Suppressor of cytokine signaling (SOCS-1) is an essential regulator of cytokine signaling. SOCS-1-deficient mice die before weaning with a complex disease characterized by fatty degeneration and necrosis of the liver. This disease is mediated by interferon (IFN) \( \gamma \) as neonatal mortality fails to occur in SOCS-1-/- mice. However, the immune system of healthy SOCS-1-/- mice is dysregulated with a reduced ratio of CD4:CD8 T cells, increased mortality in some aspects of T cell activation. SOCS-1-/- mice also die before their wild type and IFN\( \gamma \)-/- counterparts with a range of inflammatory conditions including pneumonia, gut infiltration, and skin ulceration, suggesting that SOCS-1 controls not only IFN\( \gamma \) signaling, but also other immunoregulatory factors. This study shows that T cells from SOCS-1-deficient mice display hypersensitivity to cytokines that act through the \( \gamma c \) receptor. SOCS-1 expression is induced by interleukin (IL) 2, IL-4, IL-7, and IL-15, and SOCS-1-deficient T cells show increased proliferation and prolonged survival in response to IL-2 and IL-4. Furthermore, IL-2 induced increased STAT5 phosphorylation and CD44 expression in SOCS-1-deficient T cells compared with controls. Hypersensitivity to \( \gamma c \)-dependent cytokines may contribute to abnormal T cell function, as well as the pathology observed in mice lacking SOCS-1.

Suppressor of cytokine signaling (SOCS-1) is an important negative regulator of cytokine signaling. Studies in vitro have shown that SOCS-1 can be induced by and inhibit signaling initiated by a wide range of cytokines including interleukin-2 (IL-2), IL-4, IL-6, growth hormone, leukemia inhibitory factor, prolactin, interferon \( \alpha/\beta \) (IFNa/\beta), and IFN\( \gamma \) (reviewed in Ref. 1). Studies using mice lacking SOCS-1, however, have suggested a more specific role for SOCS-1 in vivo. SOCS-1-deficient mice die before weaning with a complex neonatal disease characterized by fatty degeneration of the liver, lymphocyte activation, and hematopoietic infiltration in several tissues (2, 3). This disease is dependent on IFN\( \gamma \) as mice lacking both SOCS-1 and IFN\( \gamma \) do not develop liver disease and survive in apparent health into adult life (4). Disease in SOCS-1-/- mice is characterized by increased production of IFN\( \gamma \) resulting in higher levels of IFN\( \gamma \) in the serum, and hypersensitivity to IFN\( \gamma \) (5, 6). Although IFN\( \gamma \) plays a key role in neonatal disease in SOCS-1-/- mice, mice lacking both SOCS-1 and IFN\( \gamma \) die prematurely with a variety of inflammatory conditions including skin ulceration, pneumonia, and hematopoietic infiltration of the gut (7). This suggests that in vivo SOCS-1 not only controls IFN\( \gamma \) signaling, but also regulates other aspects of immune cell function.

Recently, we have shown that SOCS-1 influences T cell homeostasis (8). Mice lacking SOCS-1 have a decreased ratio of CD4:CD8 T cells, and expression of the activation marker CD44 is increased on T cells (5, 9, 10). Furthermore, proliferation in vivo of both CD4+ and CD8+ cells is increased in peripheral lymphoid organs of SOCS-1-/- IFN\( \gamma \)-/- mice. These defects occur not only in sick SOCS-1-/- mice but are evident also in healthy SOCS-1-/- IFN\( \gamma \)-/- mice (8). The apparent activation state of T cells in SOCS-1-deficient mice is antigen-independent. T cell receptor (TCR)-transgenic OT-I SOCS-1-/- mice, in which T cells respond specifically to an exogenous antigen, ovalbumin, still appear activated despite the absence of ovalbumin stimulation (8).

Cytokines have been shown previously to be important regulators of T cell homeostasis (reviewed in Ref. 11). Altered ratios of CD4:CD8 T cells can occur in instances where cytokine signaling is dysregulated. For example, IL-12 can increase the numbers of CD8+ cells in the thymus (12), and IFNa/\beta can promote the survival of CD8+ T cells over that of CD4+ T cells (13). Indeed, in the absence of SOCS-1, IL-12 responses are dysregulated, with IL-12 inducing increased T cell proliferation and natural killer cell activity (14). However, even in the absence of IL-12 signaling in SOCS-1-/- STAT4-/- mice, T cell function is still perturbed with a decreased CD4:CD8 ratio and increases in apparent activation (14). This suggests that, even though uncontrolled IL-12 signaling may contribute to T cell defects, other immunoregulatory factors are also important.

