Suppressors of Cytokine Signaling Proteins Are Differentially Expressed in Th1 and Th2 Cells: Implications for Th Cell Lineage Commitment and Maintenance

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Suppressors of Cytokine Signaling Proteins Are Differentially Expressed in Th1 and Th2 Cells: Implications for Th Cell Lineage Commitment and Maintenance

Charles E. Egwuagu,1 Cheng-Rong Yu, Meifeng Zhang, Rashid M. Mahdi, Stephen J. Kim, and Igal Gery

Positive regulatory factors induced by IL-12/STAT4 and IL-4/STAT6 signaling during T cell development contribute to polarized patterns of cytokine expression manifested by differentiated Th cells. These two critical and antagonistic signaling pathways are under negative feedback regulation by a multimember family of intracellular proteins called suppressor of cytokine signaling (SOCS). However, it is not known whether these negative regulatory factors also modulate Th1/Th2 lineage commitment and maintenance. We show here that CD4⁺ naïve T cells constitutively express low levels of SOCS1, SOCS2, and SOCS3 mRNAs. These mRNAs and their proteins increase significantly in nonpolarized Th cells after activation by TCR signaling. We further show that differentiation into Th1 or Th2 phenotype is accompanied by preferential expression of distinct SOCS mRNA transcripts and proteins. SOCS1 expression is 5-fold higher in Th1 than in Th2 cells, whereas Th2 cells contain 23-fold higher levels of SOCS3. We also demonstrate that IL-12-induced STAT4 activation is inhibited in Th2 cells that express high levels of SOCS3 whereas IL-4/STAT6 signaling is constitutively activated in Th2 cells, but not Th1 cells, with high SOCS1 expression. These results suggest that mutually exclusive use of STAT4 and STAT6 signaling pathways by differentiated Th cells may derive in part, from SOCS3- or SOCS1-mediated repression of IL-12/STAT4- or IL-4/STAT6 signaling in Th2 and Th1 cells, respectively. Given the strong correlation between distinct patterns of SOCS expression and differentiation into the Th1 or Th2 phenotype, SOCS1 and SOCS3 proteins are therefore Th lineage markers that can serve as therapeutic targets for immune modulation therapy. The Journal of Immunology, 2002, 168: 3181–3187.

Diff erentiation of naïve, uncommitted Th precursor cells into Th1 or Th2 cells is a complex developmental process, and understanding the underlying molecular mechanisms may provide a conceptual framework in developing immune modulation therapies against allograft rejection, autoimmune diseases, and allergic diseases. The dominant factors that control the differentiation program are now recognized to be cytokines (1). Th cells activated in the presence of IL-12 differentiate into Th1 cells, predominantly secrete IFN-γ and TNF-β and promote delayed-type hypersensitivity responses (2). In contrast, Th cells that are activated in the presence of IL-4 differentiate into Th2 cells, produce mainly IL-4 and IL-5, and promote humoral and allergic responses (2).

Mutually exclusive use of the IL-12 or IL-4 signal transduction pathway by Th1 and Th2 cells, respectively, has spurred significant interest in understanding how regulation of these pathways are coupled to the differentiation process. IL-12 or IL-4 signaling is mediated by STAT4 and STAT6, respectively (3). Because transduction of cytokine signals through STAT proteins generally results in transcriptional activation of STAT-inducible genes, it is tacitly assumed that Th cells differentiate into Th1 or Th2 phenotype because of differential activation of genes that drives them to their respective developmental state. However, recent studies showing that cytokine signaling is under negative feedback regulation by a multimember family of proteins called suppressors of cytokine signaling (SOCS) have raised the possibility that differentiation toward the Th1 or Th2 pathway may be mediated in part by the selective repression of IL-12/STAT4 or IL-4/STAT6-signaling pathways, respectively (4).

