Dendritic Cells Transduced with SOCS-3 Exhibit a Tolerogenic/DC2 Phenotype That Directs Type 2 Th Cell Differentiation In Vitro and In Vivo

Yonghai Li, Niansheng Chu, Abdolmohamad Rostami and Guang-Xian Zhang

*J Immunol* 2006; 177:1679-1688; doi: 10.4049/jimmunol.177.3.1679

http://www.jimmunol.org/content/177/3/1679

---

**References**

This article cites 53 articles, 23 of which you can access for free at: http://www.jimmunol.org/content/177/3/1679.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Dendritic Cells Transduced with SOCS-3 Exhibit a Tolerogenic/DC2 Phenotype That Directs Type 2 Th Cell Differentiation In Vitro and In Vivo

Yonghai Li, Niansheng Chu, Abdolmohamad Rostami, and Guang-Xian Zhang

Dendritic cells (DCs) are major professional APCs that initiate and modulate immune response and may instruct Th0 to differentiate into Th1 or Th2 cells (1, 2). Various DC-derived factors that induce Th cell polarization have been identified, including the Th1 cell-polarizing cytokines IL-12 and IL-23 and the Th2-regulatory T cell-polarizing cytokines IL-10 and TGF-β (2). It has been shown that DCs not only produce IL-12 and IL-23 but also express high levels of their receptors, indicating an autocrine regulatory loop (3). The positive loop by IL-12 on DCs has been proposed to cause a strong Th1 cell polarization (4, 5). IL-23 can bind to its receptors in DCs and induce IL-12 and IL-23-directed autocrine signal (5). IL-23 can bind to its receptors in DCs and induce IL-12 and IL-23-directed autocrine signal (5). SOCS-3 can bind to its receptors in DCs and induce IL-12 and IL-23-directed autocrine signal (5). SOCS-3 can bind to its receptors in DCs and induce IL-12 and IL-23-directed autocrine signal (5).

Suppressor of cytokine signaling (SOCS) proteins, a family of cytokine-induced intracellular proteins, can function as feedback inhibitors to regulate the duration or intensity of the cytokine-induced signal. SOCS-3, one member of the SOCS family, can be induced by various cytokines, such as IL-6, IL-12, and IFN-γ, and negatively regulate the actions of those cytokines as well as functions of Stat transcription factors (13–15). Overexpression of SOCS-3 down-regulates IFN-α and IFN-γ responses (16–18). SOCS-3-transgenic mice exhibit enhanced Th2 differentiation, whereas expression of dominant-negative SOCS-3 interferes with Th2 differentiation (13–15). Recently, Jo et al. (19) found that intracellular delivery of SOCS-3 significantly reduces production of inflammatory cytokines and attenuates liver apoptosis and hemorrhagic necrosis, thus effectively suppressing the devastating effects of acute inflammation. Furthermore, the SOCS signal may be important for the regulation of DC maturation (20).

Based on these observations, we hypothesized that enhanced SOCS-3 expression in DCs will block IL-12/IL-23 signaling in these cells and drive them to a tolerogenic/DC2 phenotype. In the present study we tested this hypothesis by transducing DCs with a bicistronic lentiviral vector encoding SOCS-3 and by determining the functional phenotype of these DCs. The capacity of SOCS-3-transduced DCs to drive naive CD4+ T cells toward Th2 polarization was determined in vitro and in experimental autoimmune encephalomyelitis (EAE) mice in vivo.

Materials and Methods

Animals and reagents

Female C57BL/6 mice at 7–8 wk of age were purchased from The Jackson Laboratory. Myelin oligodendrocyte glycoprotein (MOG) TCR transgenic mice were a gift from Dr. V. Kuchroo (Harvard Medical School, Boston, MA). The MOG35–55 (MEVGWYRSPFSRVVHLRNGK) peptide was synthesized at the Protein Chemistry Laboratory of the University of Pennsylvania (Philadelphia, PA) and purified by HPLC to >98% purity. A 293T
Bone marrow-derived dendritic cell preparation

