Human Suppressor of Cytokine Signaling 1 Controls Immunostimulatory Activity of Monocyte-Derived Dendritic Cells

Bangxing Hong, Wenhong Ren, Xiaotong Song, Kevin Evel-Kabler, Si-Yi Chen, and Xue F. Huang

Abstract

Dendritic cell (DC)–based tumor vaccines have only achieved limited clinical efficacy, underscoring the limitation of stimulatory strategies to elicit effective cytotoxic T lymphocyte (CTL) responses against self-tumor–associated antigens. Here, we investigate the role of human suppressor of cytokine signaling 1 (SOCS1), a feedback inhibitor of the Janus-activated kinase/signal transducer and activator of transcription signaling pathway, in regulating antigen presentation by human DCs (hDC). We find that human SOCS1 (hSOCS1)–silenced DCs have an enhanced stimulatory ability to prime self-antigen–specific CTLs in vitro and in a severe combined immunodeficient-hu mouse model. Human CTLs activated by SOCS1-silenced DCs, but not wild-type DCs, have an active lytic activity to natural antigen-expressing tumor cells. We further find that the capacity of hDCs to prime CTLs is likely controlled by SOCS1-restricted production and signaling of proinflammatory cytokines, such as interleukin-12. These results indicate a critical role of hSOCS1 in negatively regulating the immunostimulatory capacity of DCs and imply a translational potential of this alternative SOCS1 silencing strategy to develop effective DC vaccines.

Introduction

Dendritic cells (DC) are professional antigen-presenting cells with key regulatory roles in the maintenance of tolerance to self-antigens and in the activation of innate and adaptive immunity (1). They use pattern recognition receptors, such as Toll-like receptors (TLR), to recognize conserved microbial structures, such as lipopolysaccharide (LPS; ref. 2). TLR signaling promotes DC maturation by activating NF-κB, which mediates the up-regulation of antigenic peptide-loaded MHC molecules and costimulatory molecules, and expression of proinflammatory cytokines, resulting in the induction of innate and adaptive immunity (2). DCs are effective in inducing antitumor responses in mice (1, 3). However, the results of DC vaccine trials have been largely disappointing with very low rates of objective clinical responses (4). The major challenge now is to find a novel way to elicit effective T-cell responses to self-antigens preferentially expressed by tumor cells.

Several recent studies in mice suggest a critical role of suppressor of cytokine signaling 1 (SOCS1)–restricted signaling in maintaining self-tolerance and negatively regulating antigen-presenting cells. SOCS1 functions as a negative regulator of signaling by various cytokines, such as interferon (IFN)-γ, interleukin (IL)-2, IL-6, IL-7, IL-12, and IL-15, by inhibiting the Janus-activated kinases/signal transducer and activator of transcription in T cells and other immune cells (5, 6). Metcalf and colleagues (7) reported that adoptive transfer of bone marrow (BM) cells of neonatal SOCS1-deficient (−/−) mice into irradiated syngeneic mice caused a pathology characteristic of graft-versus-host disease with chronic inflammatory lesions in multiple organs of the recipients, in agreement with earlier findings (6). Hanada and colleagues (8) further showed that SOCS1−/− transgenic mice, in which SOCS1 expression had been restored in T and B cells on a SOCS1−/− background, developed only mild autoimmune diseases and that SOCS1−/− DCs were hyperresponsive to LPS and IFN-γ and triggered allogeneic T-cell expansion. Hashimoto and colleagues (9) recently revealed that silencing of SOCS1 in macrophages suppressed tumor development by enhancing antitumor inflammation. These results clearly suggested an essential role of SOCS1 in maintaining self-tolerance of hematopoietic immune cells. In a recent study, we found that murine SOCS1 critically controlled antigen presentation by DCs (10). In support of our study, Hanada and colleagues (11) discovered that DCs lacking the SOCS1 gene induced hyper-Th1 cell type immune responses. Because studies on the role of SOCS1 in regulating immune responses have been limited to mouse models, we sought to investigate the regulatory role of SOCS1 in human monocyte–derived DCs (hDC), which have been widely used in the clinic.