The SOCS family of proteins is at present composed of eight members characterized by the presence of a Src homology 2 domain and a C-terminal conserved domain called the SOCS box (5, 6). Evidence to date suggests that mRNA transcripts encoding SOCS are selectively up-regulated in response to several cytokines including IFN-γ, IL-2, IL-3, IL-4, IL-6, IL-12, IL-13, leukemia inhibitory factor, stem cell factor, GM-CSF, growth factor, leptin, and erythropoietin (7). The SOCS proteins translated from the corresponding mRNA transcripts inhibit requisite cytokine-induced signaling pathways by classical feedback circuits (5–7). The inhibitory effects derive from direct interaction of SOCS Src homology 2 domains with cytokine receptors and/or Janus kinases (JAK) leading to the recruitment of SOCS proteins to the signaling complex, their inhibition of the binding of STATs to tyrosine-phosphorylated cytokine receptors, and suppression of the catalytic activities of JAKs (8–11). Gene targeting has been used to generate SOCS1⁻/⁻, SOCS2⁻/⁻, and SOCS3⁻/⁻ mice (12–16). Deletion of SOCS1 or SOCS3 is lethal in mice; SOCS3 null mice die in utero and mice lacking SOCS1 die within 3 wk after birth. Analysis of

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2 Abbreviations used in this paper: SOCS, suppressor of cytokine signaling; HEL, hen egg lysozyme; Tg, transgenic; JAK, Janus kinase; TAMRA, 6-carboxytetramethylrhodamine; CD62L, CD62 ligand; 6FAM, 6-carboxyfluorescein.
the physiological functions of these SOCS proteins reveals requirement of SOCS3 in fetal liver erythropoiesis and placental development, whereas SOCS2 is important in postnatal growth. In contrast, SOCS1 is crucial in regulation of IFN-γ pathways. SOCS1−/− mice have defective thymocyte development, and overexpression of SOCS1 impairs pre-TCR-induced thymocyte proliferation, suggesting that inhibition of cytokine signaling has important influence on T cell differentiation (17, 18).

In this study, we have characterized the repertoires of SOCS mRNAs and proteins expressed in naive and differentiated Th cells to examine the possibility that mutually exclusive patterns of cytokine expression by Th1 and Th2 cells derive from differential expression of SOCS proteins. We show here that Th1 and Th2 cells preferentially express distinct SOCS proteins that may play a role in the selective inhibition of STAT4 or STAT6 signal transduction pathway was analyzed in Th1 cells. After 4 days, cultured cells were expanded with 40 IU/ml IL-2 (Chiron, Emeryville, CA) for 4 days, and these cells are designated as resting Th1 or Th2 cells. Some cells were restimulated at 2.5 × 10^6/ml RPMI 1640 supplemented with 50 µM 2-ME, antibiotics, and 10% FCS (complete medium) with 10 ng/ml IL-12 (Sigma, St. Louis, MO), 10 ng/ml IL-12 (Sigma, Aldrich), and 10 µg/ml anti-IL-4 Ab (BD Pharmingen, San Diego, CA) for Th1 or 0.2 µg/ml HEL, 10 ng/ml IL-4 (BD Pharmingen), 10 µg/ml anti-IL-4 Ab (BD Pharmingen), and 10 µg/ml anti-IL-12 Ab (BD Pharmingen) for Th2. After 4 days, cultured cells were expanded with 40 IU/ml IL-2 (Chiron, Emeryville, CA) for 4 days, and these cells are designated as restimulating Th1 or Th2 cells. Some cells were restimulated at 2.5 × 10^6/ml with 10% FCS and intracellular STAT4 or STAT6 expression was analyzed in Th1 cells. After 4 days, activated Th1 or Th2 cells were restimulated with HEL or anti-IL-12 Ab (BD Pharmingen), and IL-4 (BD Pharmingen) or IL-12 (BD Pharmingen) for Th2. Three days later, cells were harvested, washed, resuspended in RPMI 1640, and designated as activated Th1 or Th2 cells. The cells designated “Th” were obtained by incubating purified CD4+ cells, with HEL in the absence of the polarizing cytokines or their Abs.

