice could be dysregulated IL-15 signaling. We have also shown that SOCS1-deficient CD8 SP thymocytes proliferate in response to IL-15 in vitro more strongly than control cells (28), however, whether IL-15 also contributed to the increased frequency of CD8 SP thymocytes in SOCS1-deficient mice is not known.

To precisely define the role of SOCS1 in regulating IL-7 and IL-15 signaling during CD8+ T cell development and homeostasis in vivo, we have generated mice that are deficient for SOCS1 as well as for IL-7, IL-15, or both, and examined their T cell compartment. In addition, we have generated Rag1−/− mice lacking IL-7, IL-15, or both, and studied homeostatic proliferation of SOCS1-deficient lymphocytes adaptively transferred into these mice. Furthermore, we have crossed SOCS1−/− mice with H-Y TCR Tg mice to investigate whether SOCS1-deficient T cells required priming via TCR to acquire responsiveness to cytokines. Our results show unequivocally that the dysregulation of thymopoiesis and the loss of peripheral T cell homeostasis in SOCS1−/− mice are mediated by IL-7 and IL-15, respectively, and SOCS1 deficiency bypasses the requirement of prior antigenic stimulation for naive CD8+ T cells to acquire cytokine responsiveness. Further, we show that SOCS1 deficiency did not affect negative selection of CD8+ T cells in the thymus, however, their increased cytokine responsiveness may modulate immunological tolerance in the periphery.

Materials and Methods

Mice

SOCS1−/−IFN-γ−/− mice (27) were gifts from Dr. J. Ihle (St. Jude Children’s Research Hospital, Memphis, TN), and were backcrossed onto C57BL/6 background for >10 generations. SOCS1−/−IFN-γ−/− mice were obtained by breeding SOCS1 heterozygous parents because SOCS1−/−IFN-γ−/− mice breed poorly. IL-7−/− mice on C57BL6 background were purchased from Taconic Farms. IL-7−/− mice on C57BL6 background were a gift from Dr. P. Vieira (Pasteur Institute, Paris, France). Rag1−/− mice were purchased from The Jackson Laboratory. SOCS1−/−IL-7−/−, SOCS1−/−IL-15−/−, SOCS1−/−IL-7−/−IL-15−/−, Rag1−/−IL-7−/−, Rag1−/−IL-15−/−, and Rag1−/−IL-7−/−IL-15−/− mice were bred in our animal facility, using PCR-based screening to identify mice carrying the knocked-out alleles. H-Y TCR Tg (H-Y TCRtg) mice were obtained from Dr. P. Poussier (Sunnybrook and Women’s College Hospital, Toronto, Ontario, Canada) with permission from Dr. H. von Boehmer (Harvard Medical School, Boston, MA). SOCS1−/− mice expressing the H-Y TCR transgene in Rag1−/− and Rag1−/− backgrounds were bred in our animal facility. The longevities of various SOCS1-deficient mice are given in Table I. All experiments using mice have been performed following institutional guidelines.

Abs and reagents

Abs against mouse CD4, CD8, CD16/CD32 (Fc block) CD44, CD122 (IL-2Rβ), Ly6C, and TCRβ (H57), conjugated to FITC, PE, or biotin were purchased from BD Biosciences. T3.70 mAb was purified from hybridoma supernatant and conjugated to FITC or biotin. Streptavidin–spectral red was from Southern Biotechnology Association. The recombinant murine IL-7 and human IL-15 were purchased from R&D Systems. RPMI 1640 cell culture medium and PBS were obtained from Invitrogen Life Technologies. CFSE was purchased from Molecular Probes.

Flow cytometry

Single-cell suspensions in PBS containing 5% FBS and 0.05% sodium azide were preincubated with Fc block for 10 min. Expression of various cell surface markers was estimated by standard three-color staining using FITC-, PE-, and biotin-conjugated primary Abs followed by streptavidin–spectral red. Data acquisition and analysis were done on a FACSCalibur using CellQuest software (BD Biosciences).

Cell proliferation assays

The CFSE dye dilution assay was used to estimate the number of cell division cycles within each T cell subset following cytokine stimulation. Total lymph node (LN) cells were labeled with CFSE by incubating cells at 2×106 cells/ml in PBS containing 5 μM CFSE for 10 min at room temperature. The reaction was quenched with an equal volume of FBS. The cells were washed twice and stimulated with IL-15 (20 ng/ml) or IL-7 (10 ng/ml) for 3–5 days and then labeled for surface markers. Sequential reduction in dye content, which reflects successive cell division cycles, was followed within gated T cell subsets. To measure the proliferative response of T cells bearing the H-Y TCR, 1×105 total LN cells were stimulated in 96-well culture plates with indicated concentrations of H-Y peptide (KCSYRNRQL, custom synthesized by Sigma-Genosys), in the absence or presence of exogenously added IL-2. One microcurie of [methyl-3H]thymidine (NEN) was added per well during the last 8–10 h of a 72-h culture period. The labeled cells were harvested onto glass fiber filter mats and the incorporated radioactivity was measured in a Top Count microplate scintillation counter (PerkinElmer).

Homeostatic proliferation

Total LN cells from SOCS1−/−IFN-γ−/− mice were labeled with CFSE as described above. A total of 2×106 cells in 200 μl of PBS were injected i.v. into 6- to 8-wk-old Rag1−/−, Rag1−/−IL-7−/−, Rag1−/−IL-15−/−, or Rag1−/−IL-7−/−IL-15−/− male or female mice (27). Rag1−/−H-Y TcRtg male mice develop anal prolapse by about 4–6 months of age as observed in other colonies (Dr. P. Poussier, unpublished observation), necessitating their culling.

Table I. Longevity of different mice used in this study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Longevity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−</td>
<td>&lt;3 wk</td>
</tr>
<tr>
<td>SOCS1−/−IFN-γ−/−</td>
<td>&gt;1 yeara</td>
</tr>
<tr>
<td>IFN-γ−/−</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−IL-7−/−</td>
<td>3–5 wk</td>
</tr>
<tr>
<td>IL-7−/−</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−IL-15−/−</td>
<td>&lt;3 wk</td>
</tr>
<tr>
<td>IL-15−/−</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−IL-7−/−IL-15−/−</td>
<td>3–5 wk</td>
</tr>
<tr>
<td>IL-7−/−IL-15−/−</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−H-Y TcRtg females</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>H-Y TcRtg females</td>
<td>&gt;3 wk</td>
</tr>
<tr>
<td>SOCS1−/−Rag1−/−H-Y TcRtg females</td>
<td>6–8 wk</td>
</tr>
<tr>
<td>Rag1−/−H-Y TcRtg females</td>
<td>&gt;1 year</td>
</tr>
<tr>
<td>SOCS1−/−H-Y TcRtg males</td>
<td>&gt;6 mo</td>
</tr>
<tr>
<td>H-Y TcRtg males</td>
<td>&gt;6 mo</td>
</tr>
</tbody>
</table>

a Unless indicated specifically, the survival data given are for both males and females.

b Female SOCS1−/−IFN-γ−/− mice do not breed well and aged SOCS1−/−IFN-γ−/− mice develop skin lesions.

