Induction of Hyper Th1 Cell-Type Immune Responses by Dendritic Cells Lacking the Suppressor of Cytokine Signaling-1 Gene[1]

Toshikatsu Hanada,* Kentaro Tanaka,* Yumiko Matsumura,* Moriyasu Yamauchi,* Hitomi Nishinakamura,* Hiroyuki Aburatani,† Ryuichi Mashima,* Masato Kubo,‡ Takashi Kobayashi,* and Akihiko Yoshimura[2]*

Suppressor of cytokine signaling (SOCS1/JAB) has been shown to play an important role in regulating dendritic cell (DC) function and suppressing inflammatory diseases and systemic autoimmunity. However, role of SOCS1 in DCs for the initiation of Th cell response has not been clarified. Here we demonstrate that SOCS1-deficient DCs induce stronger Th1-type responses both in vitro and in vivo. SOCS1-deficient DCs induced higher IFN-γ production from naive T cells than wild-type (WT) DCs in vitro. Lymph node T cells also produced a higher amount of IFN-γ when SOCS1-deficient bone marrow-derived DCs (BMDCs) were transferred in vivo. Moreover, SOCS1$^{-/-}$ BMDCs raised more effective anti-tumor immunity than WT BMDCs. Microarray analysis revealed that IFN-inducible genes were highly expressed in SOCS1-deficient DCs without IFN stimulation, suggesting hyper STAT1 activation in SOCS1$^{-/-}$ DCs. These phenotypes of SOCS1-deficient DCs were similar to those of CD8α^+ DCs, and in the WT spleen, SOCS1 is expressed at higher levels in the Th2-inducing CD4$^+$ DC subset, relative to the Th1-inducing CD8α^+ DC subset. We propose that reduction of the SOCS1 gene expression in DCs leads to CD8α^+ DC-like phenotype which promotes Th1-type hyperresponses. *The Journal of Immunology,* **2005, 174: 4325–4332.**

Dendritic cells (DCs)[3] are characterized by a high capability for Ag capture and processing, migration to lymphoid organs, and expression of various costimulatory molecules for Ag-specific lymphocyte activation (1). Various DC-derived factors that induce Th-cell polarization have been identified. Well-documented examples are Th1-cell-polarizing cytokines, such as IL-12, IL-23, IL-27 (2), and type 1 IFNs (3), as well as Th2-cell-polarizing cytokines such as IL-10 and TGF-β (4, 5). Among them, IFN-γ is an important cytokine that activates macrophage and DC. It has long been assumed that the only cells producing IFN-γ in response to IL-12 are T and NK cells. However, recent studies have shown that DCs also produce IFN-γ, suggesting the presence of an autocrine-positive feedback pathway for DC activation. It has been proposed that the production of IFN-γ by DCs at the time of Ag presentation also causes a strong Th1-cell polarization (6). In mice, CD8α^+ DCs, which produce large amount of IL-12 and IFN-γ induce Th1 responses, whereas CD8α^- DCs produce lower amounts of IL-12 and preferentially induce Th2 responses (7). IFN-γ-secreting Th1-type T cell and CTL responses are necessary for effective anti-tumor immunity. Thus, DCs such as CD8α^+ DCs are suggested to be suitable for vaccination in cancer immunotherapy (8).

The suppressor of cytokine signaling-1 (SOCS1) emerged as an important physiological regulator of cytokine responses. SOCS1 is strongly induced by these cytokines and inhibits signal transduction by suppressing JAK tyrosine kinase activity (9, 10). Knockout mice studies indicate that SOCS1 is indispensable for the negative regulation of IFN-γ (11, 12). SOCS1 deficiency causes aberrant activation of T cells and NKT cells, which are proposed as the key cellular mediators of the SOCS1$^{-/-}$ disease (11, 13). Interestingly, severe T cell lymphopenia was observed in SOCS1$^{-/-}$ mice, but the reason for T cell apoptosis has not been clarified. Recently, however, it has been reported that mice generated using the Cre/loxP system, which lack the SOCS1 gene specifically in NKT and T cells do not develop any of the inflammatory pathology or suffer neonatal death (14). In contrast, SOCS1 deficiency in the hemopoietic compartment is thought to be sufficient to cause the SOCS1$^{-/-}$ disease because transfer of SOCS1$^{-/-}$ bone marrow results in premature lethality (11). These data suggest that severe inflammatory diseases caused by SOCS1 deficiency may require hemopoietic cell lineages other than T and NKT cells. We and other groups have found that SOCS1 has an important regulatory function in macrophages and DCs (15–17). SOCS1-deficient macrophages produce larger amounts of inflammatory cytokines in response to LPS than wild-type (WT) macrophages, and the LPS tolerance is impaired in SOCS1-deficient macrophages. SOCS1-deficient DCs are also hyperresponsive to IFN-γ and IL-4 (17). DCs from mice in which SOCS1 expression has been restored in

*Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; †Division of Genome Science, Research Center for Advanced Science and Technology, Tokyo University, Tokyo, Japan; and ‡Laboratory for Signal Network, Research Center for Allergy and Immunology, RIKEN, Yokohama, Japan

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[2] Address correspondence and reprint requests to Dr. Akihiko Yoshimura, Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail address: yakihiko@bioreg.kyushu-u.ac.jp

[3] Abbreviations used in this paper: DC, dendritic cell; SOCS1, suppressor of cytokine signaling-1; BMDC, bone marrow-derived DC; SPDC, splenic DC; WT, wild type; KLH, keyhole limpet hemocyanin; ODN, oligodeoxynucleotide; LN, lymph node; IRF, IFN regulatory factor.

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T and B cells on a SOCS1$^{-/-}$ background abnormally accumulate in the thymus and spleen, where they produce large amounts of cytokines such as BAFF (also called BLyS) and APRIL. These cytokines induce aberrant expansion of B cells and autoreactive Ab production. Thus, SOCS1 potentially regulates not only adaptive immunity but also innate immunity by suppressing hyperactivation of macrophages and DCs. SOCS1$^{-/-}$ DCs not only activate B cells but also can trigger allogeneic T cell expansion. These mechanisms probably mediate the onset of other SOCS1$^{-/-}$ diseases.

Because DCs are strictly regulated by cytokines, SOCS1 may be a potent regulator of DC differentiation, maturation, and activation. Here we report that SOCS1-deficient DCs initiate naive T cells to produce higher amounts of Th1 cytokines such as IFN-γ than WT DCs both in vitro and in vivo. Furthermore, we also demonstrated the possibility that SOCS1-deficient DCs become a powerful vaccine for cancer immunotherapy.

Materials and Methods

Mice

SOCS1$^{-/-}$ mice were previously established (11) and backcrossed onto the C57BL/6 background for at least nine generations. IFN-$\gamma^{-/-}$SOCS1$^{-/-}$ mice and SOCS1$^{-/-}$Tg mice, which carrying the SOCS1 gene in T and B cells but not in other type of cells, have been described previously (17). All experiments using these mice were approved by and performed according to the guidelines of the animal ethics committee of Kyushu University.

DC preparation and MLR

Bone marrow-derived DCs (BMDCs) were prepared from bone marrow suspensions from femurs and tibias in mice as described (18). Bone marrow cells were cultured in 10 ng/ml mouse GM-CSF (PeproTech) and the 10-day-cultured BMDCs were used for the experiment. Splenic DCs (SPDCs) were purified using MACS magnetic beads (Miltenyi Biotec) as described previously (19). For CFSE labeling, DCs were incubated with 10 μM CFSE (Molecular Probes) for 10 min at 37°C in PBS containing 0.1% BSA. Allogeneic MLR experiments using CD4$^+$ T cells from BALB/c mice with gamma-irradiated (3000 rad) SPDCs were described previously (17).