Bone marrow cells were flushed from the femurs and tibiae of normal, female, 7- to 8-week-old C57BL/6J mice. These cells were cultured in 100-mm petri dishes with 10 mL of RPMI 1640 medium containing 10% FBS and 200 U/mL mouse GM-CSF (PeproTech) at 37°C in 5% humidified CO₂. Nonadherent granulocytes were removed after 48 h of culture, and fresh medium was added. After 7 days of culture, CD11c⁺ DCs were purified by immunomagnetic sorting using anti-CD11c-coated magnetic beads and the autoMACS system according to the manufacturer’s instructions (Miltenyi Biotec). The purity of the sorted cells was determined by FACS analysis (>96% for CD11c⁺ cells). Mature DCs were generated in the presence of 100 ng/mL LPS.

SOCS-3 expression on DCs upon IL-12/IL-23 stimulation

DCs were plated in 12-well plates at 1 × 10⁶ cells/mL and stimulated with IL-12 (100 ng/mL), IL-23 (100 ng/mL), and LPS (100 ng/mL). One microgram of RNA from treated DCs was reverse transcribed using SuperScript2 (Invitrogen Life Technologies) according to the manufacturer’s instructions. The SOCS-3 gene was cloned with the sense primer (5′-AGTGGCACCAACAGGATGT-3′) and the antisense primer (5′-AAGTGGAGCATCATACTGATCC-3′); SOCS-3 expression in response to the above stimuli in DCs was detected by real-time PCR. Briefly, a TaqMan probe for SOCS-3 was purchased from Applied Biosystems. Real-time PCR was conducted at a final volume of 25 μL containing cDNA amplified for 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C.

Generation of SOCS-3-lentiviral vector and transduction into DCs

The SOCS-3 gene was inserted into a bicistronic lentiviral vector. The lentiviral vector particles were produced by cotransfecting the NIH 3T3 cell line. For transduction to DCs, enriched viral particles were pseudotyped with the vesicular stomatitis virus G glycoprotein. For transduction, a 10-cm-diameter dish of 293T cells, the mixture of three plasmids was added into 2× HBSS and added drop by drop onto the 293T cells. The medium was replaced after 5 h of incubation. Supernatants were collected on days 2, 3, and 4 posttransduction, and viral particles were concentrated by ultracentrifugation at 26,000 rpm for 2 h at 4°C. Pellets were dissolved in PBS, resulting in 200-fold concentration. Multiplicity of infection was determined by infecting the NIH 3T3 cell line. For transduction to DCs, enriched viral particles with a multiplicity of infection of 10 were added into DCs at day 6 of culture in the presence of 4 μg/mL Polybrene. The same steps were repeated the next day. In some experiments, the transduced DCs were purified by sorting CD11c⁺ GFP⁺ cells with FACSaria (BD Bioscience). The viability of DC cultures was monitored periodically before and after transduction using trypan blue (Sigma-Aldrich) staining. Cells with >85% viability were continued for additional experiments.

Immunohistology staining

Transduced DCs were cytopsin onto slides. After being fixed with acetone for 2 min and blocked with 1% BSA in PBS, slides were incubated with biotin-conjugated anti-CD11c mAb (BD Pharmingen) for 1 h. Afterward, rhodamine red-conjugated streptavidin (Jackson ImmunoResearch Laboratories) was added and incubated for 30 min. Slides were covered with mounting medium (Vector Laboratories), and coexpression of CD11c and GFP on the same cells was examined by confocal microscopy.

Immunoblot analysis

Transduced DCs were stimulated by LPS for 4 h and then washed twice. The next morning cells were incubated with IL-12 and IL-23 for 2 h, and then cells were centrifuged and lysed in radioimmunoprecipitation assay buffer (Santa-Cruz-Aldrich) with the addition of protease inhibitors (Roche). Lysates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were blotted with primary Abs, including anti-SOCS-3 (Santa Cruz Biotechnology), phosphorylated Stat3 and Stat3 (Santa Cruz Biotechnology), and phosphorylated Stat4 and Stat4 (Zymed Laboratories). Intensity of bands from the Western blots was quantified by densitometry using ImageJ software (NIH images scan). Signal was normalized for total Stat expression for each loading lane.