Both control and SOCS1-deficient H-Y TcRtg males developed anal prolapse by about 4–6 months of age as observed in other colonies (Dr. P. Poussier, unpublished observation), necessitating their culling.
**Results**

**SOCS1 limits the proliferative potential of thymic and peripheral CD8\(^+\) T cells exposed to IL-7 and IL-15**

We and others have previously shown that SOCS1\(^{-/-}\)IFN-\(\gamma^{-/-}\) mice generate more CD8\(^+\) T cells within the thymus and harbor more CD8\(^+\) T cells in the periphery than control IFN-\(\gamma^{-/-}\) mice due to a T cell autonomous defect (28–30) (Fig. 1A, first row, day 0). Because SOCS1\(^{-/-}\) mice die within 3 wk after birth due to unbridled IFN-\(\gamma\) signaling (26, 27), SOCS1\(^{-/-}\)IFN-\(\gamma^{-/-}\) mice are generally used for studies on the functions of SOCS1 in regulating T lymphocyte development and homeostasis (29, 30). Incubation of total thymocytes and splenocytes from SOCS1-deficient mice with IL-7 or IL-15 alone, without concomitant stimulation via TCR, resulted in preferential accumulation of CD8\(^+\) T cells after 5 days of culture (Fig. 1A). To determine whether the increase in the frequency of CD8 SP cells was absolute, or resulted from a decrease in the relative proportion of other cellular subsets, we counted the cells after 5 days and estimated the number of CD4 and CD8 cells. CD8 SP thymocytes from both SOCS1\(^{-/-}\)IFN-\(\gamma^{-/-}\) and control mice markedly increased in frequency and number in the presence of IL-15, whereas the effect of IL-7 was comparable (Fig. 1, A and B). In contrast, both IL-7 and IL-15 dramatically increased the frequency and number of peripheral CD8\(^+\) T cells from SOCS1-deficient mice compared with control mice (Fig. 1, A and B). CFSE dye dilution assay showed that the increase in the number of SOCS1-deficient CD8\(^+\) thymocytes and peripheral T cells following IL-15 stimulation resulted from massive proliferative expansion, indicated by the number and magnitude of the division cycles (Fig. 1C). IL-7 induced at least seven division cycles in peripheral CD8\(^+\) T cells from SOCS1-deficient mice.

**FIGURE 1.** SOCS1 deficiency leads to selective accumulation of CD8\(^+\) T cells in total thymocyte and splenocyte cultures stimulated with IL-7 or IL-15. A, Total thymocytes and splenocytes from SOCS1\(^{+/+}\)IFN-\(\gamma^{-/-}\) and SOCS1\(^{-/-}\)IFN-\(\gamma^{-/-}\) mice were seeded in 24-well tissue culture dishes at a concentration of 2 \(\times\) 10\(^5\) cells/ml. The cells were cultured in the presence of IL-7 (10 ng/ml), IL-15 (20 ng/ml), or left unstimulated. On day 3, half of the culture supernatant was removed and replenished with fresh medium containing twice the concentration of respective cytokines. At the end of day 5, the cells were collected, counted and stained for CD4 and CD8, and analyzed by flow cytometry. The CD4 and CD8 profiles of cells before seeding are shown on the top panel (day 0). Numbers within each quadrant or marker boundaries represent the frequency of cells. B, The number of CD4 and CD8 T cells after 5 days of culture was determined from the frequencies of CD4\(^+\) and CD8\(^+\) cells and the total cell number at the end of the culture period. Total number of CD4 and CD8 cells at the beginning of the culture is shown for comparison. C, Total thymocytes and splenocytes were labeled with CFSE before stimulation with indicated cytokines as described in A. After 5 days, cells were stained for CD4 and CD8, and the extent of CFSE dye dilution was evaluated on gated CD8\(^+\) T cells. Results shown are representative of three to five experiments in each category.
and control mice, however, these cultures contained a large population of undivided cells (Fig. 1C), suggesting that IL-7 induced proliferation of only a small fraction of cells. These in vitro results strongly implicated IL-7 and IL-15 as potential contributors to the increased frequency and number of CD8+ T cells in the thymic and peripheral compartments of SOCS1-deficient mice, but did not exclude the possibility that cytokines other than IL-7 or IL-15 could contribute to the dysregulation of T cell homeostasis. To determine the relative contribution of IL-7 and IL-15 in skewing the T cell development toward CD8 lineage and in dysregulating peripheral homeostasis in SOCS1-deficient mice, we generated SOCS1−/+IL-7−/− and SOCS1−/+IL-15−/− mice and compared them to SOCS1−/−, IL-7−/−, and IL-15−/− controls.

Both IL-7 and IL-15 contribute to the increased generation of CD8 SP thymocytes in SOCS1-deficient mice

As reported previously (28), SOCS1−/− mice exhibited accelerated T cell maturation within the thymus when compared with control thymi (60% TCRβ cells vs 17% in control mice) and harbored more SP cells (37 vs 12% for CD4 SP cells, 25 vs 3% for CD8 SP cells) (Fig. 2). In SOCS1−/− mice, the thymus was atrophied and the thymocytes were severely reduced in number (Table II). Noticeably, the frequency and the number of CD8 SP cells was elevated in the absence of SOCS1, lowering the CD4:CD8 ratio from 4.2 ± 0.9 observed in control thymi to 1.4 ± 0.2 in SOCS1-deficient mice (Fig. 2, Table II). Although thymic atrophy and decrease in the frequency of DP cells caused by SOCS1 deficiency are IFN-γ dependent, preferential development of CD8 SP cells in the absence of SOCS1 occurs independently of IFN-γ (28) (Fig. 2, Table II). Examination of SOCS1-deficient mice, which were also lacking IL-7, IL-15, or both, revealed that not only IL-7 but also IL-15 contribute to the skewed CD8+ T cell development in SOCS1−/− mice.