Priming with Ag-pulsed DC in situ

CFSE-labeled BMDCs or SPDCs were incubated in culture medium with 50 μg/ml keyhole limpet hemocyanin (KLH) for 16 h. DCs were washed three times, resuspended in PBS (5×10^6 cells of BMDCs or 1×10^6 cells of SPDCs in 40 μl) and then administered into the hind footpad. The draining lymph nodes (LNs) were removed and teased into a cell suspension on day 3 for FACS analysis. On day 5, lymphocytes were cultured with or without Ag (20 μg/ml KLH) at 1×10^6 cells/well in 96-well plates for 96 h and cytokine levels in the culture supernatant were determined by ELISA.

Tumor challenge and vaccination with peptide pulsed DC

To induce skin tumors, mice received s.c. injection of 3×10^5 B16 melanoma cells. Three days after tumor inoculation, mice were vaccinated with 1×10^6 BMDCs, pulsed with TRP2 peptide (SVYDFFVWL) (20). One week later, this vaccination was repeated. Peptide pulsation to DCs was performed as described previously (20). In brief, BMDCs cultured 9 days were loaded in Opti-MEM1 (Invitrogen Life Technologies) and pulsed with 25 μM peptide for 2 h at 37°C. Peptide-loaded DCs were washed twice in saline and injected s.c. in the right flank.

Measurement of IFN-γ production from DCs

BMDCs were cultured with the indicated concentrations of LPS (Sigma-Aldrich) or CpG oligodeoxynucleotide (ODN) (TCCATGACGTTCCTGATGCT) for 24 h. Concentrations of IFN-γ in the culture supernatants were measured by ELISA.

Flow cytometric analysis of DCs subsets

Spleen and LNs were minced in RPMI 1640 containing 10% FCS and digested with 400 U/ml collagenase type III for 20 min at 37°C. The cells were passed through a 50-μm nylon mesh. Cells were stained with FITC-conjugated anti-CD8 (53-6.7), PE-conjugated anti-CD4 (RM4-5), biotin-conjugated anti-I-A^b (AF6-120.1), and allophycocyanin-conjugated anti-CD11c (HL3) (BD Pharmingen). Biotinylated Ab staining was followed by streptavidin-PerCP Cy5.5 (BD Pharmingen). Stained cells were analyzed on a FACSCalibur (BD Biosciences) flow cytometer and FlowJo software (Tree Star). DC subsets (CD8α^+, CD4$^+$, and double negative) were isolated by a cell sorter using EPICS ALTRA cell sorter (Beckman Coulter), and the purity was $\geq$97% judged by cell surface marker expression.

RT-PCR

Total cellular RNA was isolated from DC subsets using RNeasy (Qiagen) according to the manufacturer's instructions. The expression level of GAPDH was first evaluated as an internal control using serially diluted reverse-transcribed cDNA. The expression levels of SOCS1 were then assessed using appropriate pairs of primers. Primer sequences were as follows: SOCS1, sense 5′-CGCCAACGGAACTGCTTCTTC-3′, antisense 5′-TCAGGTAGTCACGGAGTACC-3′; T-bet, sense 5′-CCCACAAGCCATTACAGG-3′, antisense 5′-AGTGATCTCTGCGTTCTGGT-3′; Eomes, sense 5′-TGAATGAACCTTCCAAGACTCAGA-3′, antisense 5′-GGCTTGAGGCAAAGTGTTGACA-3′; GAPDH, sense ACCACAGTCCATGCCATCAC-3′, antisense 5′-TCCACCACCCTGTTGCTGTA-3′. PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. For quantitative determination of SOCS1 expression, quantitative real-time PCR was performed by using ABI 7000 sequence detector system (Applied Biosystems). SOCS1 mRNA expression was assessed using the above-mentioned primers in combination with SYBR Green (Applied Biosystems). SOCS1 quantity was normalized by the levels of GAPDH using TaqMan rodent GAPDH control reagents (Applied Biosystems).

High-density oligonucleotide microarray analysis

Total RNA was extracted by standard methods using TRIzol reagent (Invitrogen Life Technologies). cRNA preparation and microarray hybridization were conducted according to the supplier (Affymetrix), using Genechip HG-U95Av2. Scanned output files were analyzed by the probe level analysis package, Microarray Suite MAS 5.0 (Affymetrix).

Statistical analysis

For statistical analysis, we performed Student's t test, and a 95% confidence limit was taken to be significant, defined as $p < 0.05$.

Results

Enhanced IFN-γ production from T cells by SOCS1-deficient DCs in allogeneic MLR

To examine the effect of SOCS1 deficiency in DCs for T cell activation, we first assessed MLR with allogeneic T cells from BALB/c mice and CD11c$^+$ SPDCs from SOCS1-deficient mice. SOCS1$^{-/-}$ DCs are expected to stimulate proliferation of allogeneic T cell more extensively than WT DCs, because SOCS1$^{-/-}$ DCs expressed higher levels of MHC and costimulators than WT-DCs (17). Unexpectedly, however, SOCS1$^{-/-}$ DCs from the spleen of SOCS1$^{-/-}$Tg mice stimulated T cell proliferation less efficiently than WT-DCs (Fig. 1*A*, *left panel*). In vitro, IFN-γ is shown to inhibit the expansion of T cells that are stimulated through their Ag receptors by promoting caspase-8-dependent apoptosis (21). Thus, we examined IFN-γ concentration in supernatants of T cells/DCs cocultures. As shown in Fig. 1*A* (*middle* and *right panels*), extremely high amounts of IFN-γ, but not IL-2, were detected in the culture supernatants of T cells and SOCS1$^{-/-}$ DCs cocultures. Impaired proliferation of T cells cocultured with SOCS1$^{-/-}$ DCs was restored by adding IFN-γ neutralizing Ab (Fig. 1*A*). Therefore, lower proliferation of T cells by coculture with SOCS1$^{-/-}$ DCs may be due to a cytostatic or cytotoxic effect of high levels of IFN-γ to T cells and/or DCs. Suppression of T cell proliferation by SOCS1$^{-/-}$ DC may be a cause of T cell lymphopenia found in SOCS1-deficient mice. Apoptosis of T cells was not enhanced by coculture with SOCS1$^{-/-}$ BMDCs compared with WT BMDCs coculture as judged by annexin V and propidium iodide staining (data not shown). IDO which degrades the essential amino acid triptophan is implicated in suppression of T cell proliferation (22), and IFN-γ is a potent inducer of IDO. It has been reported that DCs expressing IDO can suppress T cell responses