Cytokines that appear to play the most pivotal roles in controlling T cell function are those that signal through the common \( \gamma c \) chain receptor subunit (\( \gamma c \)), that is IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (15, 16). Of these IL-2, IL-4, IL-7, and IL-15 show clear roles in T cell proliferation and survival (17–21). Moreover, altered \( \gamma c \)-dependent cytokine signaling can lead to changes in the ratio of CD4:CD8 T cells within the mouse.
Excess IL-7, for example, induces a decreased CD4:CD8 ratio (22, 23), whereas the converse is evident in IL-15R-deficient mice (24).

To explore the mechanism underlying the decreased ratio of CD4:CD8 T cells and the increased proliferation and CD44 expression by T cells in mice lacking SOCS-1, responses to the γc-dependent cytokines IL-2, IL-4, IL-7, and IL-15 were investigated in T cells from healthy SOCS-1−/−/IFNγ−/− mice. Relative to control cells, T cells from mice lacking SOCS-1 displayed increased sensitivity to γc-dependent cytokines, particularly IL-2. Thus, SOCS-1 appears not only to regulate IFNγ signaling in vitro, but may also control responses to γc-dependent cytokines in peripheral T cells.

EXPERIMENTAL PROCEDURES

Generation and Maintenance of Mice

SOCS-1−/−/IFNγ−/− mice were generated as described previously on a mixed 129/Sv and C57BL/6 genetic background (2, 4). IFNγ−/− mice on an inbred C57BL/6 background (C57BL6-IFNγ−/−) were obtained from Jackson Laboratories via Monash University (25). For studies on a syngeneic genetic background, SOCS-1−/− mice were backcrossed at least 10 generations to C57BL/6 mice and then mated with IFNγ−/− mice to produce deficient for both SOCS-1 and IFNγ. These mice had an identical phenotype to SOCS-1−/−/IFNγ−/− mice generated on a mixed 129/Sv and C57BL/6 genetic background, as described previously (4). SOCS-1−/− mice were generated as described (26). Briefly, the SOCS-1 coding region was replaced by a SOCS-1 allele as described (26). Briefly, mice were genotyped by Southern blot analysis of genomic DNA obtained from tail tips as described (4). Mice had an identical phenotype to SOCS-1−/−/IFNγ−/− mice generated on a mixed 129/Sv and C57BL/6 genetic background, as described previously (4). SOCS-1−/− mice were generated as described (28). Briefly, the SOCS-1−/− mice were backcrossed onto the C57BL/6 genetic background for at least 10 generations.

Mice were genotyped for SOCS-1 and IFNγ alleles by Southern blot analysis of genomic DNA obtained from tail tips as described (4). Mice with Cre transgenic mice were genotyped for their SOCS-1 allele as described (26). Briefly, mice were genotyped by Southern blot analysis from tail tips to discriminate between the SOCS-1−/−, SOCS-1+, and SOCS-1+/− alleles. Recombination of the SOCS-1−/− allele was also confirmed by Southern blotting. Cre transgenic mice were genotyped by PCR as described (26).

Purification of T Cell Populations

Lymphoid cells were isolated from pooled inguinal, brachial, axillary, submandibular, and mesenteric lymph nodes from SOCS-1−/−/IFNγ−/− or IFNγ−/− mice. Cells were sorted into CD4+CD44−, CD4+CD44+, CD8+CD44+, or CD8+CD44+ T cell populations by FACS. Cells were stained with mAb for CD44, CD8, and CD4 as described above and then stained with hamster anti-mouse CD3 (clone 145–2C11) at 50 μg/ml. Samples were run on the FACS machine for ~30 s to obtain a baseline reading, followed by TCR stimulation and calcium flux induction after cross-linking the anti-CD3 Ab with 25 μg/ml anti-hamster IgG (BD Pharmingen). Changes in intracellular calcium concentrations were monitored for 4–6 min by calculating the ratio of the fluorescence emissions of T cells at 425 nm and 530 nm by FACS.

Statistical Analyses

Data from in vitro assays were analyzed using a two-tailed Student’s t test for independent events. Bonferroni adjustments for multiple testing were included.