T cell development is severely repressed in IL-7-deficient mice due to the lack of survival signals for DN thymocytes, whereas IL-15−/− mice exhibit nearly normal intrathymic T cell development (4, 5, 14, 21). Although thymic cellularity of IL-15-deficient mice is comparable to that of C57BL/6 mice, IL-7−/− thymi contained ~10-fold less cells compared with normal thymi (Table II) (4). Similarly, the frequency of mature SP cells and the CD4:CD8 ratio were comparable between IL-15−/− and C57BL/6 thymi, whereas IL-7-deficient thymi exhibited a slight decrease in CD8 SP cells that caused an increase in CD4:CD8 ratio (Fig. 2, Table II). Like SOCS1−/− mice, SOCS1−/+IL-15−/− mice died within 3 wk, whereas SOCS1−/+IL-7−/− and SOCS1−/+IL-7−/−IL-15−/− mice survived for slightly longer periods, possibly due to paucity of T cells, which are the principal mediators of pathology in SOCS1−/− mice (Table I and II) (26, 27). Ten- to 14-day-old SOCS1−/+IL-7−/−, SOCS1−/+IL-15−/− mice showed accelerated thymocyte maturation very similar to SOCS1−/− mice, characterized by an increase in the frequencies of TCRβ cells, and CD4 and CD8 SP cells with a concomitant decrease in DP cells (Fig. 2). Strikingly, the increase in the frequency of CD8 SP cells and the decrease in CD4:CD8 ratio caused by SOCS1 deficiency were not reversed by the lack of either IL-7 or IL-15 (Fig. 2, Table II). These observations suggested that IL-7 and IL-15 might complement each other, possibly at different developmental stages, to skew the T cell development toward CD8 lineage in the absence of SOCS1. Alternatively, T cell maturation pathways from DP to SP stages could be influenced by other cytokines that are distinct from IL-7 and IL-15 and are regulated by SOCS1. To explore these possibilities, we generated SOCS1−/+IL-7−/−IL-15−/− mice.

Like IL-7-deficient mice, mice lacking both IL-7 and IL-15 exhibited severely atrophied thymus (Table II). Within the very limited number of thymocytes that could be recovered from IL-7−/−IL-15−/− mice, the proportion of mature T cells was comparable to that of C57BL/6 mice, however the proportion of CD8 SP cells was dramatically reduced resulting in a CD4:CD8 ratio of 7.8 ± 2.6 (Fig. 2, Table II). The decrease in the frequency of CD8 SP thymocytes in IL-7−/− mice, and more severely in IL-7−/−IL-15−/− mice, underscores the importance of IL-7, and the limited capacity of IL-15, to sustain the production of mature CD8+ T cells within the normal thymus. SOCS1−/+IL-7−/−IL-15−/− mice harbored fewer thymocytes, and exhibited a strikingly severe paucity of CD8 SP thymocytes compared with IL-7−/−IL-15−/− controls (Fig. 2, Table II). These results clearly demonstrated that the accumulation of CD8 SP thymocytes in SOCS1−/− and SOCS1−/+IFN-γ−/− mice was mediated primarily by IL-7 and IL-15.

Predominance of CD8 T cells in the secondary lymphoid organs of SOCS1-deficient mice is mediated by IL-15 but not IL-7

In addition to its role in thymocyte development, IL-7 provides survival signals to naive CD4+ and CD8+ T cells in the periphery,
and IL-7−/− mice harbor 10- to 100-fold less T cells in their secondary lymphoid organs (4, 5, 8). IL-15−/− and IL-15Rα−/− mice contain fewer CD8+ T cells exhibiting memory phenotype (14, 15). Transgenic mice overexpressing IL-7 or IL-15 accumulate memory phenotype CD8+ T cells in the periphery of SOCS1-deficient mice. These observations raised the possibility that the accumulation of CD8+ T cells in the periphery of SOCS1-deficient mice could arise from increased signaling by IL-7, IL-15, or both due to prolonged survival and augmented proliferative renewal. To verify this, we examined the LN cells from SOCS1−/− IL-7−/−, SOCS1−/− IL-7+/−, and SOCS1−/− IL-7−/− IL-15−/− mice (Fig. 3). As shown before, SOCS1−/− mice showed an increase in the frequency of CD8+ T cells compared with control mice (Fig. 3). The LN of IL-7−/− and IL-7−/− SOCS1−/− mice were very small and difficult to locate. Examination of cells from a single mesenteric LN showed a CD4:CD8 ratio of 2:1 in IL-7−/− mice, whereas SOCS1−/− IL-7−/− mice exhibited an elevated frequency of TCRα+ cells with a predominance of CD8+ T cells and a CD4:CD8 ratio of <1 (Fig. 3). Transgenic mice subjected to increased number of CD8+ T cells in SOCS1-deficient mice. SOCS1−/− IL-15−/− and SOCS1−/− IL-7−/− IL-15−/− mice contained three to five times less CD8+ T cells than CD4+ T cells (Fig. 3), demonstrating that IL-15 is the principal mediator of dysregulated homeostasis of peripheral CD8+ T cells in SOCS1-deficient mice. Noticeably, IL-15−/− and IL-7−/− IL-15−/− mice, which express functional SOCS1, also showed a severe reduction in the frequency of CD8+ T cells, with a CD4:CD8 ratio of 4:1 and 10:1, respectively (Fig. 3), demonstrating the critical requirement for IL-15 in the homeostasis of CD8+ T cells. SOCS1 deficiency partially rescued the disparity in the proportions of CD4 and CD8 T cells in these mice, bringing the CD4:CD8 ratio to 3:1 and 5:1, respectively (Fig. 3), indicating minor contributions by other SOCS1-regulated cytokines in maintaining the CD8+ T cell pool.