Materials and Methods

Western blot and quantitative reverse transcription-PCR analysis of human SOCS1 expression. We first used a computer program from Dharmaco DNA Technologies (Dharmacon, Inc.) to select small interfering RNA (siRNA) sequences targeting human SOCS1 (hSOCS1): siSOCS1-1 (CACGCACUUCCGCAUUCdTdT), siSOCS1-2, and siSOCS1-3. We then cotransfected 293T cells with a siRNA oligonucleotide duplex (21 bp) or an irrelevant oligo duplex and a flag-tagged hSOCS1 expression vector (pCMV-hSOCS1) we constructed using GenePORTER reagent (Genlantis, ref. 10). The relative expression of hSOCS1 in transfected 293T cell or hDCs was evaluated by Western blotting analysis and quantitative real-time reverse transcription-PCR (RT-PCR; ref. 10).

Transfection of human monocyte–derived DCs and in vitro priming of human T cells. hDCs derived from peripheral blood mononuclear cells (PBMC) of HLA-A2+ healthy volunteers were generated as described in our previous studies (12, 13). This research was approved by the Institutional Review Board on Human Subjects. Monocyte-derived DCs were transfected with 120 nmol/L of siRNA oligonucleotides using GenePORTER. The transfected DCs were then pulsed with MAGE3 peptide (20 µg/mL) overnight. A total of 1 × 106 human T cells per well were cocultured with...
5 × 10^5 MAGE3-pulsed, transfected DC (20:1) in 0.5 mL of RPMI 1640 supplemented with 5% AB human serum, recombinant human IL (hIL)-2 (50 units/mL), and tumor necrosis factor α (TNFα; 10 ng/mL; R&D Systems). The cocultured T cells were restimulated weekly. Some cocultures were supplemented with 10 μg/mL of monoclonal anti–hIL-12 (R&D Systems) to block hIL-12 activity.

**Cytokine ELISA, enzyme-linked immunospot assays, and tetramer staining.** Levels of various proinflammatory cytokines were quantitated from the supernatant of DC cultures by ELISA analysis (BD Biosciences) according to the manufacturer's instructions. An HLA-A2–restricted human melanoma antigen MAGE3 cytototoxic T lymphocyte (CTL) peptide (FLWGPRLAV), an HLA-A2–restricted human herpesvirus EBV latent membrane protein 2 (LMP2) CTL peptide (CGLGGLTMV), a control ovalbumin (OVA) peptide (SIINFEKL), and a control human hepatitis C virus (HCV) E2 protein peptide (RLWHPYCPDI) were synthesized and purified by high-performance liquid chromatography to >95% purity by Genemed Synthesis, Inc. (14). Human MAGE3/HLA-A2 tetramers were synthesized at the Baylor College of Medicine Tetramer Core Facility (Houston, TX). Enzyme-linked immunospot (ELISPOT) assays and tetramer staining were performed as described (10).

**DC immunization of HLA-A2 transgenic mice.** HLA-A2.1 transgenic mice were purchased from The Jackson Laboratory and maintained in the pathogen-free mouse facility according to institutional guidelines. BM-derived DCs were prepared from HLA-A2.1 transgenic mice and transduced with lentiviral vector (LV), as described in our previous study (10). DCs were pulsed with peptides, stimulated with TNFα (10 ng/mL) for 24 h, and then injected into transgenic mice via a footpad. In some mice, LPS (30 μg/mouse) or murine IL-12 (1 μg/mouse; PeproTech) was administered i.p. on indicated days.

**CTL assays.** CD8+ CTL responses were assessed with a standard chromium release assay (10) or lactate dehydrogenase (LDH) release assay (Roche Diagnostics). Splenocytes pooled from two to three immunized mice were restimulated in vitro in RPMI 1640 containing MAGE3 or LMP2 peptide for 4 to 6 d. Human MAGE3, HLA-A2 melanoma cells (SK-Mel-37) and control human MAGE3, HLA-A2 melanoma cells (NA-6-Mel) were labeled with sodium 51Cr chromate solution for 90 min at 37°C. LMP2–specific CTL response was tested using LDH release assay according to the manufacturer’s instructions.