RESULTS

SOCS-1 Expression Is Up-regulated by γc-dependent Cytokines—SOCS-1 expression has been shown to be induced by a number of cytokines (reviewed in Ref. 1), including members of the γc-dependent cytokine family IL-2 and IL-4 (30–32). Recently, conditional SOCS-1-deficient mice were produced using the Cre-Lox system (26). These mice were crossed to CMV-Cre mice resulting in widespread deletion of SOCS-1. SOCS-1lox/−/CMV-Cre mice were used to assess SOCS-1 expression in response to γc-dependent cytokines. In these mice, the reporter gene hCD4 is under the regulation of the SOCS-1 promoter and thus provides a surrogate measure of SOCS-1 expression.

SOCS-1 expression was regulated in T cells by the γc-dependent cytokines IL-2, IL-4, IL-7, and IL-15 (Fig. 1). Induction of SOCS-1 expression in response to IL-4 and IL-7 was seen in both CD4+ and CD8+ cells, whereas expression was restricted to CD8+ cells in response to IL-2 and IL-15 (Fig. 1). SOCS-1 expression was most strongly induced by IL-4. Ligation of the TCR through anti-CD3 binding also strongly induced SOCS-1 expression in both T cell subsets (Fig. 1), consistent with recent data (33). This expression, however, appears to be largely because of autocrine cytokine production by these cells in response to anti-CD3, as cytokine-induced SOCS-1 expression was almost completely abrogated in the presence of neutralizing antibodies to IL-2 and IFNγ (Fig. 1).

Intracellular Calcium Flux in Response to TCR Cross-linking

Intracellular calcium flux was monitored as previously described (29). Briefly, indo-1/AM (Molecular Probes, Eugene, OR)-loaded lymph node cells were stained with mAb for CD4, CD8, and CD44 as described above and then stained with hamster anti-mouse CD3 (clone 145–2C11) at 50 μg/ml. Samples were run on the FACS machine for ~30 s to obtain a baseline reading, followed by TCR stimulation and calcium flux induction after cross-linking the anti-CD3 Ab with 25 μg/ml anti-hamster IgG (BD Pharmingen). Changes in intracellular calcium concentrations were monitored for 4–6 min by calculating the ratio of the fluorescence emissions of T cells at 425 nm and 530 nm by FACS.
SOCS-1 Regulates γc-dependent Cytokine Signaling

FIG. 1. SOCS-1 expression is induced by γc-dependent cytokines. T cells expressing the hCD4 reporter under control of the SOCS-1 promoter from SOCS-1-/-/CMV-Cre mice were treated with the indicated stimuli and analyzed by FACS.

Increased Proliferation of SOCS-1-/-/IFNγ-/- T Cells in Response to γc-dependent Cytokines—We then examined whether biological responses to γc-dependent cytokines were perturbed in T cells from SOCS-1-/-/IFNγ-/- mice compared with IFNγ-/- mice. SOCS-1-/-IFNγ-/- T cells display immune defects, including a decreased ratio of CD4:CD8 T cells and increased expression of the activation marker CD44, which complicate direct comparisons with control cells. To overcome this difficulty, T cells were sorted into the following subsets, CD4+CD44lo, CD4+CD44hi, CD8+CD44lo, and CD8+CD44hi. T cells were treated with IL-2, mIL-7, mIL-4, or mIL-15 (with endogenous mIL-2 effects blocked by a neutralizing anti-mIL-2 antibody) and proliferation was measured by [3H]thymidine incorporation (Fig. 2). SOCS-1-/-IFNγ-/- T cells were more sensitive than control cells to IL-2, with robust proliferation evident at 2–20 ng/ml, doses at which little response was observed for control IFNγ-/- T cells (Fig. 2A). The increase in proliferation was greatest in the CD8+CD44lo T cell subset. IL-4 also induced significantly greater proliferation in SOCS-1-/-IFNγ-/-CD8+ T cells compared with controls (Fig. 2B). Comparatively low levels of proliferation were induced by IL-7 and no difference in proliferation was observed between T cells from SOCS-1-/-IFNγ-/- and IFNγ-/- mice (Fig. 2C). IL-15 induced proliferation only at the highest concentration used, 200 ng/ml, and proliferation was increased modestly in SOCS-1-/-IFNγ-/-CD8+CD44lo T cells (Fig. 2D).