For signal transduction studies, Th1 and Th2 cell lines were established and maintained in IL-2 (40 IU/ml) under polarization conditions described above. However, the HEL protein and APC were substituted by anti-CD3 (0.1 µg/ml HEL) and anti-IFN-γ Ab (BD Pharmingen), and IL-4 (BD Pharmingen) or IL-12 (BD Pharmingen) for Th2. Three days later, cells were harvested, washed, resuspended in RPMI 1640, and designated as activated Th1 or Th2 cells. The cells designated “Th” were obtained by incubating purified CD4+ cells, with HEL in the absence of the polarizing cytokines or their Abs.

Isolation of naive CD4+ T cells and FACS analysis

CD4+ T lymphocytes from spleens and lymph nodes of 3A9 mice were purified as described above. The cells were stained with PE-Cy5-CD4 or CD62L, CD25, and their corresponding isotype controls. Samples were subjected to hot-start PCR in a reagent mix containing digoxigenin-11-dUTP (Roche, Indianapolis, IN) and AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). Primers used for PCR amplification are as follows. For SOCS1, 5′-CTCGAGTATGATGTACGGC-3′ and 5′-5CATCTCCAGCTGAGGGGAG-3′; for SOCS2, 5′-GGACAGTGCTGCTGGACGTG-3′ and 5′-GGACAGAGAATATTTTACCATCGGAAAT-3′; for SOCS3, 5′-TCGGCGATGTCACCACGACAGATT-3′ and 5′-GCTCTTATTAAGTGGGACATCATACTG-3′. For T-bet, 5′-TGGCTGCTAGTGTCTAACAG-3′ and 5′-TTGGCCGCTTTCCTCCCAACCA-3′. For c-maf, 5′-GTGACGAGACACATCTC-3′ and 5′-CAACTGACAGAAGCCACAC-3′; for SOCS1, 5′-TCGGCGATGTCACCACGACAGATT-3′ and 5′-GCTCTTATTAAGTGGGACATCATACTG-3′. Activation of the STAT4 or STAT6 signal transduction pathway was analyzed in Th1 and Th2 cells cultured for 2 h under starvation conditions (1% BSA, RPMI 1640). The cells were then treated with IL-12 (10 ng/ml) or IL-4 (10 ng/ml) for 15 or 30 min.

Differential SOCS expression in Th cells

For measurement of cytokines produced by the polarized cells, Th1 or Th2 cells were cultured as described above. Supernatants were collected after the second cycle of activation and assayed for cytokine secretion by ELISA, using kits obtained from Endogen (Woburn, MA).

RNAase protection assay

RNA (10 µg) was hybridized overnight with [α-32P]-UTP-radioabeled RNA probes transcribed in vitro from cDNA templates indicated in Fig. 1A. Overlapping ssRNA on hybridized dsRNAs was digested, and protected dsRNA duplexes were fractionated on denaturing-urea gels and processed for autoradiography.

Northern blot analysis

Northern blot analysis was performed with 20 µg RNA as described (20). The integrity and comparability of RNA preparations used for analysis were verified by agarose-formaldehyde gel electrophoresis; comparable amounts of 18S and 28S rRNAs were detected for all RNA preparations. Mouse β-actin, SOCS1, SOCS2, and SOCS3 cDNAs were used as hybridization probes. SOCS-specific cDNAs were kindly provided by D. Hilton (Walther and Eliza Hall Institute, Melbourne, Australia) and H. Young (National Institutes of Health, Bethesda, MD).

Western blotting and immunoprecipitation analyses

Preparation of whole cell lysates, immunoprecipitation, and immunodetection were performed as described (21). Briefly, samples (40 µg/lane) were fractionated on 4–20% gradient SDS-PAGE, and SOCS1, SOCS2, or β-actin-specific (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-pSTAT4- or anti-pSTAT6-specific (Zymed Laboratories, San Francisco, CA) polyclonal Abs were used as probes. For immunoprecipitation, 0.2 mg whole cell extract was incubated with protein G-agarose (Pharmacia Biotech, Piscataway, NJ), and anti-SOCS2 Ab for 1 h at 4°C and immunoprecipitates were washed four times in lysis buffer before electrophoresis. Preimmune serum was used in parallel as controls and signals were detected with HRP-conjugated secondary F(ab′)2 Ab (Zymed Laboratories) using the ECL system (Amersham, Arlington Heights, IL).