**Severe combined immunodeficient-hu mouse model.** siSOC51–transfected DC in vivo priming human T cells was performed in a severe combined immunodeficient (SCID)-hu mouse model as described previously (15). PBMCs were obtained from the blood of healthy HLA-A2+ and combined immunodeficient (SCID)-hu mouse model as described previously (15). PBMCs were obtained from the blood of healthy HLA-A2+ and control human MAGE3+, HLA-A2 transfecten DCs (5 × 10^5 per mouse) and autologous T cells (3 × 10^5 per mouse) were transfected with siRNA oligo and loaded with MAGE3 or LMP2 peptide as described above. The transfected DCs (5 × 10^5 per mouse) and autologous T cells (3 × 10^5 per mouse) were administered i.p. into SCID mice (Charles River) and followed by in vivo injections (i.p.) with LPS (30 μg/mouse) daily for three times. Ten days after the immunization, mice were sacrificed, and cells were recovered from the peritoneum of different groups of mice and pooled for immune assays.

**Statistical analysis.** For statistical analysis, we used Student’s t test, and a 95% confidence limit was taken to be significant, defined as P < 0.05. Results are typically presented as mean ± SE.

**Results**

**SOC51 negatively regulates hDCs in response to LPS stimulation.** To investigate the role of SOC51 in hDCs, we first identified siRNA with the ability to specifically down-regulate hSOC51 (Fig. 1A). The specificity of hSOC51 mRNA down-regulation by hSOC51 siRNA1 (siSOC51) was confirmed by the inability of a scrambled siRNA1 oligonucleotide duplex (siMut) to down-regulate hSOC51 mRNA. Human siSOC51 was therefore selected for further study. Synthetic siRNA duplexes were transfected into DCs derived from human monocytes with a transfection efficiency of ~85.5% (Fig. 1B). As verified by quantitative RT-PCR assays, the level of hSOC51 mRNA in the total DC population transfected with the siSOC51 duplexes was specifically decreased by ~60% compared with levels in mock-transfected DCs (P < 0.01; Fig. 1C). The siRNA efficiency and SOC51 RNA reduction were similar to those in our previous study using siRNA duplexes to target mouse SOCS1 (mSOC51) in the total mouse DC population (10).

We next tested whether hSOC51 regulates the expression of costimulatory molecules and MHC class I/II molecules on DCs by flow cytometric analysis. siSOC51-, siMut-, and mock-transfected DCs showed no apparent difference in their expression of costimulatory molecules CD40, CD80, CD86, and the CC chemokine receptor CCR7 before and after LPS-induced maturation (Fig. 2A). Comparable levels of MHC I molecule and MHC II molecule HLA-DR were detected on siSOC51, siMut, and mock DCs (data not shown; Fig. 2A). In contrast, we found that siSOC51 DCs were more responsive to stimulation with the TLR agonists, such as LPS, CpG, and polyinosinic acid:poly-CMP (polyI:C), than siMut or mock DCs, as indicated by drastically enhanced secretion of proinflammatory cytokines, such as IL-12p70, IL-12p40, IL-1β, IL-6, and TNF-α (Fig. 2B). These data agree with the observations in mouse DCs (9), Gingers and colleagues (16) recently reported that levels of IL-12 produced by SOCS1−/− BM-derived macrophages were comparable with those produced by wide-type macrophages in response to LPS. However, we found that human monocyte–derived DCs differentiated under the described condition were insensitive to the TLR ligand stimulation unless SOCS1 expression was down-regulated. Thus, the different species and cell type used in the studies (mouse macrophages versus hDCs) may contribute to the discrepancy (10, 17).