Homeostatic proliferation of SOCS1-deficient CD8+ T cells is severely compromised in lymphopenic mice lacking IL-7 or IL-15

We have shown previously that CD8+ but not CD4+ T cells lacking SOCS1 undergo vigorous proliferative expansion when injected into Rag1-deficient mice within 4 days, compared with wild-type CD8+ T cells which required more than 1 wk to undergo the same number of division cycles (30) (Fig. 4, top row; data not shown). Homeostatic proliferation of CD8+ T cells has been shown to be mediated primarily by IL-15, however, IL-7 can also significantly contribute to the homeostatic expansion, particularly under lymphopenic conditions (18, 34–36). To determine whether

### Table II. Thymic cellularity and CD4/CD8 ratio in various mice used in this study

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total Cell Number (×10⁶)</th>
<th>CD4:CD8 Ratio</th>
<th>CD4 SP Thymocytes</th>
<th>CD8 SP Thymocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6</td>
<td>120 ± 25</td>
<td>4.2 ± 0.9</td>
<td>14.4 ± 3.0</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>SOCS1−/−</td>
<td>3.5 ± 2</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.7</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>IFN-γ−/−</td>
<td>110 ± 20</td>
<td>4 ± 0.4</td>
<td>28.6 ± 5.2</td>
<td>8.8 ± 1.6</td>
</tr>
<tr>
<td>SOCS1−/− IFN-γ−/−</td>
<td>102 ± 26</td>
<td>1.4 ± 0.2</td>
<td>24.5 ± 6.2</td>
<td>23.5 ± 6.0</td>
</tr>
<tr>
<td>IL-7−/−</td>
<td>12 ± 8</td>
<td>5.6 ± 1.5</td>
<td>2.3 ± 1.5</td>
<td>0.36 ± 0.24</td>
</tr>
<tr>
<td>SOCS1−/− IL-7−/−</td>
<td>1.5 ± 1</td>
<td>1.8 ± 0.4</td>
<td>0.4 ± 0.3</td>
<td>0.18 ± 0.12</td>
</tr>
<tr>
<td>IL-15−/−</td>
<td>135 ± 50</td>
<td>3.6 ± 2.8</td>
<td>23.0 ± 8.5</td>
<td>6.75 ± 2.5</td>
</tr>
<tr>
<td>SOCS1−/− IL-15−/−</td>
<td>58 ± 28</td>
<td>2.8 ± 0.9</td>
<td>19.1 ± 9.2</td>
<td>7.5 ± 3.6</td>
</tr>
<tr>
<td>IL-7−/− IL-15−/−</td>
<td>6 ± 2</td>
<td>7.8 ± 2.6</td>
<td>0.8 ± 0.3</td>
<td>0.12 ± 0.04</td>
</tr>
<tr>
<td>SOCS1−/− IL-7−/− IL-15−/−</td>
<td>3.1 ± 1.6</td>
<td>15 ± 3.5</td>
<td>0.7 ± 0.3</td>
<td>0.03 ± 0.02</td>
</tr>
</tbody>
</table>

* CD4 and CD8 SP thymocyte numbers were calculated from the total thymocyte count and the frequencies of thymocyte subsets. All mice were examined 15–20 days after birth except SOCS1−/− mice, which were analyzed within 10 days after birth.

### Figure 3

Predominance of CD8+ T cells in the periphery of SOCS1-deficient mice is caused by IL-15. LN cells from 1- to 2-wk-old SOCS1−/−, SOCS1−/− IL-7−/−, SOCS1−/− IL-15−/−, and SOCS1−/− IL-7−/− IL-15−/− mice and the corresponding control mice were stained for TCR, CD4, and CD8 and analyzed by flow cytometry. Representative data from one of the several animals within each category are shown. The frequencies of T cells (TCR+), and CD4+ and CD8+ cells within the TCR-gated population are indicated.
CD8^+ T cell development and homeostasis regulation by SOCS1

The deficient CD8^+ T cell subset in lymphopenic hosts is driven by IL-15 and/or IL-7, or by other γc cytokines, we injected CFSE-labeled total LN cells into Rag1^-/-, Rag1^-/-IL-7^-/-, Rag1^-/-IL-15^-/-, or Rag1^-/-IL-7^-/-IL-15^-/- mice. Three days after injection, we recovered LN cells from the recipient mice and evaluated the fluorescence intensity of CFSE on CD4 and CD8 subsets. SOCS1-deficient CD8^+ T cells, which underwent vigorous homeostatic proliferation in Rag1^-/- mice lacking IL-7 or IL-15 (Fig. 4). Undivided cells represented 12% of the CD8^+ T cell population in Rag1^-/- recipients, whereas Rag1^-/-IL-7^-/- and Rag1^-/-IL-15^-/- mice, respectively, contained 44 and 39% of CD8^+ T cells that have not undergone even a single division cycle (Fig. 4). Homeostatic proliferation of SOCS1-deficient CD8^+ T cells was minimal in Rag1^-/-IL-7^-/-IL-15^-/- mice, which contained 74% of undivided CD8^+ T cells (Fig. 4). These findings demonstrate that absence of SOCS1 does not overcome the dependence of CD8^+ T cells on IL-7 and IL-15 for homeostatic proliferation and ruled out the possibility that cytokines other than IL-7 and IL-15 could significantly contribute to the augmented homeostatic proliferation of SOCS1-deficient CD8^+ T cells.

IL-15 mediates the acquisition of CD44^{high} phenotype in SOCS1-deficient CD8^+ T cells

In the absence of SOCS1, CD8^+ T cells in the secondary lymphoid organs of SOCS1-deficient mice acquire CD44^{high}CD122^{high}Ly6C^{high} phenotype without augmenting the expression of CD25 or CD69, resembling memory CD8^+ T cells (29, 30) (Fig. 5, left panel, top two rows). SOCS1-deficient CD8^+ T cells express these markers even before they emigrated from the thymus (Fig. 5, right panel, top two rows). Most of the peripheral CD8^+CD44^{high} cells in SOCS1-deficient mice also expressed high levels of CD62L, a characteristic of central memory T cells (T CM, 37, 38) (data not shown). To determine whether the acquisition of memory T cell markers by CD8^+ T cells in SOCS1-deficient mice is intrinsic to SOCS1 deficiency, or is dependent upon stimulation by cytokines IL-7 and IL-15, we examined the expression of certain memory T cell markers on T cells isolated from the LNs of SOCS1-deficient mice lacking either IL-7 or IL-15. Peripheral CD8^+ T cells from SOCS1^-/-IL-7^-/^- but not IL-7^-/- mice showed elevated CD44 expression (Fig. 5, left panel, column 1, rows 3 and 4). As documented in the literature, IL-15^-/- mice harbored fewer memory phenotype CD8^+ T cells expressing high levels of CD44 in the periphery (Fig. 5, left panel, column 1, row 5). SOCS1 deficiency augmented the level of CD44 expression on CD8^+ LN T cells from IL-15^-/- mice (9–33%), but not to the extent observed on CD8^+ LN T cells from SOCS1^-/-IFN-γ^-/- (32–62%) or SOCS1^-/-IL-7^-/- mice (18–86%) (Fig. 5, left panel, column 1). CD44 expression on CD8^+ thymocytes from SOCS1^-/-IL-7^-/- and SOCS1^-/-IL-15^-/- mice also showed a pattern similar to peripheral CD8^+ T cells (Fig. 5, right panel). These observations...
show that increased CD44 expression on CD8+ T cells is mediated primarily by IL-15 and not by IL-7, and that the IL-15-mediated CD44 up-regulation is regulated by SOCS1. In contrast to CD44, elevated expression of CD122 and Ly6C on SOCS1-deficient peripheral CD8+ T cells was comparable between SOCS1−/− IL-7−/− and SOCS1−/− IL-15−/− mice (Fig. 5, left and right panels). These observations suggest that the induction of CD122 and Ly6C on memory phenotype CD8+ T cells is mediated by cytokines other than IL-7 and IL-15, or by other cellular activation signals, that are regulated by SOCS1. By dissociating the up-regulation of CD44 from that of CD122 or Ly6C in IL-15-deficient mice, SOCS1 deficiency has revealed that the differentiation program leading to memory CD8+ T cell development is regulated by multiple signaling events.