Increased Survival of SOCS-1-/-IFNγ-/- T Cells in Response to γc-dependent Cytokines—Survival of T cells was compared by propidium iodide exclusion after treatment with IL-2, IL-4, IL-7, and IL-15 (Fig. 3). In general, cell survival was promoted by all 4 cytokines examined, with IL-4 and IL-7 having the strongest effect. IL-2 mainly induced survival of CD8+ cells, and survival of CD8+CD44lo cells from SOCS-1-/-IFNγ-/- mice appeared to be modestly enhanced compared with those from control IFNγ-/- mice (Fig. 3A). IL-4-induced survival was increased significantly in SOCS-1-/-IFNγ-/- T cells, and this difference was most pronounced in CD8+ cells (Fig. 3B). In contrast, there was no difference in IL-7-induced survival (Fig. 3C) and only minor increases in IL-15-induced survival were observed in SOCS-1-/-IFNγ-/-CD8+ T cells (Fig. 3D).

Increased CD44 Expression by SOCS-1-/-IFNγ-/- T Cells in Response to γc-dependent Cytokines—Mice lacking SOCS-1 express high levels of CD44 on peripheral T cells, which is most pronounced in cells of the CD8+ lineage. This occurs not only in sick SOCS-1-/- mice, but also in healthy SOCS-1-/-IFNγ-/- mice and in TCR-transgenic OT-I SOCS-1-/- mice not exposed to a specific antigen, suggesting that up-regulation occurs independently of disease, IFNγ, and specific TCR stimulation by antigen (8). CD44 up-regulation was examined on sorted CD44lo T cells from healthy SOCS-1-/-IFNγ-/- mice after γc-dependent cytokine stimulation. In control cells, only minor changes in CD44 expression were induced by treatment with these cytokines. In SOCS-1-/-IFNγ-/- T cells, however, CD44 expression was strongly induced by IL-2 and to a lesser extent IL-15, but not by IL-4 or IL-7 (Fig. 4). This was only evident in CD8+ T cells (Fig. 4, A, D, and E).

Elevated Responses to γc-dependent Cytokines Are Not Because of Increased Receptor Expression—To assess whether the changes in sensitivity to γc-dependent cytokines were due merely to changes in receptor expression, the expression of receptor subunits by SOCS-1-deficient T cells was examined (Fig. 5). The receptor subunit γc is shared by the IL-2, IL-4, IL-7, and IL-15 cytokine receptor complexes, whereas IL-2Rβ is required for signaling by IL-2 and IL-15. IL-7Ra is specific for IL-7 and IL-2Ra for IL-2 signaling. There appeared to be a higher level of IL-2Rβ expression on SOCS-1-deficient CD8+ T cells (Fig. 5). From previous studies, it is known that IL-2Rβ expression is increased on CD44lo T cells, especially from the CD8+ lineage (20, 34). When T cells were separated into CD44lo and CD44hi populations, no difference was observed in IL-2Rβ expression from CD8+ T cell subpopulations from
SOCS-1 Regulates γc-dependent Cytokine Signaling

Increased proliferation of SOCS-1−/−IFNγ−/− T cells in response to γc-dependent cytokines. T cells from SOCS-1−/−IFNγ−/− and IFNγ−/− mice were sorted into the indicated subsets and stimulated with IL-2 (A), IL-4 (B), IL-7 (C), or IL-15 (D). Cellular proliferation was monitored by [3H]thymidine incorporation after 3.5 days, n = 3, *, p < 0.05.

Increased survival of SOCS-1−/−IFNγ−/− T cells in response to γc-dependent cytokines. T cells from SOCS-1−/−IFNγ−/− and IFNγ−/− mice were sorted into the indicated subsets and stimulated with IL-2 (A), IL-4 (B), IL-7 (C), or IL-15 (D). Cellular survival was determined as a percentage of live cells (negative for propidium iodide staining) by FACS after 3.5 days, n = 3, *, p < 0.05.

Increased STAT5 Phosphorylation in Response to γc-dependent Cytokines—Given the enhanced biological responses of SOCS-1-deficient T cells to γc-dependent cytokines, biochemical studies were performed to determine whether loss of SOCS-1 resulted in increased downstream signaling after γc-dependent cytokine stimulation. As SOCS-1 acts to negatively regulate the JAK-STAT pathway, the activation status of STAT5 in response to γc-dependent cytokines was observed in cells lacking SOCS-1. STAT5 phosphorylation was enhanced in cells lacking SOCS-1 in response to IL-2 and IL-7. STAT5 phosphorylation in SOCS-1-deficient cells was both more intense and occurred in response to lower concentration of these cytokines (Fig. 6, A and B). Phosphorylation of STAT5 in re-
response to IL-15 was also more intense in SOCS-1–deficient cells, but the increase was less pronounced than for IL-2 and IL-7 (Fig. 6C). These results were also seen in T cells lacking SOCS-1 derived from SOCS-1lox/lox/lck-Cre mice, compared with SOCS-1-replete T cells from control SOCS-1lox/lox mice (data not shown).