**SOC51 negatively regulates the stimulatory ability of hDCs in priming autologous T cells in vitro.** To test if silencing SOCS1 can enhance the stimulatory potency of hDCs in priming self-antigen–specific CTLs, an HLA-A2–restricted peptide derived from human MAGE3 (14) was used as a model self-antigen. Human monocyte–derived DCs from HLA-A2+ healthy volunteers were transfected with siRNA and then pulsed with MAGE3 peptide overnight. Autologous human T cells were cocultured with MAGE3-pulsed, transfected DC in the presence of TNFα (a maturation stimulus) for 2 to 3 weeks. In cocultures with siSOC51 DCs, 13.9% of the CD8+ T cells were positive for the MAGE3 tetramer compared with only 5.4% and 4.3% in cocultures with siMut or mock DCs, respectively (Fig. 3A). Tetramer staining of unstimulated lymphocytes from the same donors showed a low level of MAGE3 tetramer staining (0.5% of the CD8+ T cells). In agreement, intracellular IFN-γ staining showed that siSOC51 DCs substantially improved MAGE3-specific CTL responses (11.18% of IFN-γ+ CD8+ T cells) compared with siMut DCs (6.9%) or mock DCs (5.7%; Fig. 3B). Furthermore, IFN-γ ELISPOT assays showed that an increased number of MAGE3-specific CTLs were activated by siSOC51 DCs (Fig. 3C). Repeated experiments from HLA-A2+ donors showed similar results. Most of the primary human T cells were dead after a 2-week coculture with DCs not pulsed with antigens. Collectively, these results indicate that hSOC51-silenced DCs have enhanced immunostimulatory ability to prime self-antigen–specific CTLs. Because SOCS1-silenced DCs produce enhanced amounts of IL-12 (Fig. 2B), a key cytokine in the activation of CTL responses (17), and IL-12 signaling is restricted by SOCS1 (18), we examined the role of IL-12 in priming CTLs. Figure 3B and C shows that anti–IL-12 antibody blocking abrogated the enhanced ability of siSOC51 DCs to stimulate...
MAGE3-specific CTLs. Moreover, anti–IL-12 blocked the immunostimulatory activity of siSOCS1 DCs in a dose-dependent manner, as shown by the increasing concentration of anti–IL-12 (1, 5, and 10 μg/mL) led to enhanced inhibitory effects on siSOCS1 DCs (Supplementary Fig. S1). Anti–IL-6 was also found to block the immunostimulatory activity of siSOCS1 DCs, but the inhibitory effect was less prominent than anti–IL-12, as evidenced by that a higher concentration of anti–IL-6 (2 μg/mL) failed to achieve the inhibitory effect mediated by 1 μg/mL of anti–IL-12 in the siSOCS1 DC blocking assay (Supplementary Fig. S1). The result highlighted importance of IL-12 in siSOCS1 DCs priming CTLs.

We next determined whether activated T cells possess tumor lytic effector function by using natural MAGE3+ human tumor cells as target cells for CTL assays. Previous studies indicated that the human melanoma cell lines, such as MAGE3-transfected NA8-MEL and SK23-MEL, inefficiently processed and presented the MAGE3271-279 epitope due to inappropriate proteasomal cleavage at the COOH terminus of the antigenic peptide (19) and therefore failed to be recognized by MAGE3271-279-specific CTL line (20). However, a recent study showed that the melanoma cell line SK-Mel-37 could be recognized and killed by CTL responses triggered by MAGE3271-279–pulsed, Akt-expressed DCs (21), implicating that SK-Mel-37 does present MAGE3271-279 epitope, albeit less efficiently, on the cell surface. Consistent with the study, our result showed that whereas human T cells activated by siMut or mock DC showed a very weak cytolytic activity to natural SK-Mel-37 cells, the T cells activated by siSOCS1 DCs had a strong cytolytic activity against the MAGE3+, HLA-A2+ SK-Mel-37 cells (Fig. 4A and B). We further...
found that the tumor lytic activity of T cells in the coculture with siSOCS1 DCs was significantly compromised by anti–hIL-12 antibody treatment (Fig. 4A and B). The tumor cytolytic activity was specifically mediated by CTLs because the activated human T cells only had a background cytolytic activity against the HLA-A2, MAGE3 melanoma cells (NA-6-Mel; Supplementary Fig. S2A). Repeated experiments from different donors showed similar results.

Due to the inefficiency of SK-Mel-37 in processing and presenting MAGE3-271-279 (19, 20), it is not excluded that the observed siSOCS1 DC–induced CTL activity against SK-Mel-37 may be caused by some level of nonspecific activation of lytic effectors (CTL or NK) to which SK-Mel-37 might be more sensitive than the other cell lines. To verify the ability of siSOCS1 DC in inducing antigen-specific CTL response, we pulsed the transfected DCs with EBV-LMP2 CTL peptide and cocultured the DCs with autologous human T cells as described above. We found that activated T cells by siSOCS1 DCs exhibited a superior CTL activity against LMP2-pulsed, HLA-A2’ T2 cell or renal carcinoma A498 cell (Supplementary Fig. S2B and C). Once again, siSOCS1 DC–induced, T-cell–mediated tumor lytic activity was significantly compromised by addition of anti–hIL-12 antibody (Fig. 4C and D).