**Acquisition of CD44high phenotype by SOCS1-deficient CD8+ T cells does not require prior antigenic stimulation**

SOCS1-deficient mice contained more CD8+ T cells exhibiting CD44highCD122high memory phenotype compared with controls (Fig. 5, data not shown), and these cells responded vigorously to IL-15 in vitro (30), suggesting that SOCS1 deficiency has selectively augmented the expansion of Ag-experienced memory T cells. Even though purified CD8+CD44low naive T cells from SOCS1-deficient mice did not proliferate in vitro in response to IL-15, the possibility that these cells may respond to other combinations of cellular activation signals in vivo has not been ruled out. To address this issue, we bred SOCS1−/− and H-Y TCRtg mice (31), which allows simultaneous comparison of Ag-activated and naive CD8+ T cells in the same animal. H-Y TCR recognizes the male-specific H-Y Ag presented by the H-2Dd haplotype of C57BL/6 mice. H-Y TCR, composed of α and β TCR transgenes (αF and βF), can be detected on the T cell surface with T3.70 mAb against αF (40). The H-Y TCR is highly Ag specific and is known to be non-cross-reactive to self or environmental Ags (41). In female mice, because positive selection occurs in the absence of Ag and allelic exclusion of TCRα is leaky (42), nearly 50% of the CD8+ T cells in the periphery do not bear the Tg H-Y TCR (40). We hypothesized that if CD8+ T cells that accumulated in SOCS1-deficient T cells arose from true memory cells, only the non-Tg T cells will exhibit the memory phenotype in SOCS1−/− H-Y TCRtg female mice.

**SOCS1−/− H-Y TCRtg females do not survive for >3 wk of age**

(Table I). Therefore, we examined CD8+ T cells from SOCS1−/− H-Y TCRtg females at 12 and 18 days after birth, and evaluated the distribution of CD44 expression on T3.70+ TCR transgenic and T3.70+ nontransgenic CD8+ T cells. Whereas H-Y TCRtg females showed a normal CD4:CD8 ratio of 2:1, SOCS1−/− H-Y TCRtg mice harbored nearly three times more CD8+ T cells than CD4+ cells (60 vs 21%; Fig. 6A). The frequency of T3.70+ Tg T T cells was comparable between H-Y TCRtg and SOCS1−/− H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%). However, in SOCS1-deficient H-Y TCRtg females, the T3.70+ CD8+ Tg T cells were increased in frequency by 2-fold (9 vs 5%), whereas the T3.70+ CD8+ cells from H-Y TCRtg female mice (14 vs 16%).
T3.70−CD8+ and T3.70+CD8+ T cells contributed to the premature death of SOCS1−/−H-Y TCRtg female mice (Table I). These observations show that cellular activation and the induction of CD44high phenotype on CD8+ T cells in SOCS1-deficient mice can occur without the prior TCR engagement, presumably by cytokines. Therefore, SOCS1 is essential to prevent naïve T cells from acquiring cytokine responsiveness without prior antigenic stimulation.

Acquisition of CD44high phenotype by naïve SOCS1-deficient CD8+ T cells requires help from activated T cells

In Rag-deficient female H-Y TCRtg mice, rearrangement of endogenous TCRβ genes fails to occur, therefore all CD8+ T cells in Rag1−/−H-Y TCRtg female mice are CD44low naïve T3.70+ T cells (43). Because CD8+T3.70− cells that are reactive to environmental Ags do not arise in the Rag1−/− background, SOCS1−/−Rag1−/−H-Y TCRtg mice survived for a longer duration but still succumbed to the classical pathology of SOCS1-deficient mice by 8 wk (Table I, data not shown). Comparison of 6-wk-old, female Rag1−/− and SOCS1−/−Rag1−/−H-Y TCRtg mice showed identical frequencies of T3.70+ T cells (85 vs 78%, Fig. 7), however, the frequency of CD8+T3.70+ cells was markedly decreased in SOCS1−/−Rag1−/−H-Y TCRtg mice as the majority of T3.70+ cells in SOCS1−/−Rag1−/−H-Y TCRtg mice were CD8− (64 vs 11%, Fig. 7). SOCS1-sufficient, female Rag1−/−H-Y TCRtg mice also harbored a substantial proportion of CD8+T3.70+ cells that are neither CD4+ nor NK1.1+, and represented less than one-third of the total T3.70+ cells (26%, Fig. 7, data not shown). The nature of CD8+T3.70+ cells, mechanisms that underlie their preferential accumulation in SOCS1-deficient mice and whether they are directly responsible for the premature death of female SOCS1−/−Rag1−/−H-Y TCRtg mice are questions that remain to be addressed.

Compared with 18-day-old SOCS1−/−H-Y TCRtg mice (Fig. 6, bottom panels), CD44 expression on CD8+T3.70+ cells from SOCS1−/−Rag1−/−H-Y TCRtg mice remained low even at 6 wk of age (Fig. 7, bottom panels). Unlike CD8+T3.70− cells in female H-Y TCRtg or SOCS1−/−H-Y TCRtg mice, CD8+T3.70+ from both Rag1−/−H-Y TCRtg and SOCS1−/−Rag1−/−H-Y TCRtg females did not dramatically up-regulate CD44 (Figs. 6 and 7). These observations suggest that certain type of “bystander” help from Ag-activated T cells might underlie the Ag-independent activation of naïve CD8+T3.70+ T cells in SOCS1−/−H-Y TCRtg females (Fig. 6B).