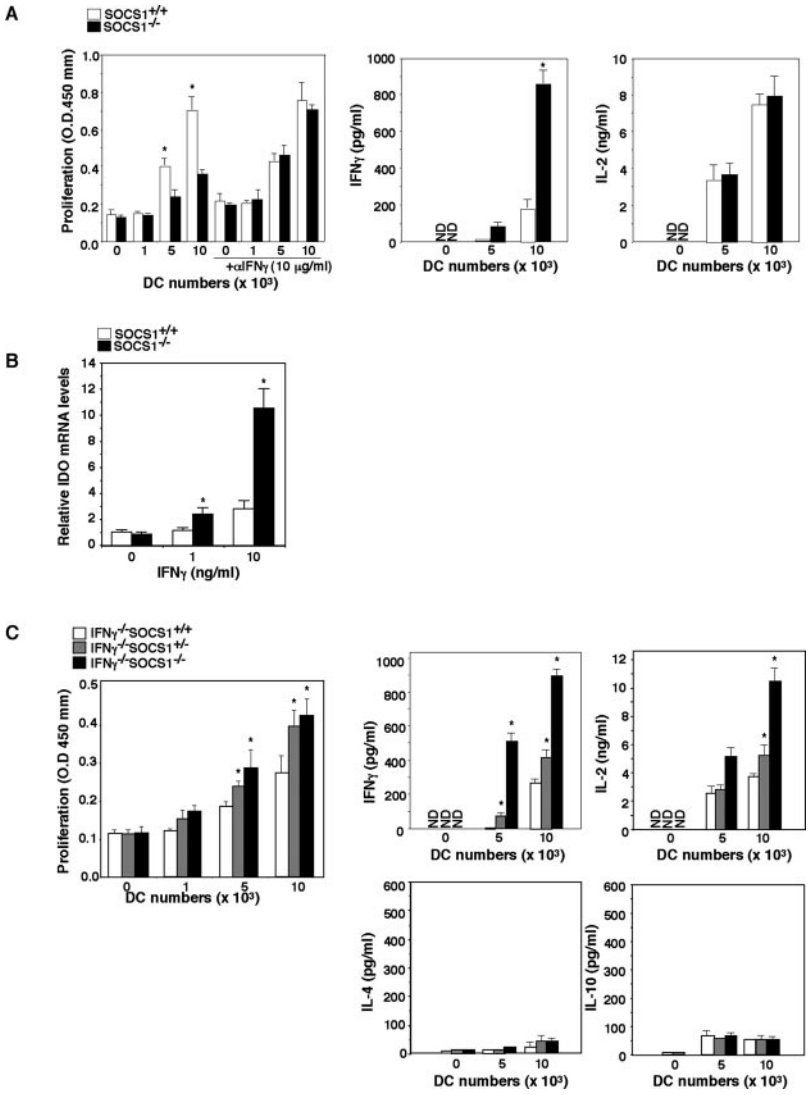

FIGURE 1. SOCS1-deficient DCs induce strong Th1 responses in MLR. Proliferative capacity of allogeneic T cells from BALB/c mice cocultured with DCs from spleens of SOCS1$^{+/+}$ (□) and SOCS1$^{-/-}$ (■) mice in MLR assays with or without anti-IFN-γ-neutralizing Ab. *A*, IFN-γ and IL-2 concentration in the culture medium were measured by ELISA. IDO mRNA expression levels in DCs from SOCS1$^{+/+}$ (□) and SOCS1$^{-/-}$ (■) mice were assessed by real-time PCR. IDO expression levels were normalized by internal GAPDH expression. *B*, The data indicate means ± SD of triplicate samples of one representative experiment of three independent experiments. MLRs were performed in cocultured with DCs from SOCS1$^{+/+}$, SOCS1$^{+/-}$, and SOCS1$^{-/-}$ on an IFN-γ$^{-/-}$ background. *C*, IFN-γ, IL-2, IL-4, and IL-10 concentrations in the culture supernatants were determined by ELISA.

and promote tolerance (22). As shown in Fig. 1*B*, IDO expression induced by IFN-γ stimulation was higher in SOCS1$^{-/-}$ DCs than in WT-DCs. The ability to produce high amounts of IDO in SOCS1$^{-/-}$ DCs may be one of the possible cause of suppression of T cell proliferation.

To eliminate the effect of IFN-γ from DCs, we performed MLR experiments using DCs from an IFN-γ$^{-/-}$ background. In contrast to the normal IFN-γ genetic background, IFN-γ$^{-/-}$SOCS1$^{-/-}$ DCs augmented T cell proliferation, and this effect was dependent on the expression level of SOCS1 in DCs (Fig. 1*C*). The levels of Th1-type cytokines such as IFN-γ and IL-2 were much higher in the culture supernatants of T cells cocultured with SOCS1$^{-/-}$ DCs than those cocultured with WT-DCs, whereas the level of Th2-type cytokines such as IL-4 and IL-10 were not different in these MLRs. These results indicate that SOCS1-deficient DCs induce stronger Th1-type responses in vitro compared with WT DCs.

SOCS1$^{-/-}$ DCs induce strong Th1-type responses in vivo

Next, to assess whether SOCS1$^{-/-}$ DCs strongly initiate Th1 polarization in vivo, BMDCs from WT and SOCS1$^{-/-}$Tg mice were pulsed with KLH for 2 h and then transferred into separate footpads of the same C57BL/6 mice. Although the levels of CD80, CD86, and CD40 were similar between WT and SOCS1$^{-/-}$ BMDCs after KLH pulse, class II MHC molecules were significantly up-regulated in SOCS1$^{-/-}$ BMDCs regardless of Ag loading (Fig. 2*A*). The migration rate of CFSE-labeled SOCS1$^{-/-}$ BMDCs to the draining popliteal LNs was also identical to that of SOCS1$^{+/+}$ BMDCs (Fig. 2*B*) and the size of each LNs was indistinguishable 3 days after injection (data not shown), when the DCs population is thought to peak (23). However, 5 days after injection, the popliteal LN on the side in which SOCS1$^{-/-}$ BMDCs were injected was swollen more extensively than that receiving the SOCS1$^{+/+}$ BMDCs injection (Fig. 2*C*). We compared CD4-, CD8-, and B220-positive cells in BMDC-immunized LNs. There was no difference in the population of these cells between SOCS1$^{-/-}$ BMDC and WT BMDC immunizations, although much more cells were obtained from SOCS1$^{-/-}$ BMDC-immunized LN than from WT BMDC-immunized LN (data not shown). Therefore, both WT BMDCs and SOCS1$^{-/-}$ BMDCs have similar phenotype for increasing the number of T and B cells; however, SOCS1$^{-/-}$ BMDCs possess stronger potential than WT DCs.

Then we examined the Ag-specific response of T cells from these LNs. Single-cell suspensions were prepared from the popliteal LNs and cultured in the presence or absence of KLH for 4 days. As shown in Fig. 2*C*, IFN-γ and IL-2 were not strongly induced from KLH-restimulated LN cells by WT DC-immunization, probably because we used immature BMDCs, while significantly higher levels of these Th1-type cytokines, especially IFN-γ