RT-PCR analysis

RNA (5 µg), SuperScript II Reverse Transcriptase (Life Technologies, Gaithersburg, MD), and oligo(dT)24-nucleotides used for first-strand synthesis as previously described (21). Samples were subjected to hot-start PCR in a reagent mix containing digoxigenin-11-dUTP (Roche, Indianapolis, IN) and AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). Primers used for PCR amplification are as follows. For SOCS1, 5′-CTCGAGTATGATGTACGGC-3′ and 5′-5CATCTCCAGCTGAGGGGAG-3′; for SOCS2, 5′-GGACAGTGCTGCTGGACGTG-3′ and 5′-GGACAGAGAATATTTTACCATCGGAAAT-3′; for SOCS3, 5′-TCGGCGATGTCACCACGACAGATT-3′ and 5′-GCTCTTATTAAGTGGGACATCATACTG-3′. Amplification was conducted for 35 cycles of 30 s each at 95°C, 60°C, and 72°C, followed by a final 10-min extension at 72°C. Under this condition, amplification was within the linear portion of the Taq amplification curve. First-strand synthesis using each mRNA sample but no reverse transcriptase was performed to control for possible DNA contamination of mRNAs used as target for PCR amplification; failure to obtain RT-PCR products with any of the PCR amplifiers confirmed the absence of contaminating DNA templates. PCR-amplified fragments were fractionated on 1.5% agarose gels. PCR products from naive cells were transferred onto nylon membranes, and signal detection was by the radioimmunoassay method as recommended for the ECL detection system (Amersham).
SOCS1, SOCS2, or SOCS3 plasmid cDNA were assayed and used to construct the standard curves.

**Results**

**Generation of Ag-specific Th1 or Th2 cell lineage**

Recent studies have shown that SOCS proteins inhibit IL-4 signaling and the cross-talk between IFN-γ and IL-4 signaling pathways in hematopoietic cells (22, 23). Because these pathways have been implicated in Th cell differentiation and lineage commitment, we hypothesized that SOCS genes might be differentially expressed in Th cells and may contribute to establishment of stable Th1 or Th2 phenotype. To examine these possibilities, we generated HEL-specific Th1 and Th2 cells from the spleen and lymph nodes of HEL-specific TCR Tg mice and characterized them by RPA and ELISA cytokine assays. As shown in Fig. 1, the Th1 cells express relatively high levels of IFN-γ but undetectable amounts of IL-4 or IL-5 mRNAs, whereas the Th2 cells contain relatively high levels of IL-4 and IL-5 mRNAs. Nonpolarized Th cells produced lower levels of all three cytokines. Adaptive transfer of the Th1 cells into Tg mice expressing the HEL protein in the ocular lens produced delayed-type hypersensitivity-like ocular inflammatory disease, whereas large numbers of the Th2 cells induced eosinophilic inflammation only in immunodeficient recipients (19), further underscoring the fact that polarized T cells used in this study have the requisite phenotype expected of Th1 or Th2 lineage.

**SOCS1, SOCS2, and SOCS3 genes are differentially expressed in Th1 and Th2 cells**

We then examined the repertoire of SOCS family members expressed in HEL-stimulated nonpolarized (Th), Th1, and Th2 cells by Northern blot analysis. We found significantly higher levels of SOCS1 and SOCS2 mRNA transcripts in Th1 cells than in Th2 cells (Fig. 2A). In contrast, we detected a considerably higher level of the SOCS3 mRNA transcript in Th2 cells, with very little SOCS3 expression in Th1 cells. Nonpolarized Th cells, consisting of both Th1 and Th2 cells, produced low levels of all three SOCS transcripts. These results were obtained in three separate experiments that used independently derived Th, Th1, and Th2 cells.