**SOCS1 negatively regulates the stimulatory ability of hDCs in SCID-hu mice.** To additionally evaluate the in vivo immunostimulatory capacity of hSOCS1-silenced DCs, we used a SCID-hu mouse model (15). Groups of SCID mice were xenotransplanted with human HLA-A2’ or HLA-A2’ monocyte-derived DCs that were transfected with siSOCS1, siMut, or mock and loaded with MAGE3 or EBV-LMP2 peptide, and autologous T cells, followed by in vivo injections with LPS for three times, as administration of LPS was shown to boost the immunostimulatory efficacy of siSOCS1 DC (10) owed to the large number of proinflammatory cytokines it induces, many of which are regulated by SOCS1. Mice immunized with siSOCS1 DCs showed much lower efficiency in killing the irrelevant peptide HCV E2 peptide-pulsed, HLA-A2’ T2 cell or renal carcinoma A498 cell (Supplementary Fig. S2B and C). Consistently, mice immunized with siSOCS1 DCs also showed an enhanced LMP2–specific T-cell response compared with siMut DC–immunized or

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**Figure 2. SOCS1 negatively regulates human monocyte–derived DCs in response to LPS stimulation.** A, flow cytometric analysis. Surface representative costimulatory molecules and MHC molecules on transfected hDCs with or without LPS stimulation (100 ng/mL) for 24 h were analyzed by flow cytometric analysis. Histograms are representative of four independent experiments. B, enhanced production of proinflammatory cytokines by siSOCS1-transfected DCs. Cytokines secreted by transfected DCs after LPS (100 ng/mL), CpG (2.5 μmol/L), or poly(I:C) (1 μg/mL) stimulation for 24 h were measured by ELISA assays. The data are representative of three independent assays. *, P < 0.01 versus hSOCS-siRNA DCs.
mock DC–immunized mice (Fig. 5B). The T-cell responses were antigen specific because HLA-A2\(^+\) DCs that were transfected with siSOCS1 and loaded with MAGE3 did not induce MAGE3-specific T-cell responses (data not shown). Intracellular IFN-\(\gamma\) staining also showed the enhanced T-cell responses induced by siSOCS1 DCs (data not shown). The results of the SCID-hu mouse experiments indicate that hSOCS1-silenced DCs have an enhanced ability to prime CTL responses in vivo. Collectively, our results indicate that hSOCS1-silenced DCs may possess a unique ability to fully activate CTLs that have an active lytic effector function against natural tumor cells.

**SOCS1 negatively regulates the stimulatory ability of DCs in humanized HLA-A2.1 mice.** We used humanized HLA-A2.1 transgenic mice to further define SOCS1 regulatory function in DCs. BM-derived DCs from HLA-A2.1 transgenic mouse were transduced with LV-mSOCS1 siRNA or LV-green fluorescent protein (GFP) siRNA, which was previously generated (10), and pulsed with A2-restricted MAGE3 peptide. After maturation with TNF\(\alpha\), transduced DCs were administered into the transgenic mice twice at a weekly interval. After each DC immunization, the mice were stimulated in vivo thrice with either LPS or a low dose of recombinant IL-12 cytokines. Here, LPS served as a positive control for in vivo stimulation. Consistent with our previous study (10), with in vivo LPS stimulations, 70 IFN-\(\gamma\) spots per 2 \(\times\) 10\(^5\) T cells were detected in siSOCS1 DC–immunized spleen compared with only 20 or 10 IFN-\(\gamma\) spots per 2 \(\times\) 10\(^5\) T cells in siGFP DC–immunized or mock DC–immunized spleen (Fig. 6A). In vivo LPS stimulations also preferentially augmented the CTL responses.