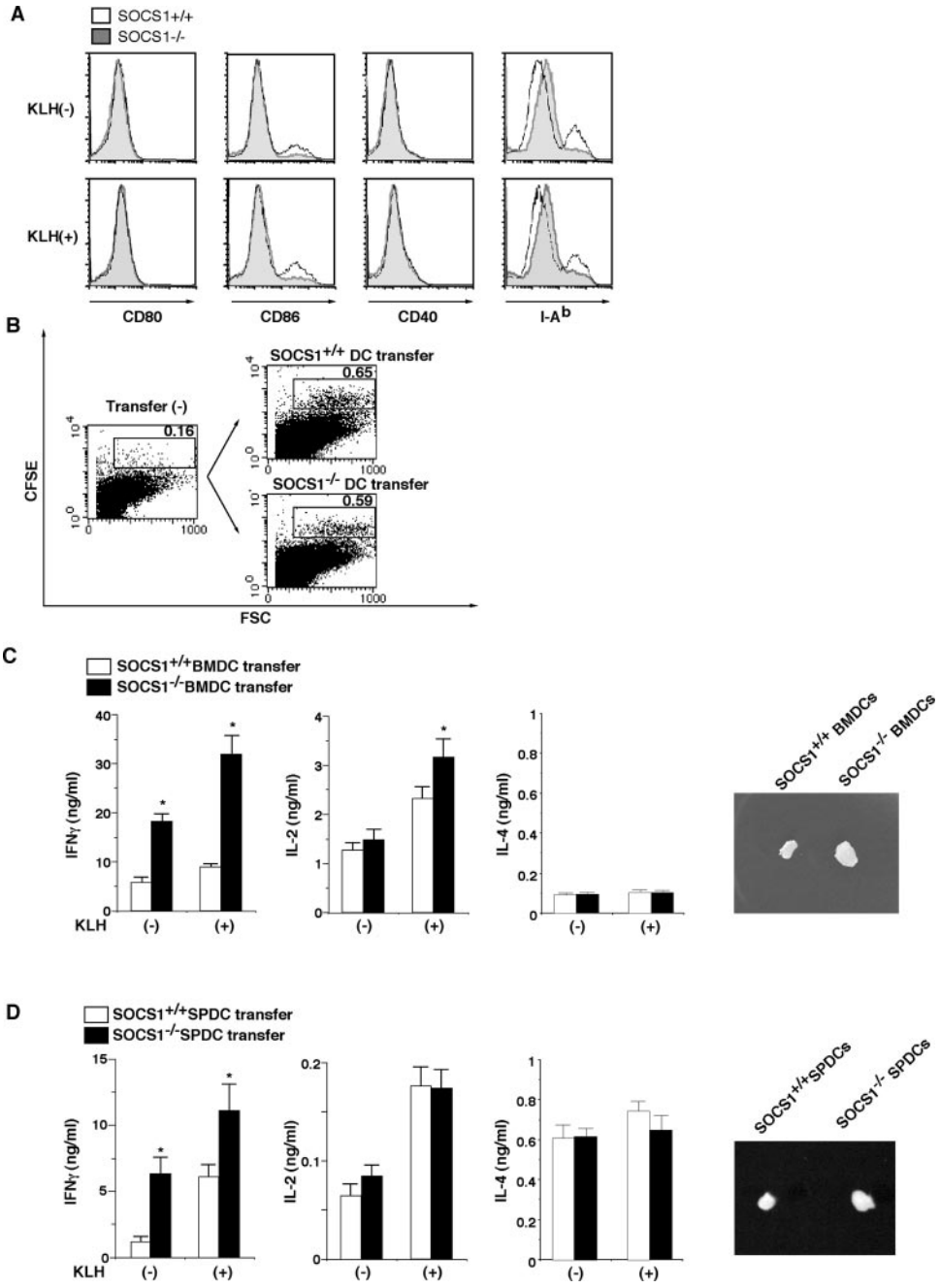

FIGURE 2. SOCS1$^{-/-}$ DCs induce Th1-type responses in vivo. A, Flow cytometric analysis of CD80, CD86, CD40, and I-A^b expression on BMDCs from SOCS1$^{-/-}$Tg mice and WT littermates cultured in the presence or absence of KLH for 2 h. KLH-pulsed and CFSE-stained BMDCs from SOCS1$^{-/-}$Tg mice and WT littermates were injected into the footpad of C57BL/6 mice. *B*, Three days after injection, flow cytometric analysis was performed to evaluate DC migration into the draining LN. Lymphocytes from the draining LN immunized by WT or SOCS1$^{-/-}$ BMDCs (*C*) or SPDCs (*D*) for 5 days were cultured for 96 h in the presence or absence of KLH. The concentrations of cytokines were determined by ELISA. Right images represent the draining LNs on 5 days after injections of WT or SOCS1$^{-/-}$DCs.

were induced in lymphocytes from the LNs of SOCS1$^{-/-}$ BMDC-immunized side. Th2-type cytokine, IL-4 was not significantly induced in both cases (Fig. 2*C*). Similar experiments were performed using SPDCs from WT and SOCS1$^{-/-}$ Tg mice. Again, the popliteal LN immunized with SOCS1$^{-/-}$ SPDCs were more swollen than that immunized with WT-SPDCs. Lymphocytes from SOCS1$^{-/-}$ SPDC-immunized LN with KLH produced higher amount of IFN-γ production than those from WT SPDC-immunized LN (Fig. 2*D*). However, IL-2 and IL-4 levels were not significantly different. These data suggest that SOCS1-deficeint SPDCs could specifically promote IFN-γ production from T cells in vivo. These results indicated that SOCS1-deficient DCs induced strong T cell proliferation as well as hyper Th1 response in vivo.

SOCS1$^{-/-}$ DC is a highly potent anti-tumor vaccine

IFN-γ-secreting Th1 cells are believed to be critical for long-lasting anti-tumor immunity (24). Because SOCS1$^{-/-}$ DCs can induce strong Th1-type responses, we examined whether SOCS1-deficiency potentiates the anti-tumor ability of DCs used for therapeutic vaccination against the melanoma, B16 melanoma Ag peptide (TRP2)-loaded BMDCs were injected s.c. on days 3 and 10 after injection of B16 melanoma cells (Fig. 3*A*). Unlike preventive models in which DCs are injected before tumor challenge, normal DCs were not very effective in this therapeutic model. We observed reduction of tumor size and a slight improvement of recipient survival by introduction of WT-BMDCs, but these effects were independent of melanoma Ag (Fig. 4, *B* and *C*). However, strong reduction of tumor size was observed when the Ag peptide-pulsed SOCS1$^{-/-}$ BMDCs were given (Fig. 3*B*). The mice immunized with TRP2-pulsed SOCS1$^{-/-}$ BMDCs also survived longer than mice immunized with WT-BMDCs (Fig. 3*C*). Peptide-unloaded BMDCs were not effective. These data indicate that BMDCs lacking SOCS1 expression represent a strong possibility for creating a powerful vaccine adjuvant for tumor-reactive immunity in vivo.

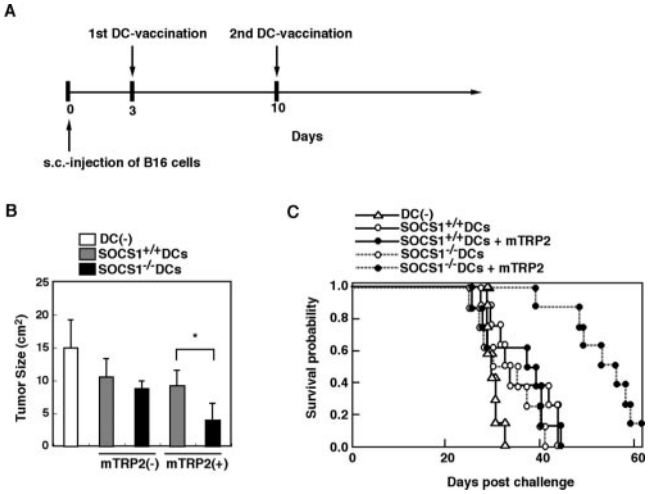

FIGURE 3. Analysis of therapeutic immunity against a lethal s.c. challenge with B16 melanoma cells, and induced by vaccination using mTRP2-loaded BMDCs from SOCS1$^{-/-}$Tg mice and WT littermates ($n = 8$). *A*, Vaccination schedule. *B*, Tumor size is assessed by measuring two perpendicular diameters of skin tumor on 26 days after tumor challenge ($n = 8$). *C*, Kaplan-Meier survival curves are depicted as time after tumor challenge ($n = 8$).