In concert with results of our mRNA analyses, the level of SOCS1 protein detected in Th1 cells by Western blot analysis is significantly higher than in Th2 cells (Fig. 2B). Conversely, we detected SOCS3 protein in Th2 but not in Th1 cells. Although we could not detect SOCS2 protein in either Th1 or Th2 cells by Western blot analysis (not shown), it was detected in both cell types by immunoprecipitation, albeit with higher amounts in Th1 cells (Fig. 2B). The low SOCS2 protein level is surprising considering that Th1 cells express high levels of SOCS2 mRNA transcripts. This may suggest that the SOCS2 protein is either unstable in Th1 cells or that its steady-state concentration is regulated at the level of translation. Together, our protein and mRNA data provide the first evidence that SOCS are differentially expressed in Th1 and Th2 cells.

**Quantitative PCR analysis of SOCS1, SOCS2, and SOCS3 expression in Th1 and Th2 cells**

We determined the abundance of SOCS1, SOCS2, and SOCS3 in Th1 and Th2 cells by real-time quantitative PCR assay. In Fig. 3, we show real-time PCR amplification profiles of mouse SOCS1, SOCS2, and SOCS3 cDNAs from resting Th, Th1, or Th2 cells. Standard curves generated from the SOCS cDNA dilution series showed excellent linearity indicating precise, quantitative relationship between cDNA copy number, and fluorescence signal intensity within the dynamic range of the assay (data not shown). Assuming that a typical mammalian cell contains 10 pg RNA, our real-time PCR data indicate that Th1 cells contain ~1276 copies of SOCS1 transcripts per cell, as compared with only 246 copies in a Th2 cell. A similar analysis revealed a value of 1225 and 320 copies of SOCS2 transcripts in the Th1 and Th2 cell, respectively. In contrast, 1297 copies of SOCS3 transcripts are detected per Th2 cell and only 56 copies in Th1 cell. The relatively high levels of SOCS expression in Th1 and Th2 cells may be sufficient to influence phenotype-specific cytokine signaling and maintenance of the corresponding differentiated state.

**Effects of SOCS expression on IL-12 and IL-4 signaling in Th1 and Th2 cells**

Given the differential patterns of SOCS expression in Th1 and Th2 cells, we predicted that IL-4 signaling would be inhibited in Th1
cells by the relatively high levels of SOCS1 and SOCS2 expression in these cells. Conversely, we expected marked inhibition of IL-12 signaling in Th2 cells by high constitutive expression of SOCS3. To test this hypothesis, we established long term Th1 and Th2 cell lines as described above. Before use in our signaling studies, we verified that these cells do indeed retain their respective phenotypes by examining expression patterns of transcription factors that characterize Th1 or Th2 lineage. Consistent with published reports (2, 3), Th1 cells expressed high levels of T-bet, whereas Th2 cells expressed relatively higher levels of GATA-3 and c-maf than did Th1 cells (Fig. 4A). Analysis of these cells by real-time PCR verified that they retain their expected patterns of SOCS expression, with Th1 cells expressing relatively higher levels of SOCS1 and SOCS2, whereas Th2 cells contain higher levels of SOCS3 (Fig. 4B). We then examined activation of STAT6 and STAT4 after treatment of the cells with either IL-4 or IL-12 by Western blotting. In Th2 cells, IL-4 induced strong tyrosine phosphorylation of STAT6, whereas activation of STAT6 in Th1 cells is barely detected (Fig. 4C). We also detected activated STAT6 in untreated Th2 but not in Th1 cells, suggesting that constitutive and inducible IL-4/STAT6 signals in Th1 cells may be inhibited in part by high SOCS1 and SOCS2 expression. In contrast, IL-12 induced high levels of tyrosine-phosphorylated STAT4 in Th1 but not Th2 cells, suggesting that the high constitutive SOCS3 expression may inhibit IL-12/STAT4 pathway in these cells (Fig. 4D). Although these results clearly show that expression of specific SOCS correlates with activation of specific STAT signaling pathways, this does not necessarily constitute proof that the specific SOCS members mediate the relevant pathways in vivo.