SOCS1 deficiency modulates thymic maturation but does not compromise negative selection

In male H-Y TCRtg mice, the DP compartment is severely depleted and CD8+T3.70+ Tg T cells undergo negative selection resulting in reduced thymic cellularity (39) (Fig. 8). CD8 SP thymocytes expressing Tg TCR that mature within the thymus express low levels of CD8 and are tolerant to the H-Y Ag (39). SOCS1 deficiency further reduced the thymic cellularity in 5-wk-old male H-Y TCRtg mice (14.3 ± 3.2 × 106 thymocytes in H-Y TCRtg vs 9 ± 2.5 × 106 cells in SOCS1−/−H-Y TCRtg males compared with 91 ± 22 × 106 thymocytes in non-Tg controls). The DP thymocyte compartment was completely absent in 5-wk-old SOCS1−/− H-Y TCRtg males, and female SOCS1−/−H-Y TCRtg mice also showed a marked reduction of DP thymocytes (Figs. 8 and 2), possibly resulting from dysregulated IFN-γ signaling as documented in non-TCR Tg SOCS1−/− mice (28). SOCS1−/−H-Y TCRtg males exhibited a nearly 2-fold increase in the frequency of CD8+ T cells, all expressing Tg TCR (35 vs 19%; Fig. 8), however, the total number of CD8 SP thymocytes was comparable between SOCS1−/−H-Y TCRtg males and controls (3.15 ± 0.6 × 106 vs 2.7 ± 0.9 × 106 cells). These observations suggested that depletion of the DP compartment in SOCS1−/− H-Y TCRtg males led to a proportional increase in CD8 SP thymocytes, but did not compromise the negative selection process.

![FIGURE 7](https://www.jimmunol.org/content/doi/10.4049/jimmunol.88.02.0436.supp/1)
TCR" males showed significantly higher levels of total anti-DNA IgGs than SOCS1"/"H-Y TCR" controls (Fig. 9D). However, SOCS1"/"H-Y TCR" males displayed a significantly lower anti-DNA Ab level than SOCS1"/"IFN-γ"/" mice. Further investigations are needed to determine whether the paucity of CD4" T cells and the highly restricted TCR repertoire of H-Y TCR" males underlie the diminished levels of autoantibodies in SOCS1"/"H-Y TCR" males.

Discussion

Previous studies have shown that SOCS1" mice generate more CD8" T cells within the thymus and accumulate them in secondary lymphoid organs (28–31). In vitro studies using thymocytes and peripheral T cells have strongly implicated IL-7 and IL-15 as potential cytokines that contribute to the dysregulated T lymphocyte homeostasis in SOCS1-deficient mice (28–31) (Fig. 1). In addition to IL-2, IL-7, and IL-15, several other γc and non-γc cytokines could influence the T lymphocyte compartment by providing survival signals to activated T cells (46). Because most of these cytokines elicit SOCS1 gene expression, and overexpression of SOCS1 could inhibit their signaling in various experimental systems (23), it is possible that cytokines other than IL-7 and IL-15 could also contribute to the dysregulation of T lymphocyte homeostasis in SOCS1"/" mice. The present study using SOCS1"/"IL-7"/" and SOCS1"/"IL-15"/" mice establish that IL-7 and IL-15 are absolutely required to dysregulate the CD8" T cell compartment in SOCS1"/" mice, with predominant roles in thymic development and peripheral T cell homeostasis, respectively.

IL-7 has been implicated in providing critical survival signals during differentiation of DP thymocytes to CD8 SP thymocytes (6, 7). Consistent with a role for SOCS1 in regulating IL-7 signaling during lineage commitment, Chong et al. (31), have shown that the differentiation of signaled DP cells (CD4"CD8low" into CD8 SP cells in vitro was augmented in the absence of SOCS1. IL-15 alone did not increase the frequency or number of CD8 SP cells in this system, nonetheless, addition of IL-2 and IL-15 augmented the generation of CD8 SP cells stimulated by IL-7. In addition to contributing to the lineage differentiation process, IL-7 and IL-15 might contribute to the survival and proliferation of CD8 SP thymocytes before emigration to the periphery (28, 47). Mice expressing transgenic IL-7 or Bcl-xL generate more CD8 SP thymocytes (16, 48), and IL-15Ra" mice contained fewer CD8 SP cells (15). We have shown that SOCS1-deficient CD8 SP thymocytes express higher levels of the prosurvival protein Bcl-xL, and proliferate more strongly in response to IL-7 or IL-15 when compared with cells from control mice (28). Therefore, increased IL-7 and IL-15 signaling due to SOCS1 deficiency could lead to increased production of CD8 SP cells by 1) facilitating differentiation of DP thymocytes toward CD8 lineage, and 2) increasing the survival and proliferation of differentiated CD8 SP thymocytes. Results presented in Fig. 2, that deficiency of either IL-7 or IL-15 alone failed to prevent the increased generation of CD8 SP thymocytes in SOCS1"/" mice, whereas SOCS1"/"IL-7"/"IL-15"/" thymus contained very few CD8 SP thymocytes, demonstrate that both IL-7 and IL-15 contributed to the increased frequency of CD8 SP thymocytes in SOCS1"/" mice. Strikingly, IL-7"/"IL-15"/" thymus showed a severe paucity of CD8 SP cells compared with IL-7"/" or IL-15"/" thymus, revealing a critical requirement for either IL-7 or IL-15 to sustain optimal CD8 T cell development, and substantiating the in vitro studies (6, 7).