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**Figure 3.** Enhanced potency of SOCS1-silenced hDCs to induce antigen-specific T-cell response in vitro. Human monocyte–derived DCs from HLA-A2\(^+\) donors were transfected with siSOCS1 oligo, pulsed with MAGE3 peptide, and cocultured with autologous T cells for 2 wk in the presence or absence of anti–hIL-12 antibodies. MAGE3-PE tetramer\(^+\) T-cell percentages (A), intracellular IFN-\(\gamma\) T-cell percentages (B) in the gated CD8\(^+\) T-cell populations, and IFN-\(\gamma\) ELISPOT numbers (C) in the cocultures are shown from one of four independent experiments. Unstimulated samples from the same donors were used as controls. *, \(P < 0.01\), siMut DCs versus siSOCS1 DCs; **, \(P < 0.01\), siSOCS1 DCs versus siSOCS1 DCs + anti–hIL-12.
against the natural SK-Mel-37 in siSOCS1 DC–immunized mice compared with those in control DC–immunized mice, as shown in Fig. 6B. However, in vivo IL-12 stimulation was more effective than LPS stimulation to enhance the immunostimulatory potency of siSOCS1 DCs, as manifested by a higher frequency of IL-12–stimulated T-cell–producing IFN-γ in response to MAGE3 stimulation than LPS-stimulated T cells (239 versus 70 IFN-γ+ spots per 2 × 10^5 T cells; Fig. 6A) and more robust CTL responses against the natural SK-Mel-37 in IL-12–stimulated, siSOCS1 DC–immunized mice (P < 0.01; Fig. 6B). In vivo IL-12 stimulation also preferentially boosted the stimulatory ability of siSOCS1 DC compared with boosting control DCs, as evidenced by that, with in vivo IL-12 stimulations, 239 IFN-γ+ spots per 2 × 10^5 T cells were detected in siSOCS1 DC–immunized splenocytes but only 10 or 9 IFN-γ+ spots per 2 × 10^5 T cells in control DC–immunized splenocytes (Fig. 6A). IL-12 preferentially stimulating siSOCS1 DC was also shown by more active cytolytic response against SK-Mel-37 triggered by siSOCS1 DC immunization compared with immunization by siGFP or mock DCs (Fig. 6B). Furthermore, the CTL response is HLA-A2 restricted, as the isolated splenocytes from either LPS-stimulated or IL-12–stimulated, siSOCS1 DC–immunized mice failed to kill HLA-A2− NA-6-Mel tumor cell line, as shown in Supplementary Fig. S3A. These results agree with the results shown in Fig. 4.

To confirm the superior ability of siSOCS1 DC in inducing antigen-specific immune response in the mouse model, groups of HLA-A2.1 transgenic mice were immunized with LMP2-pulsed, transduced DCs followed by in vivo stimulation of LPS or IL-12 as described above. As shown in Fig. 6C, in comparison with siGFP or mock DC immunization, siSOCS1 DC immunization enhanced the frequency of IFN-γ+ T cells in response to LMP2 stimulation under in vivo administration of either LPS or IL-12 and augmented LMP2-specific CTL response against HLA-A2+, EBV-transferred LCL under in vivo administration of either LPS or IL-12 (Fig. 6D). Consistent with the above result, in vivo IL-12 stimulation was more effective than LPS stimulation in elevating the immunostimulatory potency of siSOCS1 DCs (Fig. 6C and D). siSOCS1 DC immunization induced the vigorous CTL response against the LCL line, which is LMP2 specific, as the splenocytes from siSOCS1 DC–immunized, LPS-stimulated or IL-12–stimulated mice failed to kill HLA-A2+ A498 tumor cells (Supplementary Fig. S3B).

**Discussion**

The results of this study, for the first time, show a critical role of SOCS1 in negatively regulating the immunostimulatory ability of hDCs to prime antigen-specific CTLs. Significantly, we found
that hSOCS1-silenced DCs had a unique ability to fully activate human CTLs that possessed a robust lytic function against natural antigen-expressing tumor cells. It has been frequently observed in the clinic and laboratory studies that self-antigen–specific T cells can be activated by DC vaccination or in vitro sensitization, as determined by various immune assays, such as tetramer staining and ELISPOT assays. However, although such activated T cells can effectively kill artificial antigen-pulsed tumor cells or tumor cells modified to overexpress self-antigens, they usually show a weak cytolytic activity against natural tumor cells, which has been considered to be a main reason for the poor efficacy of current tumor vaccines (22, 23). Our results suggest that enhanced antigen presentation provided by SOCS1-silenced DCs may be required to fully activate self-reactive, low-affinity T cells and endow them with active lytic effector function against natural tumor cells.