SOCS genes are constitutively expressed in naive nonpolarized CD4+ lymphocytes

To determine whether differential regulation of the SOCS is intrinsic to Th cells or derives from effects of cytokines that polarize Th cells to the Th1 or Th2 phenotype, we analyzed SOCS expression in naive nonpolarized CD4+ T cells. Fresh CD4+ cells from the spleen and lymph nodes were isolated on a cell sorter as described in the text.

**FIGURE 3.** Quantitative detection of SOCS mRNA transcripts in Th cell types by real-time PCR. Amplification plots of the relative fluorescence intensity vs cycle number are shown for GAPDH, SOCS1, SOCS2 and SOCS3 target cDNAs. The dynamic range of each assay covers 1–10,000 cDNA copies. Bars show the number of copies (per cell) of SOCS1, SOCS2, and SOCS3 as calculated in the text.
in Materials and Methods. More than 99% of the sorted cells express cell surface CD4. FACS analysis of the cells further reveal that they express high levels of CD62L and low levels of CD25, features associated with a naive phenotype (Fig. 5). Analysis of cDNAs from these cells for the expression of SOCS1, SOCS2, or SOCS3 gene by quantitative RT-PCR reveals constitutive expression of SOCS1, SOCS2, and SOCS3 mRNA transcripts (Fig. 6). However, in contrast to significant amounts of mRNAs expressed in Th, Th1, and Th2 cells that allow their detection by the relatively insensitive Northern blot assay, the levels of SOCS transcripts in naive Th cells are very low; their detection required at least 35 cycles of PCR amplification (Fig. 6A). Of particular note is the relatively low abundance of SOCS1 transcripts detected by real-time PCR in the naive nonpolarized cells (Fig. 6B).

FIGURE 4. Analysis of transcriptional activators and repressors in Th1 and Th2 cell lines. A, Detection of lineage-specific transcription factors T-bet, GATA-3, c-maf by RT-PCR; B, quantitative detection of SOCS mRNA transcripts in activated Th1 or Th2 cell lines by real-time PCR. Amplification plots of the relative fluorescence intensity vs cycle number for GAPDH, SOCS1, SOCS2, and SOCS3 target cDNAs. Bars show the number of copies of each SOCS per cell. Shown are detection by Western blotting of pSTAT6 (C) and pSTAT4 (D) proteins in Th1 and Th2 whole cell extracts (25 μg) before and after treatment of the cells with either IL-4 or IL-12.

FIGURE 5. Three-color flow cytometric analysis of sorted CD4⁺CD62LhighCD25⁻ naive T cells. CD4⁺ cells were stained with PE-Cy5-CD4, FITC-CD62L, or PE-CD25 Ab. A and C, Isotype controls.
JAKs, STATs, Tec, p95

receptors (IL-4, IL-6, IL-10, IFN-

signaling is dependent on activated STAT1 and tyrosine phosphor-

expression of SOCS1 in Th1 cells. Thus, similar to T-bet, SOCS1 is a Th1 lineage marker and presumably promotes commitment to the Th1 phenotype.

Although the roles of either SOCS2 or SOCS3 in lymphoid cell development have not been established, the preferential use of SOCS3 in Th2 but not Th1 cells is suggestive of its role in the maintenance of the Th2 cell lineage. Therefore IL-12/STAT4 sig-

mRNA, SOCS3 mRNA is expressed at relatively high levels in nonpolarized Th cells, suggesting that this SOCS member may also contribute to the initial phase of the differentiation process. Constitutive expression of SOCS2 in naive and differentiated Th cells is particularly intriguing in that this SOCS member functions primarily to regulate growth through its inhibitory effects on growth hormone-induced STATS-dependent gene transcription. Given that IL-2 effects on Th cells are mediated by STAT5-de-

involvement in Th differentiation process and/or maintenance of the polarized patterns of cytokine expression in Th1 and Th2 cells.