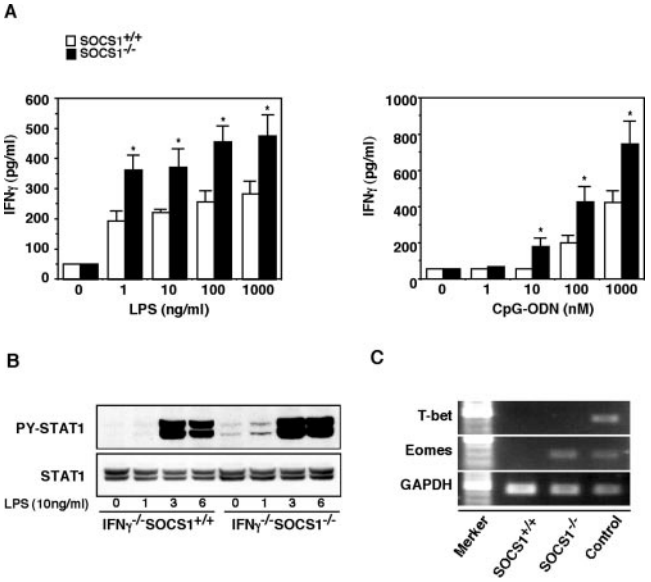

FIGURE 4. IFN-γ production and STAT1 activation in DCs in response to TLR ligands. WT and SOCS1$^{-/-}$ BMDCs (1×10^6/ml) were stimulated with the indicated amount of LPS or CpG-ODN for 24 h, then IFN-γ in the culture supernatant was measured by ELISA (*A*), and STAT1 activation was detected by immunoblotting with anti-phosphorylated STAT1 Ab (*B*). RT-PCR analysis for mRNA expression of T-bet and Eomes in BMDCs from SOCS1$^{-/-}$Tg mice and WT littermates. The mRNA of T cells stimulated by anti-CD3 and anti-CD28 Abs were used for positive control. GAPDH, internal control. Experiments were repeated twice with similar results.

Hyperexpression of IFN-inducible genes in SOCS1$^{-/-}$ DCs

To define the molecular basis for hyper Th1 skewing by SOCS1$^{-/-}$ DCs, we examined whether SOCS1 affects the adjuvant effect of TLR signaling in DCs. Although the levels of LPS and CpG-ODN-induced up-regulation of activation markers and IL-12 production were not altered between WT and SOCS1$^{-/-}$ BMDCs (data not shown), IFN-γ production as well as STAT1 activation was more strongly augmented in SOCS1$^{-/-}$ BMDCs compared with WT-BMDCs (Fig. 4*A*). It has long been assumed that the only cells producing IFN-γ in response to IL-12 are T and NK cells. However, recent studies have shown that DCs also produce IFN-γ, which induces strong Th1-cell polarization by an autocrine-paracrine positive feedback pathway during T-DC interaction (6). Therefore, hyperproduction of IFN-γ or hyperactivation of IFN-signaling in SOCS1$^{-/-}$ DCs may account for hyperinduction of Th1 cells. In consistent with this notion, microarray analysis revealed that IFN-inducible genes were up-regulated in SOCS1-deficient BMDCs compared with WT-BMDCs (Table I). Furthermore, STAT1 was constitutively tyrosine-phosphorylated in IFN-γ$^{-/-}$SOCS1$^{-/-}$ BMDCs when they were cultured in GM-CSF (Fig. 4*B*). Several genes on the list, such as IFN-activating gene 203 and IFN regulatory factor (IRF)-7 are implicated in Th1 responses. Therefore, hyperactivation of the IFNs-STAT1 pathway

Table I. *List of IFN-inducible genes whose expression levels were more than two times higher in SOCS$^{-/-}$ DC than IFN-γ$^{-/-}$ DC*[a]

Unigene ID	Short Name	Mean: IFNγ$^{-/-}$SOCS1$^{+/+}$	IFNγ$^{-/-}$ SOCS1$^{-/-}$	Fold Change	*P* Value
Mm,8180	Sca-1	105.2	2755.6	4.8	0.00002
Mm,951	IFN-induced protein with tetratricopeptide repeats 3	8.7	309.8	4.8	0.00002
Mm,3233	IRF-7	87.1	690.4	4.5	0.00002
Mm,200270	Hypothetical protein LOC211528	8.7	68.8	4.2	0.00002
Mm,2038	IFN-induced protein with tetratricopeptide repeats 2	11.9	213.4	4.1	0.00002
Mm,16091	Chemokine ligand 7	7.2	160.0	4.0	0.00003
Mm,27182	2′-5′ oligoadenytate synthetase-life 2	34.4	802.4	3.8	0.00002
Mm,30756	AW261480	19.5	247.1	3.7	0.00002
Mm,180157	28 kDa IFN-responsive protein	72.3	1194.4	3.5	0.00003
Mm,213137	Chemokine ligand 2	51.1	712.8	3.5	0.00002
Mm,24045	Viperin	20.5	291.0	3.2	0.00002
Mm,8718	IFN-induced protein with tetratricopeptide repeats 1	26.0	221.1	3.1	0.00002
Mm,231914	IFN-activated gene 205	83.2	767.3	2.0	0.00002
Mm,29008	IFN-inducible GTPase	21.0	137.9	2.5	0.00003
Mm,212870	IFN-activated gene 204	13.8	65.5	2.4	0.00002
Mm,21036	IL-18 binding protein	26.5	116.5	2.4	0.00002
Mm,23991	IFN-induced guanylate-binding protein 2	22.4	178.6	2.4	0.00005
Mm,19028	IFN-stimulated protein Isg20	17.3	02.8	2.1	0.00002
Mm,18530	IFN-activated gene 203	26.7	124.5	2.1	0.00002
Mm,33902	IFN-γ-induced GTPase	22.2	89.9	2.1	0.00002
Mm,24769	IFN-γ-inducible protein	88.6	392.8	2.0	0.00002

[a] Mean: expression intensity of cDNA.