We further showed that the superior ability of hSOCS1-silenced DCs to prime antigen-specific CTLs was likely due to the enhanced production and signaling of proinflammatory cytokines such as IL-12 (signal 3), which is different from SOCS1−/− mice through unrestricted IFN-α/β signaling (16). This conclusion is based on the following observations: (a) hSOCS1-silenced DCs produce enhanced levels of IL-12 in response to stimuli, (b) antibody blocking of IL-12 compromises the immunostimulatory capability of SOCS1-silenced DCs, and (c) in vivo administration of a low dose of IL-12 more efficiently enhances antigen-specific CTL responses induced by SOCS1-silenced DCs than those induced by wild-type DCs. The argument that hSOCS1-silenced DCs had an enhanced IL-12 signaling is mainly derived from observation (c) and in agreement with our previous finding in which murine SOCS1-silenced DCs cotransduced with Ad-IL-12 induced significantly more potent antigen-specific T-cell responses than Ad-IL-12–transduced wild-type DCs, and knock-out of IL-12 receptor deprived the Ad-IL-12–transduced, SOCS1-silenced DCs of the superior stimulatory ability (24). The argument is also supported by the study of Nishimura’s group that murine SOCS1 negatively regulated IL-12 signaling (18). However, we should point out that this study does not rule out the roles of other proinflammatory cytokines, such as IFN-γ and IL-6, in the induction of CTLs (11). Indeed, addition of anti–IL-6 into the DC/T-cell coculture also blocked the immunostimulatory function of siSOCS1 DCs, albeit with a lower efficiency (Supplementary Fig. S1), implying that the other proinflammatory cytokines overexpressed by siSOCS1 DC also play a certain role in up-regulation of DC function.

Early studies suggested the effect of IL-12 on induction of immune response in combination with immunization (17, 25). However, our experimental result showed that siGFP or mock DC immunization in combination with IL-12 stimulation only induced a modest CTL immune response (Figs. 4 and 6). To investigate the possible deviation, we examined the effect of IL-12 administration on distribution of antitumor effector cells in MAGE3-pulsed, siGFP DC–immunized mice. As shown in Supplementary Fig. S4, in vivo IL-12 stimulations led a high frequency of IFN-γ+ T cells to respond to MAGE3 stimulation in siGFP DC–immunized lymph nodes compared with those in immunized mice without IL-12 stimulation, whereas IL-12 stimulation only led a low frequency of IFN-γ+ T cells to be detected in siGFP DC–immunized spleen, although the frequency is also higher than in those splenocytes without IL-12 stimulation. With in vivo IL-12 stimulation, siSOCS1 DC–immunized lymph nodes persistently exhibited much higher frequency of IFN-γ+ cells compared with siGFP DC–immunized lymph nodes (data not shown). The result confirmed the effect of IL-12 on induction of immune response in combination with immunization and is also in agreement with the previous studies that IL-12–activated antitumor effector cells preferentially accumulated in peripheral lymph nodes from spleen or in spleen in spite of inducing splenomegaly (18). As the IL-12–stimulated, siSOCS1 DC–immunized mice harbor active antigen-specific T cell not only in the lymph nodes but also in the spleen (Fig. 6; data not shown), the result supported that silencing SOCS1 in combination with administration of IL-12 would lead to DC more potent in inducing antitumor immune responses and also implied a critical role of SOCS1-restricted

Figure 5. SOCS1-silenced DCs more potently prime T-cell responses in SCID-hu mouse model. Groups of SCID mice (four mice per group) were immunized with hDCs that were transfected with siRNA oligos and pulsed with MAGE3 peptide (A) or LMP2 peptide (B) and autologous T cells. Ten days after immunization, cells were recovered from the peritoneum and subjected to IFN-γ ELISPOT assays. IFN-γ+ spot numbers specific for MAGE3 or LMP2 after subtracting the background spots of the SCID mice immunized with siSOCS1 oligo DCs without peptide pulsing are presented from one of two assays. *, P < 0.01, siMut-DCs versus sihSOCS1-DCs.
IL-12 signaling in DCs for the induction of antigen-specific T-cell responses.

In summary, our results provide evidence that SOCS1 restricts the signaling of IL-12 in DCs, underscoring the importance of cytokine signaling in determining the efficacy of DC-based tumor therapy. The SOCS1 silencing approach has the ability to enhancing an antigen-specific immune response induced by DCs loaded with tumor-associated antigens, which would be more attractive than blocking of CTLA4 on T cells to non-discriminately overactivates self-reactive T cells (26). Overall, this study implies a translational potential of this generally applicable SOCS1 silencing approach to develop more effective tumor vaccines.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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References


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Human Suppressor of Cytokine Signaling 1 Controls Immunostimulatory Activity of Monocyte-Derived Dendritic Cells

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