In vitro assay of coculture system

The coculture system was set up with MOG-specific CD4⁺ T cells and FACS-sorted transduced DCs by gate of CD11c⁺ GFP⁺ cells. CD4⁺ T cells were purified from the spleen of naïve MOG TCR transgenic mice using anti-CD4 coupled magnetic beads (Miltenyi Biotec). The purity of both CD4⁺ T cells and DCs was ≥95% as determined by FACS. A total of 1.5×10⁵ purified MOG-reactive CD4⁺ T cells and 0.5×10⁶ transduced DCs were cocultured in the presence of 25 μg/mL MOG35-55. Peptide. This concentration of peptide was chosen because it was optimal for T cell stimulation in our preliminary study (data not shown). After coculture for 5 days, the supernatant was collected for cytokine analysis. The cytokines produced by CD4⁺ T cells were identified by intracellular staining. For proliferation, purified MOG-reactive CD4⁺ T cells and transduced DCs were placed in a 96-well plate. Cells were cultured in triplicate with 25 μg/mL MOG35-55, 25 μg/mL OVA, or without Ag. After 60 h of incubation, the pulses were collected for 12 h with 1 μCi of ³H. Cells were harvested and counts read using a beta counter.

Immunophenotype by FACS and cytokine assay by intracellular staining and ELISA

Cells, including MOG-reactive CD4⁺ T cells and DCs, were stained with a combination of mAbs against the following Ags: CD4, CD11c, CD40, CD80, CD86, and MHC class II. The appropriate isotype was used as control mAb. Cells were incubated at 4°C for 20 min, washed, and analyzed by FACS. Intracellular staining of cytokines was conducted using Cytofix/Cytoperm kits (BD Pharmingen) following the manufacturer’s recommendations. Briefly, cells were cultured in the presence of MOG35-55 (25 μg/mL) for 5 days. GolgiStop, a protein transport inhibitor containing brefeldin A, was added during the last 4 h of culture. After surface staining of CD4⁺ cells, were thoroughly resuspended and fixed in Cytofix/Cytoperm solution for 20 min. Cells were permeabilized with 1× Perm/Wash solution (BD Pharmingen) and stained for intracellular cytokines using PE-conjugated anti-mouse cytokine mAbs (IFN-γ clone XMG1.2, TNF-α clone MP6-XT22, and IL-4 clone 11B11; BD Pharmingen). FlowJo software was used to analyze data. The cytokines in supernatants were analyzed by ELISA in accordance with the manufacturer’s instructions (BD Pharmingen).

Th differentiation driven by transduced DCs in vivo

The CD11c⁺ GFP⁺ cells were purified by FACS sorting 48 h posttransduction. The purified DCs were pulsed with MOG35-55 (25 μg/mL) overnight, washed twice, and injected s.c. into each hind footpad of recipient mice at 0.5×10⁶ cells in 50 μL of sterile PBS, following a protocol described previously (21, 22). The viability of injected cells was >85%. Seven days after injection the recipient mice were sacrificed, and mononuclear cells from draining popliteal and inguinal lymph nodes were harvested. Cells were cultured for lymphocyte proliferative responses to MOG35-55. Supernatants were collected after 72 h of culture for cytokine analysis.

Induction of EAE and SOCS-3-DC treatment

To confirm the immunoregulatory effect of SOCS-3-transduced DCs in vivo, we administered these cells in EAE mice. Female 8- to 10-week-old C57BL/6J mice were each injected s.c. with 200 μg MOG35-55 in CFA containing 4 mg/mL Mycobacterium tuberculosis over two sites at the back. Two hundred nanograms of pertussis toxin (List Biological Laboratories) was given i.p. on days 0 and 2 postimmunization (p.i.), SOCS-3-transduced