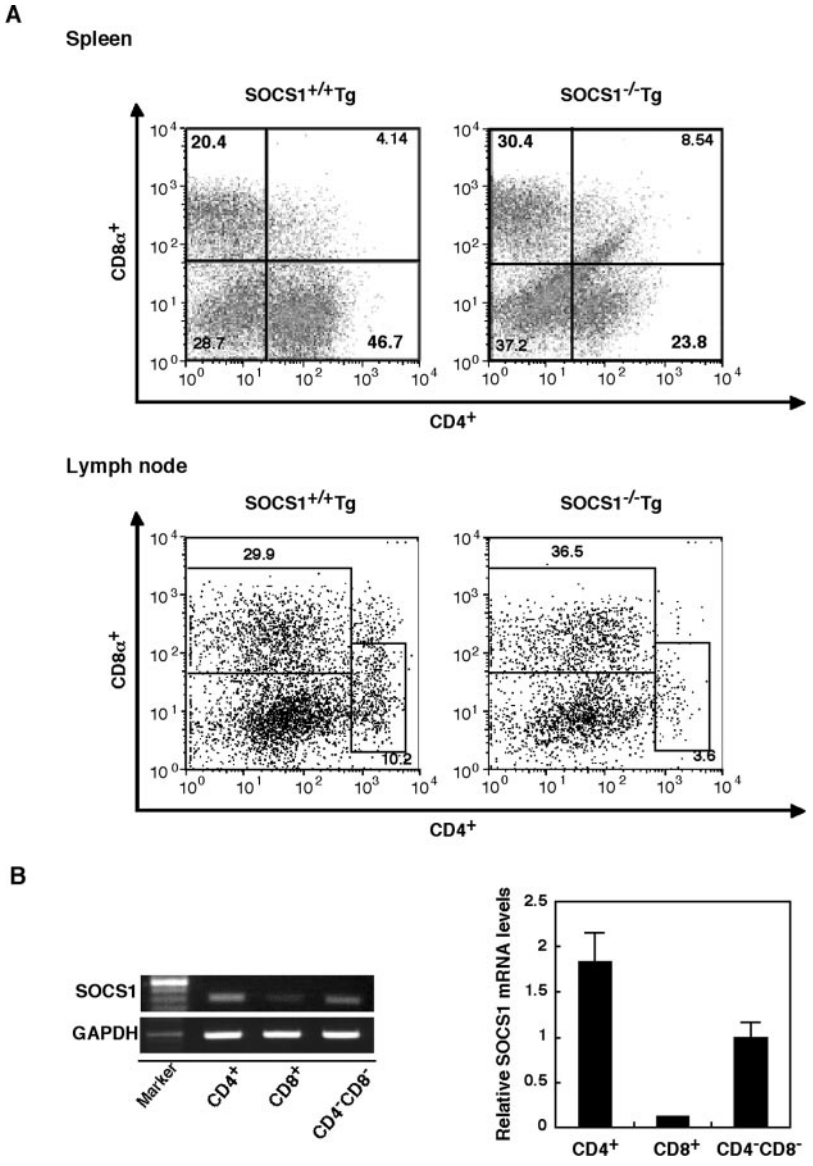

FIGURE 5. *A*, Profiles of spleen and LN DC subsets in SOCS1$^{+/+}$Tg and SOCS1$^{-/-}$Tg mice are shown. CD4$^+$ DCs, CD8α^+ DCs, and double-negative DCs from WT spleens were sorted using a EPICS ALTRA cell sorter. SOCS1 mRNA expression levels in each subset were assessed by real-time PCR. SOCS1 expression levels were normalized to internal GAPDH expression. The data indicate means ± SD of triplicate samples from one representative experiment of three independent experiments.

in DCs could be a mechanism of hyperinduction of Th1 responses in SOCS1$^{-/-}$ DCs.

To define the molecular mechanism of hyper IFN-γ production in SOCS1$^{-/-}$ DCs, we examined the expression of transcription factors, T-bet and Eomes, which are related to regulation of IFN-γ expression (25). As shown in Fig. 4*C*, T-bet was not detected in both WT and SOCS1$^{-/-}$ BMDCs. However, Eomes expression was only detected in SOCS1$^{-/-}$ BMDCs. These results imply that Eomes may be a key molecule to produce high amount of IFN-γ in SOCS1$^{-/-}$ DCs.

SOCS1 regulates CD8α$^+$DC differentiation

Secondary lymphoid organs in mice have at least three subsets of classical CD11c^{high} DCs, including CD8α$^+$, CD4$^+$ and double-negative DCs, as well as a population of CD11c^{low} DCs with plasmacytoid morphology (26). Among them, CD8α$^+$ DCs produce high levels of IL-12 and induce Th1 responses (7). CD8α$^+$ DCs are also able to produce IFN-γ upon IL-12 stimulation (27). DC-derived IFN-γ, together with IL-12, may also be important in activation of DCs in an autocrine manner, and lead to Th1 responses. Thus, we examined CD8α$^+$DCs from SOCS1$^{-/-}$Tg and WT mice. The proportion of CD8α$^+$ DCs in splenic and LN DCs of SOCS1$^{-/-}$Tg mice was significantly higher than that from normal C57BL/6 and SOCS1$^{+/+}$Tg mice (Fig. 5*A*; data for C57BL/6 mice not shown). We also compared the expression levels of SOCS1 in WT CD8α$^+$, CD4$^+$, and double-negative DCs. As shown in Fig. 5*B*, SOCS1 levels were significantly lower in CD8α$^+$ DCs, than those in CD4$^+$ and double-negative DCs. Thus, the Th1-skewing nature of CD8α$^+$ DCs may be partly explained by the reduced expression of SOCS1.

Discussion

In this paper, we demonstrated that SOCS1-deficient DCs could induce a strong Th1 response both in vitro and in vivo. It has been reported that distinct DC subsets participate in Th1 and Th2 skewing (8, 28–31). In particular, CD8α$^+$ DC produce high amount of IL-12 and IFN-γ, which are crucial cytokines for Th1 skewing of T cells, and induce Th1 responses (7). Th1 development can be induced in vivo by administration of Ag-loaded CD8α$^+$ DCs, into syngeneic mouse footpads (8). Interestingly, the phenotypes of SOCS1$^{-/-}$ DCs are similar to those of splenic CD8α$^+$ DCs from WT mice. Moreover, in WT mice, the expression levels of SOCS1 in CD8α$^+$ DC were extremely low compared with CD4$^+$ or double-negative DCs (Fig. 5). Therefore, we propose that SOCS1

plays an important role to determine the Th1-inducing phenotype of DCs. In this regard, it is interesting that Eomes, a recently identified transcription factor that regulates IFN-γ expression was detected in SOCS1$^{-/-}$ BMDCs. These results imply that Eomes may be a key molecule to produce high amount of IFN-γ in SOCS1$^{-/-}$ DCs (Fig. 4*C*). Our data raise a possibility that Eomes may play an important role in determining CD8α^+ DC phenotype.

Then, how does SOCS1 regulate DC phenotypes? Up-regulation of STAT1 activation and IFN-γ production in SOCS1$^{-/-}$ DCs are likely mechanisms of strong induction of Th1 response by SOCS1$^{-/-}$ DCs. CD8α^+ DCs are also shown to produce IFN-γ upon IL-12 stimulation in vivo (27). Possibly, CD8α^+ DC and SOCS1$^{-/-}$ DCs produce IL-12 and IFN-γ in an autocrine fashion, creating a positive feedback loop (6). In addition, a high amount of IFN-γ produced by SOCS1$^{-/-}$ DCs may directly promote the development of Th1 cells.

IFN-γ produced from DCs may have a profound effect on T cell proliferation. As shown in Fig. 1*A*, SOCS1$^{-/-}$ DCs rather suppressed T cell proliferation, while SOCS1$^{-/-}$IFN-$\gamma^{-/-}$DCs enhanced T cell proliferation. One plausible mechanism is the expression of IDO. We found that DCs from SOCS1$^{-/-}$Tg mice expressed higher levels of IDO (Fig. 1*B*), which may suppress T cell proliferation. IFN-$\gamma^{-/-}$SOCS1$^{-/-}$ DCs expressed similar levels of IDO as control IFN-$\gamma^{-/-}$ DCs did (data not shown). Thus, IDO is a candidate of T cell lymphopenia found in SOCS1-deficient mice.