TCR Signaling Is Not Enhanced in SOCS-1–Deficient T Cells—T cell activation occurs through the specific interaction of the TCR and antigen presented by major histocompatibility complex molecules upon antigen presenting cells. This interaction results in a number of downstream signaling events to induce specific gene activation through the transcription factors NFκB, NFAT, and AP-1. These signaling events include kinase cascades activating the mitogen-activated protein kinase pathway and increases in intracellular calcium flux. Interestingly, studies in vitro have identified several components implicated in the TCR signaling pathway, namely Syk, CD3ζ, and Tec, as potential binding partners of SOCS-1 (35, 36). In addition, reconstitution of the TCR signaling pathway using overexpression of Syk and a chimeric CD8/CD3ζ protein in 293T cells was inhibited by the addition of SOCS-1 (35). SOCS-1, therefore, may negatively regulate signals directly downstream of the TCR. In this case, SOCS-1-deficient cells may be partially activated in the absence of cytokine, and may be more responsive to mitogenic signals.

To determine whether increased sensitivity of SOCS-1-deficient cells to γc-dependent cytokines was secondary to defective
SOCS-1 Regulates γc-dependent Cytokine Signaling

TCR signaling, we examined calcium flux in response to TCR ligation, an early event in T cell activation, in T cells lacking SOCS-1. T cells were gated upon their expression of the markers CD4, CD8, and CD44 and intracellular calcium flux monitored in response to anti-CD3 ligation of the TCR. There was no difference in the performance of each T cell subset to stimulation of the TCR (Fig. 7). It seems unlikely, therefore, that increased responses to γc-dependent cytokines is secondary to unregulated signaling through the TCR.

DISCUSSION

T cells from healthy SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice display features of T cell activation including enhanced expression of CD44 and increased T cell proliferation in vivo (8). Classical activation of T cells occurs through antigen stimulation of the TCR. Despite having apparent features of activation, however, T cells from healthy SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice show little change in CD25 and CD69 expression, and exhibit no significant effector function such as cytotoxic activity (8). Increases in activation marker expression could reflect dysregulated TCR signaling in SOCS-1-deficient cells, but we found no evidence that SOCS-1 regulates signals directly downstream of the TCR.

In addition, T cells from TCR-transgenic SOCS-1<sup>-/-</sup> mice show increases in CD44 expression in the absence of specific antigen stimulation of the TCR (8).

Cytokines that signal through the γc receptor are critical for normal T cell development and function of the immune system (15) and hence are prime candidates for mediating these T cell defects. γc-dependent cytokines share common receptor subunits and exhibit redundancy in many of their activities, however, they also exhibit non-redundant functions. For example, IL-2, IL-4, IL-7, and IL-15 are all important for T cell proliferation, however, IL-2 is a particularly potent T cell mitogen, IL-4 is a key cytokine mediating Th2 functions of CD4<sup>+</sup> cells, IL-7 is critical for the survival of thymocyte progenitors and naïve T cells, and IL-15 is important for the homeostasis of memory CD8<sup>+</sup> T cells (reviewed by Ref. 15). We have demonstrated that in peripheral T cells, SOCS-1 is induced by stimulation with γc-dependent cytokines. To assess responses to γc-dependent cytokines in the absence of SOCS-1, we measured proliferation, survival, CD44 expression, and STAT activation in SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> T cells in response to IL-2, IL-4, IL-7, and IL-15.

The major finding from this study is that T cells lacking SOCS-1 are hypersensitive to IL-2. Using in vitro approaches, we showed that IL-2 treatment was able to induce immune changes similar to those seen in SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice in vivo. T cells from healthy SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice displayed increases in proliferation, up-regulation of CD44, and slight increases in survival in response to IL-2. Furthermore, IL-2-induced proliferation of CD8<sup>+</sup> cells was much stronger than that of CD4<sup>+</sup> cells, suggesting that this response to IL-2 may contribute to the perturbed CD4/CD8 T cell ratio in SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice.