FIGURE 1. Inducible expression of SOCS-3 in DCs by LPS, IL-12, and IL-23. Purified CD11c⁺ DCs at day 8 of culture were incubated with LPS (100 ng/mL), IL-12 (100 ng/mL), and IL-23 (100 ng/mL) at the indicated time points, mRNA expression of SOCS-3 was assayed by real-time PCR. Data presented as the mean values of triplicate cultures ± SD. Dashed line represents the base level of SOCS-3 in rest DCs. One representative experiment of two is shown.
DCs were pulsed overnight with MOG\textsubscript{35-55} peptide (25 \mu g/ml), washed, and injected i.v. (2 \times 10^6/0.2 ml PBS each mouse). DCs transduced with GFP only served as control. The viability of these cells was >85%. A clinical scoring system with a scale of 0 to 5, with 0.5 points for intermediate signs, was used as follows (23): 0, normal; 1, flaccid tail, abnormal gait; 2, hind leg weakness or severe ataxia; 3, minimal hind leg movement; 4, hind leg and forelimb paralysis; and 5, moribund or dead. Mice were examined daily by two blinded observers for signs of EAE. To determine the suppressive effects of SOCS3-transduced DCs on different phases of EAE, these DCs were injected at EAE induction (+3 and +7 days p.i.) or disease onset (11, 14, and 17 days p.i.), and mice were sacrificed at days 21 or 24 p.i., respectively. Splenocytes were isolated for analysis of MOG-induced proliferative responses and cytokine production. All work was performed in accordance with the guidelines for animal use and care at Thomas Jefferson University, Philadelphia, PA.

**Statistics**

Mann-Whitney U test was used for comparison of average clinical scores, and ANOVA was used for other parameters among different groups. All tests were two sided.

**Results**

*Inducible expression of SOCS-3 in DCs by LPS, IL-12, and IL-23*

To determine whether SOCS-3 is constitutively or inducibly expressed in DCs, we first examined SOCS-3 expression in DCs stimulated with LPS, IL-12, IL-23, or without stimulation. As shown in Fig. 1, the SOCS-3 gene was expressed at relatively low...
levels in nonstimulated DCs. IL-23 induced a high level of SOCS-3 expression after 2 h that persisted even after 6–12 h, whereas IL-12 induced a noticeably lower level of SOCS-3 than IL-23. LPS induced SOCS-3 expression that was 22 times higher than in the first hour, but by 6 h SOCS-3 expression returned to the base level. These data indicate that SOCS-3 is inducible upon inflammatory stimulation and may imply an important regulatory role of SOCS-3 in DC function.

High efficiency of SOCS-3 transduction in DCs

To transduce SOCS-3 in DCs, we inserted SOCS-3 into a lentiviral vector. We used a bicistronic lentiviral vector characterized by an internal ribosome entry site and a central polypurine tract sequence element. The central polypurine tract element can enhance the ability of nuclear import and increase the efficiency of gene transfer. In the presence of a single internal ribosome entry site, two genes, in this case SOCS-3 and GFP, can be transduced by the same promoter and then translated into two proteins. Transduction efficiency can be monitored by GFP expression in transduced cells.

To verify that SOCS-3 and GFP proteins can be expressed by our constructed vectors, we prepared viral particles and transduced them into DCs. The expressed SOCS-3 protein was determined by Western blotting. As shown in Fig. 2A, a single band was detected by anti-SOCS-3 mAb in SOCS-3-transduced DCs (lane 3), but not in nontransduced or GFP-transduced DCs (lanes 1 and 2).

We next determined the transduction efficiency in SOCS-3-transduced DCs. Transduced DCs were stained with biotin-conjugated anti-CD11c mAb, and the colocalization of GFP and CD11c was confirmed by confocal microscopy (Fig. 2C). Transduction efficiency was ~63.7% as determined by FACS using GFP as readout (Fig. 2D). Taken together, these data demonstrate that SOCS-3 was constitutively expressed in transduced DCs with our bicistronic lentiviral vector.

Impaired IL-12/Stat4 and IL-23/Stat3 signaling in SOCS-3-transduced DCs

To study the influence of overexpressed SOCS-3 on IL-12/Stat4 and IL-23/Stat3 signaling, DCs transduced with SOCS-3 plus GFP and control DCs transduced with GFP only were stimulated with IL-12, IL-23, or medium only. Expression of total and phosphorylated fractions of Stat3 and Stat4 was analyzed by Western blotting. As shown in Fig. 3, IL-12 and IL-23 markedly increased a fraction of phosphorylated Stat4 and Stat3, respectively. Compared with DCs transduced with GFP-lentiviral vector, SOCS-3-transduced DCs contained noticeably lower levels of phosphorylated Stat4 (~22% activity) induced by IL-12 and phosphorylated Stat3 (~31% activity) induced by IL-23. In contrast, no difference was found in total Stat3 or Stat4 expression between SOCS-3-transduced and GFP-transduced DCs (Fig. 3).