In this study, we also showed that SOCS1 is required for normal SPDC development. CD8α^+ DCs significantly increased in SOCS1$^{-/-}$Tg mice. We also showed that population of plasmacytoid DCs is increased in the spleen of SOCS1$^{-/-}$Tg mice (17). In contrast, other DC subsets, CD4$^+$ and double-negative DCs decreased in these mice. These results indicate that SOCS1 is required for proper development of DC subsets. Studies using targeted gene-disrupted mice indicated that several genes are involved in DC subset determination. It has been reported that IFN consensus sequence binding protein/IRF-8$^{-/-}$ (32–34) mice exhibit a defect in CD8α^+ DCs. On the contrary, IRF-2$^{-/-}$ (35), RelB$^{-/-}$, and TNFR-associated factor 6$^{-/-}$ (36) exhibited an almost complete absence of CD4$^+$ DCs. These observations indicate that IFN signals and/or TNFR-associated factor 6-NF-κB signals were required for the development of DC subsets. SOCS1 is also one of the crucial regulators of IFN signaling. SOCS1$^{-/-}$ DCs might be more sensitive to IFN-α/IFN-β as well as IFN-γ signaling. Our microarray data support this idea because IFN-inducible genes were up-regulated in SOCS1$^{-/-}$ DCs even in the IFN-$\gamma^{-/-}$ background. Furthermore, STAT1 was constitutively tyrosine-phosphorylated in IFN-$\gamma^{-/-}$SOCS1$^{-/-}$ BMDCs when they were cultured in GM-CSF. The expression level of SOCS1 was significantly low in the CD8α^+ DC subset. Therefore, SOCS1 may regulate DC subset development by modulating IFN/STAT1 signaling. This might also provide a divergence of susceptibility to IFNs in the respective DC subsets.

SOCS1 may also regulate other cytokines that are important for DC functions. IL-15 is reported to be an important cytokine to influence DC function. IL-15$^{-/-}$ DCs reduced IL-12 and IFN-γ production in response to LPS and IL-12, respectively, although the number of CD8α^+ and CD8α^- DCs were normal (37). SOCS1 is supposed to be a negative regulator of IL-15 signaling (38–40). Therefore, hypersignaling of IL-15 may augment the function of SOCS1$^{-/-}$ DCs to produce IFN-γ.

From Th1-skewing properties of SOCS1-deficient DCs, we have demonstrated that SOCS1 is a possible candidate molecule for augmentation of DC vaccination in cancer immunotherapy. IFN-γ-secreting Th1 cells are likely to be critical for more effective and long-lasting anti-tumor immunity (24). Indeed, strong tumor-specific Th1 responses were elicited when SOCS1$^{-/-}$ DCs were loaded with MHC class II binding melanoma peptides. Thus, DCs in which SOCS1 gene expression is knocked down by RNA interference or antisense oligonucleotide may lead to polarized Th1 responses and be beneficial for cancer immunotherapy. Indeed, recently, Shen et al. (41) reported that silencing of SOCS1 gene by small interferring RNA (siRNA) technology in Ag-presenting DCs strongly enhanced Ag-specific anti-tumor immunity. They showed that DCs transfected with SOCS1 siRNA were more responsive to LPS or IFN-γ than were DCs with control siRNA. Ag (OVA) peptide-pulsed SOCS1-siRNA treated DCs activate OVA-specific CTLs more strongly than the control DCs. These data indicate that SOCS1-deficeint DCs can strongly activate both CD4$^+$ and CD8$^+$ T cells. Naka et al. (13) have reported that the abnormal activation of NKT cells were observed in SOCS1-deficient mice and lead to lethal pathological changes in the liver. Hyperactivation of NKT cells may also be due to the aberrantly activated SOCS1$^{-/-}$ DCs. Recent studies also showed that DCs pulsed with α-GalCer are able to effectively activate Vα14 NKT cells in vivo and induce protective anti-tumor immunity in situ (42–44). It is possible that stimulation of NKT cells via SOCS1 knocked-down-DCs will also provide a therapeutic benefit in patients with several cancers.

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Disclosures

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References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245*.
2. Trinchieri, G. 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nat. Rev. Immunol. 3:133*.
3. Kadowaki, N., S. Antonenko, J. Y. Lau, and Y. J. Liu. 2000. Natural interferon α/β-producing cells link innate and adaptive immunity. *J. Exp. Med. 192:219*.
4. Groux, H., M. Bigler, J. E. de Vries, and M. G. Roncarolo. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4$^+$ T cells. *J. Exp. Med. 184:19*.
5. Zeller, J. C., A. Panoskaltsis-Mortari, W. J. Murphy, F. W. Ruscetti, S. Narula, M. G. Roncarolo, and B. R. Blazar. 1999. Induction of CD4$^+$ T cell alloantigen-specific hyporesponsiveness by IL-10 and TGF-β. *J. Immunol. 163:3684*.
6. Frucht, D. M., T. Fukao, C. Bogdan, H. Schindler, J. J. O'Shea, and S. Koyasu. 2001. IFN-γ production by antigen-presenting cells: mechanisms emerge. *Trends Immunol. 22:556*.
7. Pulendran, B., J. Lingappa, M. K. Kennedy, J. Smith, M. Teepe, A. Rudensky, C. R. Maliszewski, and E. Maraskovsky. 1997. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand- treated mice. *J. Immunol. 159:2222*.
8. Maldonado-Lopez, R., C. Maliszewski, J. Urbain, and M. Moser. 2001. Cytokines regulate the capacity of CD8α^+ and CD8α^- dendritic cells to prime Th1/Th2 cells in vivo. *J. Immunol. 167:4345*.
9. Sakamoto, H., H. Yasukawa, M. Masuhara, S. Tanimura, A. Sasaki, K. Yuge, M. Ohtsubo, A. Ohtsuka, T. Fujita, T. Ohta, et al. 1998. A Janus kinase inhibitor, JAB, is an interferon-γ-inducible gene and confers resistance to interferons. *Blood 92:1668*.
10. Yasukawa, H., H. Misawa, H. Sakamoto, M. Masuhara, A. Sasaki, T. Wakioka, S. Ohtsuka, T. Imaizumi, T. Matsuda, J. N. Ihle, and A. Yoshimura. 1999. The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. *EMBO J. 18:1309*.
11. Marine, J. C., D. J. Topham, C. McKay, D. Wang, E. Parganas, D. Stravopodis, A. Yoshimura, and J. N. Ihle. 1999. SOCS1 deficiency causes a lymphocyte-dependent perinatal lethality. *Cell 98:609*.
12. Alexander, W. S., R. Starr, J. E. Fenner, C. L. Scott, E. Handman, N. S. Sprigg, J. E. Corbin, A. L. Cornish, R. Darwiche, C. M. Owczarek, et al. 1999. SOCS1 is a critical inhibitor of interferon γ signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell 98:597*.
13. Naka, T., H. Tsutsui, M. Fujimoto, Y. Kawazoe, H. Kohzaki, Y. Morita, R. Nakagawa, M. Narazaki, K. Adachi, T. Yoshimoto, et al. 2001. SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated cross-talk inhibition of IFN-γ and IL-4 signaling in vivo. *Immunity 14:535*.

