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Regulation of Cytokine-Driven Functional Differentiation of CD8 T Cells by Suppressor of Cytokine Signaling 1 Controls Autoimmunity and Preserves Their Proliferative Capacity toward Foreign Antigens

Sheela Ramanathan,*,†,[1] Stephanie Dubois,*,† Julien Gagnon,*,† Chantal Leblanc,*,† Sanjeev Mariathasan,‡ Gerardo Ferbeyre,§ Robert Rottapel,¶ Pamela S. Ohashi,¶ and Subburaj Ilangumaran*,†,[1]

We have previously shown that naive CD8 T cells exposed to IL-7 or IL-15 in the presence of IL-21 undergo Ag-independent proliferation with concomitant increase in TCR sensitivity. In this study, we examined whether CD8 T cells that accumulate in suppressor of cytokine signaling 1 (SOCS1)-deficient mice because of increased IL-15 signaling in vivo would respond to an autoantigen expressed at a very low level using a mouse model of autoimmune diabetes. In this model, P14 TCR transgenic CD8 T cells (P14 cells) adoptively transferred to rat insulin promoter-glycoprotein (RIP-GP) mice, which express the cognate Ag in the islets, do not induce diabetes unless the donor cells are stimulated by exogenous Ag. Surprisingly, SOCS1-deficient P14 cells, which expanded robustly following IL-15 stimulation, proliferated poorly in response to Ag and failed to cause diabetes in RIP-GP mice. SOCS1-deficient CD8 T cells expressing a polyclonal TCR repertoire also showed defective expansion following in vivo Ag stimulation. Notwithstanding the Ag-specific proliferation defect, SOCS1-null P14 cells produced IFN-γ and displayed potent cytolytic activity upon Ag stimulation, suggesting that SOCS1-null CD8 T cells underwent cytokine-driven functional differentiation that selectively compromised their proliferative response to Ag but not to cytokines. Cytokine-driven homeostatic expansion in lymphopenic RIP-GP mice allowed SOCS1-null, but not wild-type, P14 cells to exert their pathogenic potential even without Ag stimulation. These findings suggest that by attenuating cytokine-driven proliferation and functional differentiation, SOCS1 not only controls the pathogenicity of autoreactive cells but also preserves the ability of CD8 T cells to proliferate in response to Ags. ***The Journal of Immunology*****, 2010, 185: 357–366.**

CD8 T lymphocytes are implicated in the initiation, progression, and perpetuation of the pathogenic process and cause tissue damage in several autoimmune diseases (1–4). However, the cellular and molecular mechanisms that enable potentially autoreactive CD8 T cells to be activated by weak autoantigens remain unclear. Lymphopenia and chronic inflammatory conditions are known to promote autoimmune diseases (5–8). Lymphopenia induces homeostatic expansion of CD8 T lymphocytes driven by IL-15 and IL-7 (9, 10). IL-21, a proinflammatory cytokine produced by NKT and Th17 cells (11, 12), has been implicated in the homeostatic expansion of CD8 T cells in the NOD mouse model of type 1 autoimmune diabetes (T1D) (13). Genetic ablation of the IL-21R chain IL-21Rα protected the NOD mouse from disease development, which was attributed to defective Th17 response and inefficient T cell infiltration of the pancreas (14, 15).

IL-21 can synergize with IL-7 or IL-15 to stimulate Ag-independent proliferation of naive CD8 T cells (16, 17). We have shown that naive CD8 T cells exposed to IL-21 in the presence of IL-7 or IL-15 display increased Ag sensitivity with increased proliferation and effector functions (18). We refer to the increased Ag responsiveness of cytokine-prestimulated CD8 T cells as "cytokine priming" (19). In the current study, we investigated whether synergistic stimulation by homeostatic and inflammatory cytokines in vivo would lead to the triggering of autoreactive CD8 T cells by weak autoantigens. For this purpose, we used *Socs1*$^{-/-}$ mice lacking suppressor of cytokine signaling 1 (SOCS1) that are rescued from IFN-γ–induced perinatal lethality (20, 21). These mice display an enlarged T cell compartment with selective accumulation of CD8 T cells that display CD44^{hi}CD122^{hi} memory-like phenotype, resulting from deregulated homeostatic proliferation driven by IL-15 (22–24). SOCS1 is strongly induced in CD8 T cells following stimulation with IL-7, IL-15, or IL-21

*Immunology Division, Department of Pediatrics, Faculty of Medicine and Health Sciences, University of Sherbrooke and †Centre de Recherche Clinique Etienne-Le Bel, Centre Hospitalier de l'Université de Sherbrooke, Sherbrooke; §Department of Biochemistry, University of Montreal, Montreal, Quebec; ¶Ontario Cancer Institute, University Health Network, Toronto, Ontario, Canada; and ‡Department of Translational Oncology, Genentech, South San Francisco, CA 94080

[1]S.R and S.I. share senior authorship.