Low expression of MHC class II and CD86 in SOCS-3-transduced DCs

We next evaluated the influence of SOCS-3 on DC maturation status by determining the expression of costimulatory molecules (CD40, CD80, and CD86) and MHC class II on transduced DCs. CD86 and MHC class II were down-regulated in SOCS-3-transduced DCs, but not in GFP-transduced DCs. As shown in Fig. 4, 71.2% (mean fluorescence intensity (MFI) = 73 ± 18) of nontransduced DCs and 63.1% (MFI = 54 ± 15) of GFP-transduced DCs expressed CD86, whereas only 49.6% of SOCS-3-transduced DCs expressed this molecule (MFI = 25 ± 6). For MHC class II, 31.4% of SOCS-3-transduced DCs (MFI = 31 ± 10) were positive, whereas 45.5% (MFI = 70 ± 21) in GFP-transduced DCs and 53.7% (MFI = 92 ± 18) in nontransduced DCs were positive (Fig. 4A). Statistical analysis of the percentages and MFI values of CD86 and MHC class II in SOCS-3-transduced DCs showed a significant difference compared with GFP-transduced and nontransduced DCs (Fig. 4B). No differences in CD40 and CD80 were observed among these three groups (data not shown). There was no deleterious effect on cell viability before or after DCs manipulation as determined by trypan blue staining.

Altered cytokine production in SOCS-3-transduced DCs

We then investigated the effects of overexpressing SOCS-3 on the cytokine profile of LPS-stimulated DCs by assaying the supernatants collected from transduced DCs. As shown in Fig. 5A, SOCS-3-transduced DCs produced reduced amounts of IFN-γ (p < 0.01) and IL-12 p70 (p < 0.05) as compared with GFP-transduced or nontransduced DCs. In contrast, a significant increase of IL-10 was found in SOCS-3-transduced DCs. No significant difference for TNF-α was observed among the three groups. Further, mRNA expression of the IL-12/IL-23 subunits p40, p35, and p19 was significantly decreased in SOCS-3-transduced DCs as compared with GFP-transduced DCs (all p < 0.05; Fig. 5B).

Effect of transduced DCs on Th phenotype in vitro: a coculture system

To define Th differentiation of CD4+ T cells primed by transduced DCs, MOG-specific CD4+ T cells purified from MOG Tg mice and CD11c-GFP-transduced DCs were mixed at a ratio of 3:1. As shown in Fig. 6A, low proliferative responses were observed in cocultured cells without Ag stimulation and with control Ag OVA. MOG-reactive CD4+ T cells primed by SOCS-3-transduced DCs exhibited significantly lower MOG-induced proliferative response as compared with those primed by GFP-transduced DCs (p < 0.05).

We then assayed the secreted cytokines in the supernatant of the above coculture. Significantly reduced production of IFN-γ, TNF-α, and IL-17, as well as higher levels of IL-4 and IL-10, were found in cocultures of T cells and SOCS-3-transduced DCs (Fig. 6B). To more accurately study cytokine production by CD4+ T cells cocultured with SOCS-3-transduced DCs, we performed intracellular cytokine staining for IFN-γ and IL-4, two important cytokines in Th1 and Th2. Within the CD4+ subset, a significantly decreased frequency of IFN-γ but an increased frequency of IL-4.
were found in the coculture with SOCS-3-transduced DCs. The percentages of IFN-γ+/CD4+ cells (mean ± SEM) were as follows: 25.0 ± 3% in the coculture with GFP-transduced DCs and 13.5 ± 4% in the coculture with SOCS-3-transduced DCs (p < 0.05), whereas the percentages of IL-4+ CD4+ cells were 24.2% ± 2% vs 31% ± 1.4% (p < 0.05) (Fig. 6C). The percentage of TNF-α+ CD4+ T cells is 9.2% ± 2.1% in the coculture with GFP-transduced DCs vs 2.5% ± 1.5% in the coculture with SOCS-3-transduced DCs (p < 0.05) (Fig. 6D). Taken together, these data indicate a Th2 shift induced by SOCS-3-transduced DCs.