14. Chong, M. M., A. L. Cornish, R. Darwiche, E. G. Stanley, J. F. Purton, D. I. Godfrey, D. J. Hilton, R. Starr, W. S. Alexander, and T. W. Kay. 2003. Suppressor of cytokine signaling-1 is a critical regulator of interleukin-7-dependent CD8$^+$ T cell differentiation. *Immunity 18:475*.
15. Nakagawa, R., T. Naka, H. Tsutsui, M. Fujimoto, A. Kimura, T. Abe, E. Seki, S. Sato, O. Takeuchi, K. Takeda, et al. 2002. SOCS-1 participates in negative regulation of LPS responses. *Immunity 17:677*.
16. Kinjyo, I., T. Hanada, K. Inagaki-Ohara, H. Mori, D. Aki, M. Ohishi, H. Yoshida, M. Kubo, and A. Yoshimura. 2002. SOCS1/JAB is a negative regulator of LPS-induced macrophage activation. *Immunity 17:583*.
17. Hanada, T., H. Yoshida, S. Kato, K. Tanaka, K. Masutani, J. Tsukada, Y. Nomura, H. Mimata, M. Kubo, and A. Yoshimura. 2003. Suppressor of cytokine signaling-1 is essential for suppressing dendritic cell activation and systemic autoimmunity. *Immunity 19:437*.
18. Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods 223:77*.
19. Fukao, T., D. M. Frucht, G. Yap, M. Gadina, J. J. O'Shea, and S. Koyasu. 2001. Inducible expression of Stat4 in dendritic cells and macrophages and its critical role in innate and adaptive immune responses. *J. Immunol. 166:4446*.
20. Schreurs, M. W., A. A. Eggert, A. J. de Boer, J. L. Vissers, T. van Hall, R. Offringa, C. G. Figdor, and G. J. Adema. 2000. Dendritic cells break tolerance and induce protective immunity against a melanocyte differentiation antigen in an autologous melanoma model. *Cancer Res. 60:6995*.
21. Refaeli, Y., L. Van Parijs, S. I. Alexander, and A. K. Abbas. 2002. Interferon γ is required for activation-induced death of T lymphocytes. *J. Exp. Med. 196:999*.
22. Mellor A. L., and D. H. Munn. 2004. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat. Rev. Immunol. 4:762*.
23. Ritchie, D. S., I. F. Hermans, J. M. Lumsden, C. B. Scanga, J. M. Roberts, J. Yang, R. A. Kemp, and F. Ronchese. 2000. Dendritic cell elimination as an assay of cytotoxic T lymphocyte activity in vivo. *J. Immunol. Methods 246:109*.
24. Thurner, B., I. Haendle, C. Roder, D. Dieckmann, P. Keikavoussi, H. Jonuleit, A. Bender, C. Maczek, D. Schreiner, P. von den Driesch, et al. 1999. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J. Exp. Med. 190:1669*.
25. Pearce E. L., A. C. Mullen, G. A. Martins, C. M. Krawczyk, A. S. Hutchins, V. P. Zediak, M. Banica, C. B. DiCioccio, D. A. Gross, C. A. Mao, et al. 2003. Control of effector CD8$^+$ T cell function by the transcription factor Eomesodermin. *Science 302:1041*.
26. Kapsenberg, M. L. 2003. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat. Rev. Immunol. 3:984*.
27. Ohteki, T., T. Fukao, K. Suzue, C. Maki, M. Ito, M. Nakamura, and S. Koyasu. 1999. Interleukin 12-dependent interferon γ production by CD8α^+ lymphoid dendritic cells. *J. Exp. Med. 189:1981*.
28. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol. 18:767*.
29. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. CD8α^+ and CD8α^- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med. 189:587*.
30. Pulendran, B., J. L. Smith, G. Caspary, K. Brasel, D. Pettit, E. Maraskovsky, and C. R. Maliszewski. 1999. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl. Acad. Sci. USA 96:1036*.
31. Maldonado-Lopez, R., and M. Moser. 2001. Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin. Immunol. 13:275*.
32. Tsujimura, H., T. Tamura, and K. Ozato. 2003. Cutting edge: IFN consensus sequence binding protein/IFN regulatory factor 8 drives the development of type I IFN-producing plasmacytoid dendritic cells. *J. Immunol. 170:1131*.
33. Schiavoni, G., F. Mattei, P. Sestili, P. Borghi, M. Venditti, H. C. Morse III, F. Belardelli, and L. Gabriele. 2002. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8α^+ dendritic cells. *J. Exp. Med. 196:1415*.
34. Aliberti, J., O. Schulz, D. J. Pennington, H. Tsujimura, C. Reis e Sousa, K. Ozato, and A. Sher. 2003. Essential role for ICSBP in the in vivo development of murine CD8α^+ dendritic cells. *Blood 101:305*.
35. Ichikawa, E., S. Hida, Y. Omatsu, S. Shimoyama, K. Takahara, S. Miyagawa, K. Inaba, and S. Taki. 2004. Defective development of splenic and epidermal CD4$^+$ dendritic cells in mice deficient for IFN regulatory factor-2. *Proc. Natl. Acad. Sci. USA 101:3909*.
36. Kobayashi, T., P. T. Walsh, M. C. Walsh, K. M. Speirs, E. Chiffoleau, C. G. King, W. W. Hancock, J. H. Caamano, C. A. Hunter, P. Scott, et al. 2003. TRAF6 is a critical factor for dendritic cell maturation and development. *Immunity 19:353*.
37. Ohteki, T., K. Suzue, C. Maki, T. Ota, and S. Koyasu. 2001. Critical role of IL-15-IL-15R for antigen-presenting cell functions in the innate immune response. *Nat. Immunol. 2:1138*.
38. Ilangumaran, S., S. Ramanathan, J. La Rose, P. Poussier, and R. Rottapel. 2003. Suppressor of cytokine signaling 1 regulates IL-15 receptor signaling in CD8$^+$CD44^{high} memory T lymphocytes. *J. Immunol. 171:2435*.
39. Ilangumaran, S., S. Ramanathan, T. Ning, J. La Rose, B. Reinhart, P. Poussier, and R. Rottapel. 2003. Suppressor of cytokine signaling 1 attenuates IL-15 receptor signaling in CD8$^+$ thymocytes. *Blood 102:4115*.
40. Cornish, A. L., M. M. Chong, G. M. Davey, R. Darwiche, N. A. Nicola, D. J. Hilton, T. W. Kay, R. Starr, and W. S. Alexander. 2003. Suppressor of cytokine signaling-1 regulates signaling in response to interleukin-2 and other γ c-dependent cytokines in peripheral T cells. *J. Biol. Chem. 278:22755*.
41. Shen, L., K. Evel-Kabler, R. Strube, and S. Y. Chen. 2004. Silencing of SOCS1 enhances antigen presentation by dendritic cells and antigen-specific anti-tumor immunity. *Nat. Biotechnol. 22:1546*.
42. Toura, I., T. Kawano, Y. Akutsu, T. Nakayama, T. Ochiai, and M. Taniguchi. 1999. Cutting edge: inhibition of experimental tumor metastasis by dendritic cells pulsed with α-galactosylceramide. *J. Immunol. 163:2387*.
43. Kawano, T., T. Nakayama, N. Kamada, Y. Kaneko, M. Harada, N. Ogura, Y. Akutsu, S. Motohashi, T. Iizasa, H. Endo, et al. 1999. Antitumor cytotoxicity mediated by ligand-activated human Vα24 NKT cells. *Cancer Res. 59:5102*.
44. Dhodapkar, K. M., B. Cirignano, F. Chamian, D. Zagzag, D. C. Miller, J. L. Finlay, and R. M. Steinman. 2004. Invariant natural killer T cells are preserved in patients with glioma and exhibit antitumor lytic activity following dendritic cell-mediated expansion. *Int. J. Cancer. 109:893*.