Recently, a positive regulatory role has been attributed to SOCS1 in positive selection of CD4 SP cells based on the observation that the development of CD4 SP cells from DP cells is

Hyperresponsiveness to cytokines caused by SOCS1 deficiency may modulate peripheral tolerance mechanisms

Even though the thymi of male H-Y TCR" mice are small and atrophied, the peripheral compartments of these mice display normal numbers of Thy-1" cells, most of which are CD4"CD8" or CD4"CD8low with very few CD4"CD8" cells (39). The CD8"/"T3.70" Tg T cells express CD44, which is indicative of prior antigenic stimulation, however, they do not respond to stimulation by the male-specific Ag or by the H-Y peptide in vitro, which could be reversed by the addition of IL-2 (44) (Fig. 9B). Despite displaying identical frequencies of CD8"/"T3.70" Tg T cells as their littermate controls (18 vs 19%, Fig. 9A, middle panels), SOCS1"/"H-Y TCR" males harbored nearly two times more cells in their LNs (26 ± 4.2 × 106 cells in H-Y TCR" versus 40 ± 6.8 × 106 cells in SOCS1"/"H-Y TCR" males). Expression of CD44 was elevated in peripheral CD8"/"T3.70" Tg T cells from SOCS1-deficient males (Fig. 9A, right panels), suggesting their activation and expansion in the periphery. However, total LN cells from SOCS1-deficient mice exhibited negligible proliferative response to the H-Y antigenic peptide (Fig. 9C), which was restored by the addition of IL-2, indicating that SOCS1 deficiency did not alter the anergic state of these cells. Unlike control cells, the SOCS1-deficient LN cells responded to IL-2 alone, and the reversal of anergy to the H-Y peptides by IL-2 was markedly elevated in SOCS1-deficient cells (Fig. 9C). These observations suggest that hyperresponsiveness to cytokines caused by SOCS1 deficiency could modulate the anergic state of T3.70" Tg T cells in H-Y TCR" males.

SOCS1 is implicated in regulating systemic autoimmunity caused by increased production of anti-DNA autoantibodies (45). We observed that sera from 12- to 15-wk-old SOCS1"/"H-Y TCRtg males.
impaired in SOCS1<sup>−/−</sup> Rag1<sup>−/−</sup> AND TCR Tg mice (49). Whereas this contention may be true for a small subset of thymocytes, which express low-affinity TCRs similar to the AND TCR, studies on SOCS1<sup>−/−</sup> mice with a polyclonal TCR repertoire suggest that a positive regulatory role for SOCS1 is unlikely for most CD4 T cells. Comparison of our results on the SOCS1<sup>−/−</sup> mice which harbor a polyclonal TCR repertoire and the data from SOCS1<sup>−/−</sup> Rag1<sup>−/−</sup> AND TCR Tg mice show that 1) in the AND Tg model, SOCS1 deficiency causes an increase in the frequency and number of DP thymocytes, whereas non-TCR Tg SOCS1<sup>−/−</sup> mice display depletion of the DP subset; 2) in the AND Tg mice, SOCS1 deficiency causes a decrease in the frequency and number of CD4 SP thymocytes, whereas in non-TCR Tg SOCS1-deficient mice, the frequency of CD4 SP is increased (which can be relative due to the decrease in the proportion of DP cells) though not the absolute cell count; 3) in terms of absolute cell number, total thymocytes and CD4 SP cells display a 2-fold reduction in SOCS1<sup>−/−</sup> AND TCR Tg mice whereas the non-TCR Tg SOCS1<sup>−/−</sup> mice, which exhibit a >30-fold decrease in total thymocyte number, display only a 10-fold decrease in CD4 SP cells; 4) paradoxically, IFN-γ deficiency restored the frequency and number of DP and CD4 SP thymocytes in both models; and 5) both models show an increase in CD8 SP cell frequency, which we have attributed to increased IL-7 and IL-15 signaling in the present study. It is likely that IL-7 and IL-15 may be required for the MHC-II-dependent development of CD8 SP cells in the
SOCS1−/−Rag1−/− and TCR Tg model. Collectively, these data support a definitive role for SOCS1 in regulating CD8 SP thymocyte development. By restraining IFN-γ signaling, SOCS1 regulates the differentiation of CD4 SP thymocytes, however, further studies using different TCR Tg models, which differ in affinity, are needed to dissect the role of SOCS1 in the development of CD4 SP thymocytes.

In the peripheral T cell compartment, clearly IL-15 but not IL-7 is critical to sustain the frequency of CD8+ T cells (Fig. 3). IL-15−/− mice contained at least four times less CD8+ cells than CD4− cells in LNs, which was also observed in IL-15Ra−/− mice (15). IL-7−/− IL-15−/− mice contained an even lower frequency of CD8+ cells (Fig. 3), possibly because fewer naive CD8+ T cells would survive in the periphery due to the absence of the survival cytokine IL-7 (50). SOCS1 deficiency only partially restored the frequency of CD8+ cells in IL-15-deficient mice, however, this rescue was unlikely to be mediated by IL-7 because such rescue also occurred in IL-7−/− IL-15−/− mice. IL-15 alone can induce proliferation of CD8+ CD44high memory T cells (51, 52), and naive CD8+ CD44low cells show negligible proliferation in response to IL-15 even in the absence of SOCS1 (30). Recently, Zeng et al. (53) have shown that IL-7 or IL-15 can stimulate naive CD8+ T cells to divide, but only in the presence of another γc cytokine IL-21. We have observed that SOCS1-deficient CD8+ T cells are stimulated very strongly by IL-7 or IL-15 in the presence of IL-21 (J. Gagnon, S. Ramanathan, C. Leblanc, R. Therrien, and S. Ilan gumaran, unpublished data). Markedly reduced numbers of CD8+ T cells in IL-15−/−, SOCS1−/−/IL-15−/−, and IL-15Ra−/− mice (Ref. 15) and Fig. 3) could therefore result from the lack of IL-15R signaling on both naive and memory CD8+ T cells. We have also noticed that IL-21 can synergize with IL-2 to induce substantial proliferation of SOCS1-deficient CD8+ T cells (J. Gagnon, S. Ramanathan, C. Leblanc, R. Therrien, and S. Ilan gumaran, unpublished data), and such cytokine combinations could account for the partial recovery of CD8+ T cell frequency in IL-15-deficient SOCS1−/− mice. Based on these observations, we conclude that 1) IL-15 is a critical regulator of the pool size of the peripheral CD8 compartment; 2) SOCS1 is an indispensable regulator of this pool; and 3) even in the absence of SOCS1, other cytokines including IL-7 can only have a minimal effect on the homeostasis of the CD8+ T cell compartment.