**Effect of SOCS-transduced DCs on directing Th differentiation in vivo**

We further determined the effect of SOCS-3-transduced DC on Th differentiation in vivo. Purified transduced DCs were incubated overnight with MOG35–55 (25 μg/ml), washed, and adoptively transferred into the footpads of naïve mice by s.c. injection. Draining lymph nodes were harvested after 7 days, and Ag-specific cytokine production was determined. As shown in Fig. 7, lymph node cells from mice receiving GFP-transduced DCs produced low levels of IL-4 and IL-5 and high levels of IFN-γ. In contrast, SOCS-3-transduced DCs primed a Th2-dominant response, i.e., increased IL-4 and IL-5 and decreased IFN-γ production. This finding was confirmed by analyzing the intracellular cytokine production in CD4+ T cells. Compared with GFP-transduced DCs, SOCS-3-transduced DCs were able to prevent Th1 polarization and induce Th2 by increasing the percentage of IL-4+ cells (1.8 ± 0.7% vs 3.5 ± 1.0%; n = 3; p < 0.05) and reducing the percentage of IFN-γ-producing cells (33.1 ± 4.1% vs 19.0 ± 3.0%; n = 3; p < 0.05) (Fig. 7).

**SOCS-3-transduced DCs inhibited development of clinical EAE**

Finally, we investigated the in vivo effect of SOCS-3-transduced DCs on clinical EAE. Control mice receiving GFP-transduced DCs exhibited characteristic signs of EAE starting on day 12 p.i. In contrast, mice receiving SOCS-3-transduced DCs at the induction phase of EAE (−3 and 7 days p.i.) developed significantly lower clinical scores compared with control mice injected with GFP-transduced DCs (p < 0.05) (Fig. 8A). Similar suppressive effects were observed in mice receiving SOCS-3-transducing DCs at disease onset (11, 14, and 17 days p.i.) (Fig. 8B). No significant difference was found in the mice receiving transduced DCs at peak EAE (18, 19, and 20 days p.i.) (data not shown). MOG-induced lymphocyte proliferation and production of the Th1 cytokines IFN-γ and IL-17 were suppressed in splenocytes of mice receiving SOCS-3-transduced DCs (p < 0.01 and 0.05, respectively).
whereas the level of MOG-induced Th2 cytokine IL-4 was significantly up-regulated in the splenocytes of these mice \( (p \leq 0.05; \text{Fig. 8, C and D}) \).

**Discussion**

The present study provides evidence that DCs overexpressing SOCS-3, an important suppressor of cytokine signaling, exhibit a tolerogenic/DC2 phenotype that promotes Th2 differentiation and have therapeutic potential for Th1 cell-mediated autoimmune diseases such as EAE/multiple sclerosis.

Negative regulation of signal transduction pathways is necessary for an appropriate cellular and physiological response to cytokine stimulation. In DCs, proinflammatory cytokines such as IL-12 and IL-23 trigger their extracellular signals to the nucleus through activated STAT proteins, and the duration and the intensity of the cytokine-induced signal are under feedback regulation by a newly described family of intracellular proteins called SOCS. Among them, SOCS-3 has been suggested to be a potent inhibitor of IL-12- and IL-6-induced signaling \((24, 25)\) and to regulate DC maturation \((20)\). SOCS-3 can be induced by infectious agents and
proinflammatory cytokines, including IL-12 (24, 26), suggesting that SOCS-3 may act in a classical negative feedback loop to attenuate proinflammatory signaling. However, the role of SOCS-3 in regulating the signaling pathway of IL-23, another important proinflammatory cytokine in the IL-12 family (27), is not known. Our current study shows that, in DCs, SOCS-3 is not only induced by IL-12 and a bacterial product LPS, consistent with previous studies, but also upon stimulation with IL-23, suggesting a negative feedback for IL-23 response.