A major issue that must be resolved relates to the relative contributions of cytokine and TCR signaling in differential regulation of SOCS expression in Th1 and Th2. The fact that SOCS expression is relatively low in naive Th cells and is up-regulated in re-

response to activation or cytokine signaling suggests that the Th cell differentiation process and expression of SOCS proteins are coordi-

ately regulated. On one hand, the up-regulation of SOCS ex-

pression by stimulation with the HEL protein in nonpolarized Th cells suggests that TCR signaling contributes to SOCS induction. In contrast, the preferential up-regulation of SOCS1 expression in Th1 but not Th2 cells after IL-12 stimulation argues for a role of cytokine signaling in SOCS regulation. Our data further suggest that high constitutive SOCS3 expression may be the default pattern of SOCS expression in CD4+ cells as naive Th cells, nonpolarized activated Th cells, and Th2 cells express relatively high levels of SOCS3 and very low levels of SOCS1. The functional consequences of elevated expression of SOCS3 may be to drive the majority of CD4+ toward a Th2 pattern of cytokine expression. In view of the significant increase of SOCS3 expression in response to Ag stimulation, it is tempting to speculate that a major impact of TCR signaling is transcriptional activation of the SOCS3 gene of naive CD4+ cells. However, cytokines can override the effects of SOCS3 during Th differentiation. In fact, a major effect of IL-12 signaling in nonpolarized Th cells is to down-regulate SOCS3 ex-

pression in cells committed to the Th1 lineage. In contrast to Th2,
Th1 cells by IFN-γ/H9253 phosphorylation of STAT1, thereby preventing autoregulation of Th1 cell maintenance. These family members may also regulate Th cell lineage commitment and main roles that SOCS proteins play in the termination of immune or regulatory factors in Th cell differentiation and, given the critical processes that are refractile to the effects of this cytokine on gene transcription and cell cycle regulation.

In summary, our data show that SOCS mRNAs and proteins are differentially expressed in Th1 and Th2 cells. The polarization expression of SOCS proteins in differentiated Th lymphocytes suggests that IL-12/STAT4- and IL-4/STAT6-signaling pathways may be repressed, at least in part, by a SOCS3- or SOCS1-mediated mechanism, respectively. Thus, Th cells that constitutively repress STAT6 signals become Th1 cells, and those that inhibit STAT4 activities develop into the Th2 phenotype. Our findings provide impetus for further investigations into the roles of negative regulatory factors in Th cell differentiation and, given the critical roles that SOCS proteins play in the termination of immune or cytokine responses, it is likely that other members of the SOCS family may also regulate Th cell lineage commitment and maintenance. These findings are of interest not merely in the context of Th differentiation but also because SOCS1 and SOCS3 proteins may be repressed, at least in part, by SOCS3- or SOCS1-mediated negative regulatory proteins.

In summary, our data show that SOCS mRNAs and proteins are differentially expressed in Th1 and Th2 cells. The polarization expression of SOCS proteins in differentiated Th lymphocytes suggests that IL-12/STAT4- and IL-4/STAT6-signaling pathways may be repressed, at least in part, by a SOCS3- or SOCS1-mediated mechanism, respectively. Thus, Th cells that constitutively repress STAT6 signals become Th1 cells, and those that inhibit STAT4 activities develop into the Th2 phenotype. Our findings provide impetus for further investigations into the roles of negative regulatory factors in Th cell differentiation and, given the critical roles that SOCS proteins play in the termination of immune or cytokine responses, it is likely that other members of the SOCS family may also regulate Th cell lineage commitment and maintenance. These findings are of interest not merely in the context of Th differentiation but also because SOCS1 and SOCS3 proteins may be repressed, at least in part, by SOCS3- or SOCS1-mediated negative regulatory proteins.

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