Upon activation, CD8+ T cells up-regulate several cell surface molecules, of which CD44, CD122, and Ly6C remain elevated in memory cells whereas others such as CD25 and CD69 are down-regulated upon memory cell differentiation (54). Ag-independent homeostatic expansion of memory CD8+ T cells is accompanied by the up-regulation of memory markers without concomitant expression of activation markers CD25 and CD69 (55–57). IL-15 and IL-2 have been shown to act on Ag-activated CD8+ T cells into distinct differentiation pathways of central (Tcm) and effector memory T cells, respectively, with a distinct pattern of cell surface molecules (37, 58). A majority of CD8+ T cells in SOCS1−/− mice exhibit a CD44high/CD62Llow/CD25low central memory phenotype (30), consistent with their in vivo expansion by IL-15 (58). Elevated expression of CD44 in SOCS1-deficient CD8+ T cells is driven essentially by IL-15 because CD44 expression on CD8+ T cells from SOCS1−/−/IL-15−/− mice was markedly diminished (Fig. 4). In contrast, expression of CD122 and Ly6C on CD8+ T cells from SOCS1−/−/IL-15−/− mice remained high (Fig. 4). It is possible that the up-regulation of CD122 and Ly6C are maintained by cytokines other than IL-15, or their induction may be integral to the memory cell differentiation process that their continued expression may not require sustained stimulation by cytokines or Ag. Whereas CD122 expression confers to memory CD8+ T cells sensitivity to low concentrations of IL-15, and the levels of CD62L and CCR7 influence the homing properties of Tcm and TEM memory cells (59), the functional significance of elevated Ly6C expression on memory CD8+ T cells remains to be investigated.

IL-15 induces primarily CD8+ CD44high memory T cells to divide (51, 52), therefore, CD8+ T cells that accumulate in SOCS1-deficient mice (30) are likely to be Ag-experienced, memory cells. Cornish et al. (29) have reported using OT-I Tg mice that the OT-I Tg cells exhibit an activated phenotype without prior Ag stimulation. The H-Y TCR is known to be nonreactive to environmental Ags (41) and the H-Y TCR female mice allow simultaneous examination of both Ag-stimulated and Ag-inexperienced cells within the same animal. Rapid acquisition of CD44high phenotype by non-Tg T cells, but only slow acquisition by Tg T cells in SOCS1-deficient mice, clearly show that Ag-experienced T cells gain early responsiveness to IL-15, possibly resulting from up-regulated IL-2Rβ expression. Interestingly, CD8+ T370+/H-Y TCR Tg cells rapidly became CD44high in SOCS1-deficient mice but not in SOCS1−/− Tg mice which do not harbor the non-Tg T cells that are reactive to environmental Ags. Moreover, T370+/CD4+ Tg cells, which developed in SOCS1−/− H-Y TCR mice, were absent in SOCS1−/− Rag1−/− H-Y TCR Tg mice (Figs. 6 and 7). Because activated CD4+ Tg cells secrete IL-21 (60) that can synergize with IL-15 or IL-7 to stimulate naive CD8+ T cells (53), it is possible that the naive Tg CD8+ T cells in SOCS1−/− H-Y TCR mice might have been activated by IL-15 and/or IL-7 in conjunction with IL-21 secreted by activated CD4+ T cells. It is also likely that CD4+ Tg cell help might have contributed to the activated phenotype of T3.70+/CD8+ T cells in SOCS1−/− H-Y TCR mice, because these cells do not up-regulate CD44 in SOCS1−/− Rag1−/− H-Y TCR Tg mice (Figs. 6 and 7). These results strongly support the notion that SOCS1 regulates T cell homeostasis by controlling cytokine responsiveness of both memory and naive CD8+ T cell compartments.

We have examined the effect of SOCS1 deficiency on the negative selection of CD8+ T cells in the male H-Y TCR mouse model (61, 62). In mice deleted for the tumor suppressor PTEN in T cells, for example, negative selection is impaired with a consequent accumulation of CD8+ H-Y TCR Tg T cells and failure of immunological tolerance (62). Our results show that SOCS1-deficient male H-Y TCR mice, despite showing accelerated maturation of CD8 SP thymocytes, exhibited reduced thymic cellularity and a CD8+ T3.70+ T cell frequency comparable to that of controls (Fig. 8), indicating that SOCS1 deficiency did not interfere with the negative selection process. These observations corroborate with the report that staphylococcal enterotoxin B-mediated negative selection in fetal thymic organ cultures was not impaired by SOCS1 deficiency (31). In this context, it is noteworthy that SOCS1 deficiency affects the negative selection of AND TCR Tg CD4+ thymocytes, allowing their abnormal differentiation into CD8+ cells (49). This finding is in contrast to the normal deletion of CD4+ VB8+ cells in SOCS1-deficient thymic lobes treated with staphylococcal enterotoxin B ex vivo (31). It remains to be investigated whether the apparent different outcomes of negative selection in the H-Y and AND TCR Tg mice in the absence of SOCS1 are intrinsic to the TCRβ models studied.

SOCS1−/− H-Y TCRβ males survived for >6 mo without displaying the characteristic pathology that occurs in the absence of SOCS1, suggesting that SOCS1 deficiency did not interfere with the anergic state of CD8+ T3.70+ Tg T cells in these mice. SOCS1-deficient CD8+ T3.70+ Tg T cells remained unresponsive to stimulation by the H-Y peptide, however, they responded to IL-2 and displayed significantly elevated response to stimulation.
by H-Y peptide plus IL-2 in vitro (Fig. 9C). These observations suggest that hyperresponsiveness to cytokines caused by SOCS1 deficiency could potentially modulate the anergic state of T3.70+ Tg T cells and lead to the breakdown of tolerance. A possible explanation for the increased survival of SOCS1−/−/H-Y TCRtg males could be the virtual absence of CD4+ T cells, which may limit the amount of IL-2 available to the anergic CD8+ T3.70+ Tg T cells in vivo. In this context, it is noteworthy that CD8+ T3.70+ Tg T cells from female mice also require help from CD4+ T cells for efficient activation (43). Inadequate T cell help may also underlie the delayed accumulation of anti-DNA autoantibodies in male H-Y TCRtg mice lacking the SOCS1 gene.

In conclusion, we have shown that IL-7 and IL-15 are crucial for the development of CD8+ T cells within the thymus, and SOCS1 regulates this process by regulating both cytokines. Second, IL-15 is primarily responsible for the dysregulated homeostasis of CD8+ T cells in the periphery of SOCS1-deficient mice, and any contribution by other cytokines to this dysregulation is only secondary or synergistic to IL-15. Third, by uncoupling the up-regulation of CD44 and CD122 or Ly6C on CD8+ T cells in IL-15-deficient mice, SOCS1 deficiency has revealed that multiple signals contribute to the memory T cell differentiation program. Fourth, Ag-independent activation of naive T cells is exacerbated in SOCS1-deficient mice, possibly by a bystander mechanism that is dependent on Ag-activated T cells. Finally, increased cytokine availability and cytokine hyperresponsiveness caused by SOCS1 deficiency could modulate immunological tolerance mechanisms, and under permissive environments, might facilitate the development of autoimmunity.

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References