Transmembrane signaling for IL-12 and IL-23 is mediated by proteins of the Janus kinase and Stat pathway. IL-12 and IL-23 activate a similar spectrum of Jak/Stat molecules: Jak2, Tyk2, Stat3, Stat4, Stat5, and Stat1 (3, 28). However, IL-12 mainly signals through activation of a Stat4/Stat4 homodimer, whereas IL-23 mainly activates a Stat3/Stat4 heterodimer (27, 29). Secretion of IL-12 by DCs leads to induction of T-bet and IFN-γ and, in turn, participates in a positive feedback loop via IL-12 receptor β1 and β2 (4, 5). IL-23 can bind to splenic DCs and drive IL-12 and
SOCS-3 induces DC2 phenotype in SOCS-3-transduced DCs potently polarizes the immune system to Th2 responses, demonstrated by decreased production of the inflammatory cytokines TNF-α and IFN-γ and increased IL-4 production by CD4+ T cells. A significant decrease in IL-17 production in cocultured T cells indicates a low production of biologically active IL-23 (12, 47, 48) of SOCS-3-transduced DCs. Such a decrease can also be due to high IL-4 production, which inhibits differentiation of IL-17-producing CD4+ T cells (49). The shift from Th1 to Th2 was confirmed by adoptive transfer of SOCS-3-transduced DCs in vivo. The combination of Th2-promoting properties and poor proliferation of Ag-reactive T cells suggests that SOCS-3-transduced DCs possess a "tolerogenic"/DC2 phenotype and would thus be useful in the treatment of Th1-mediated autoimmune diseases.

Although systemic administration of anti-inflammatory cytokines, such as IL-4 and IL-10, could serve as a useful immunotherapy for autoimmune diseases, there are several reasons why treatment by adoptively transferred cells delivering these cytokines might be a more effective and promising approach. First, because of the chronic nature of autoimmune diseases and the short half-life of cytokines, a frequent administration of exogenous cytokine...
is required with limited effect. In contrast, gene transduction offers a unique way of providing long-term delivery of immunomodulatory molecules in vivo that can antagonize the chronic inflammatory processes (50, 51). Second, the systemic distribution of exogenous cytokines will affect multiple target systems/organs, including the cardiovascular system, liver, spleen, and bone marrow, and can lead to deleterious side effects. For example, administration of IL-4 may cause severe side effects including cardiac inflammation and necrosis, hepatitis, hepatic necrosis, and even death (52, 53). In contrast, local delivery of DC2-type cytokines (low IL-12 and high IL-10) by DCs during APC-T cell interaction would specifically affect the targeted cells (46), thus avoiding the side effects of systemic administration. Because Ag-specific T cells are not easily available for in vitro manipulation, especially in humans, gene-modified DCs provide a practical means to guide the differentiation of these T cells in vivo. Finally, a highly expressed upstream regulatory factor for Th1/Th2 differentiation, such as SOCS-3 in the present study, results in a combination of therapeutic products, including high levels of endogenous, autoantigen-induced IL-4 and IL-10, but low levels of the proinflammatory cytokines IFN-γ, TNF-α, and IL-17 as shown in Fig. 6. Adoptive cellular gene transduction used in our present study as a treatment for chronic inflammatory diseases is thus clearly advantageous over the injection of exogenous IL-4, IL-10, or any other single cytokine or a mixture of multiple cytokines/cytokine antagonists.

In conclusion, our study demonstrates the suppressive effect of SOCS-3-transduced DCs on EAE and provides evidence that these DCs exhibit a tolerogenic/DC2 phenotype that selectively suppresses Th1 and induces Th2 differentiation in vitro and in vivo. Manipulation of autogenous DCs with SOCS-3 could, therefore, be a potential therapy for Th1 cell-mediated autoimmune disorders such as EAE and multiple sclerosis.

Acknowledgments
We thank Dr. Bogoljub Ciric for critical discussions and Katherine Regan for editorial assistance.

Disclosures
The authors have no financial conflict of interest.

References