Consistent with a role for SOCS-1 in the negative regulation of IL-2 signaling, several studies have shown a biochemical interaction between SOCS-1 and the IL-2 signaling pathway. SOCS-1 is postulated to act via two separate (although not necessarily mutually exclusive) mechanisms to inhibit JAK activity. The first is by direct binding to a phosphotyrosine residue in the activation loop of the JAK followed by inhibition of kinase activity (37), and the second is by targeting the JAKs for degradation via the ubiquitin-proteasome pathway (38). SOCS-1 has been shown to bind to IL-2Rα and can inhibit IL-2 signaling if overexpressed in IL-2-responsive cells (39). SOCS-1 has also been shown to associate with and inhibit the kinase activity of JAK1 and JAK3, through which the γc-dependent cytokines signal (30). Moreover, IL-2 signaling is regulated by proteasomal degradation possibly by targeting JAK1 or JAK3 to ubiquitin-mediated degradation (30, 39).

Interestingly, hIL-2 treatment in mice results in phenotypic changes similar to SOCS-1<sup>-/-</sup> mice, including fatty degeneration and necrosis of the liver, tissue infiltration by hematopoietic cells, and thrombocytopenia (40). Therefore, uncontrolled IL-2 signaling, in addition to effects on T cell regulation, may contribute to these IFNγ-dependent pathologies in SOCS-1<sup>-/-</sup> mice.

T cell proliferation and survival were also increased in SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> T cells in response to IL-4. This finding is consistent with previous studies that have found SOCS-1 to regulate IL-4, both in vitro and in vivo (3, 41–43). Although not as potent as IL-2 for inducing proliferation, IL-4 strongly sustained the survival of SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> T cells. Small increases in T cell proliferation, survival, and up-regulation of CD44 were observed also in SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> CD8<sup>+</sup> T cells in response to IL-15. IL-15 was shown to be an important factor controlling the survival of CD8<sup>+</sup> T cells with the generation of mice lacking IL-15 or IL-15Rs, which have reduced numbers of memory CD8<sup>+</sup> cells (24, 44). In addition, mice lacking the IL-15Ra subunit have an increased CD4/CD8 ratio, whereas IL-15-transgenic mice have a reduced CD4/CD8 ratio (24, 45).

Biological responses to IL-7 were unaltered in peripheral T cells from SOCS-1<sup>-/-</sup>IFN<sub>γ</sub><sup>-/-</sup> mice. This contrasts with the hypersensitivity of thymocytes to IL-7 in SOCS-1-deficient mice (26) and overexpression studies where IL-7 responses are inhibited by SOCS-1 (46, 47). Interestingly, SOCS-1 was induced after IL-7 treatment in T cells and T cells lacking SOCS-1 showed increased STAT5 phosphorylation, although no changes in biological responses were apparent. The lack of increased biological sensitivity to IL-7 in this study may reflect differences in the role of IL-7 in the thymus compared with the periphery (48–50) and/or may imply that deregulated responses to IL-7 exist in biological responses that were not examined. IL-7 was effective at supporting the survival of T cells in vitro, but was a very poor proliferative stimulus. It has been noted that IL-7 is unable to induce the proliferation of isolated T cells in vitro (51), possibly because a specific microenvironment is required for the appropriate display of IL-7 to unregulated signaling through the TCR.

Changes in intracellular calcium concentrations were monitored by calculating the ratio of the fluorescence emissions at 425 and 530 nm by FACS.

**Fig. 7.** Intracellular calcium flux in response to TCR ligation is not increased in SOCS-1-deficient T cells. Indo-1-loaded lymph node cells were incubated with anti-CD3, analyzed by FACS briefly to obtain a baseline reading, and then anti-CD3 antibody was cross-linked with an anti-hamster antibody at the indicated time point to induce TCR signaling. Changes in intracellular calcium concentrations were monitored by calculating the ratio of the fluorescence emissions at 425 and 530 nm by FACS.

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responding cells (52). STAT5 phosphorylation, however, was induced strongly in these cells. Previous work has shown that IL-7-induced proliferation and survival are not mediated through STAT5, but rather through the phosphatidylinositol 3-kinase/protein kinase B pathway, whereas IL-7-induced T cell differentiation in the thymus requires STAT5 (53, 54). It is possible, therefore, that SOCS-1 may regulate only the STAT5-dependent functions of IL-7, which have yet to be defined in mature, peripheral T cells.

Collectively, these data indicate that increased signaling by γc-dependent cytokines can induce T cell changes in vitro similar to those seen in SOCS-1−/−/IFN-γ−/− mice in vivo. The most profound changes were seen in response to IL-2, suggesting that hypersensitivity to this cytokine in the absence of SOCS-1 may contribute to the immune defects seen in these mice.

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