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Address correspondence and reprint requests to Dr. Subburaj Ilangumaran and Dr. Sheela Ramanathan, Immunology Division, Department of Pediatrics, Faculty of Medicine and Health Sciences, University of Sherbrooke, 3001 North 12th Avenue, Sherbrooke, Quebec J1H 5N4, Canada. E-mail addresses: Subburaj.Ilangumaran@Usherbrooke.ca and Sheela.Ramanathan@Usherbrooke.ca

Abbreviations used in this paper: AINR, Ag-induced nonresponsiveness; BLN, brachial lymph node; GP, glycoprotein; ILN, inguinal lymph node; LCMV, lymphocytic choriomeningitis virus; MLN, mesenteric lymph node; n.a., not applicable; P14 cell, CD8 T cell expressing the transgenic P14 TCR; poly(I:C), polyinosinic:polycytidylic acid; RIP, rat insulin promoter; SOCS1, suppressor of cytokine signaling 1; T1D, autoimmune type 1 diabetes.

(17). In vitro, SOCS1-deficient CD8 T cells show increased Ag-independent proliferation stimulated by IL-15 in the presence of IL-21 (17). A variety of innate immune stimuli induce the expression of IL-15 in macrophages and dendritic cells and IL-21 in NKT cells (12, 25). SOCS1-deficient macrophages, dendritic cells, and NKT cells have been shown to respond strongly to agents that stimulate the innate immune response (26–29). Therefore, we hypothesized that, in SOCS1-deficient mice, increased responsiveness to synergistic stimulation by endogenous IL-15 and IL-21 might lower the TCR signaling threshold presumably via cytokine priming and trigger potentially autoreactive CD8 T cells.

To test the above hypothesis, we generated P14 TCR transgenic mice lacking SOCS1 (*Socs1*$^{-/-}$ P14 mice). CD8 T cells bearing the transgenic P14 TCR (P14 cells) recognize the gp33 peptide, spanning aas 33–41 of the glycoprotein (GP) Ag of lymphocytic choriomeningitis virus (LCMV), restricted by the MHC class I molecule H-2D^{b} (30). Transgenic mice expressing the LCMV GP Ag under the rat insulin promoter ([RIP]-GP mice) are widely used to study cellular mechanisms underlying the activation of autoreactive CD8 T cells (30–34). We have chosen the RIP-GP/P14 model of T1D because, unlike the RIP-mOVA/OT-I system, the P14 cells are "ignorant" of the transgenic LCMV GP because of the low-level expression of the Ag (23, 34). Because of this ignorance, RIP-GP/P14 double-transgenic mice or RIP-GP mice with adoptively transferred P14 cells do not develop disease without LCMV infection or gp33 immunization with concomitant activation of dendritic cells or induction of an inflammatory response (31–33). We have substituted peptide immunization with in vitro stimulation of P14 cells with gp33 prior to adoptive transfer to induce T1D in the RIP-GP mice. We reasoned that SOCS1-deficient P14 cells, exposed to the stimulatory cytokine milieu in vivo, would show increased TCR sensitivity that would allow them to recognize the limiting amounts of gp33 in the islets and cause diabetes without peptide immunization or prior in vitro stimulation by gp33. Contrary to this expectation, SOCS1-deficient P14 cells failed to induce diabetes even after peptide stimulation. We show that in the absence of SOCS1, cytokine-driven functional differentiation compromises the Ag-induced proliferation of CD8 T cells. However, these cells become pathogenic following lympohopenia-driven homeostatic expansion.

Materials and Methods

Mice

Socs1$^{+/-}$*Ifng*$^{-/-}$ mice (20), obtained from Dr. J. Ihle (St. Jude Children's Research Hospital, Memphis, TN), were backcrossed to *Ifng*$^{-/-}$ mice in C57BL/6 background (The Jackson Laboratory, Bar Harbor, ME) for >10 generations in our animal facility. RIP-GP and P14 TCR transgenic mice (30) were bred in our animal facility to generate RIP-GP/P14 mice. *Ifngra*$^{-/-}$ and *Rag1*$^{-/-}$ mice in C57BL/6 background and pmel-1 TCR transgenic mice (35) were purchased from The Jackson Laboratory. *Socs1*$^{-/-}$ P14, *Socs1*$^{-/-}$*Ifng*$^{-/-}$P14, *Socs1*$^{-/-}$*Ifngra*$^{-/-}$P14, *Socs1*$^{-/-}$pmel-1, and *Rag1*$^{-/-}$RIP-GP mice were generated in our animal facility. All experiments were carried out with the approval of institutional ethical committee.

Abs and reagents

Abs against mouse CD4, CD8, CD16/CD32, CD25, CD44, CD62L, CD122, TCR vα2 (recognizes the P14 TCR), Ly6C, CD28, OX-40, 4-1BB, GITR, CTLA-4, and programmed death-1 conjugated to FITC, PE, or biotin were purchased from BD Pharmingen Biosciences (Palo Alto, CA) or eBioscience (San Diego, CA). Streptavidin-spectral red was from Southern Biotechnology Associates (Birmingham, AL). Recombinant cytokines IL-2, IL-7, IL-15, and IL-21 were purchased from R&D Systems (Minneapolis, MN). Polyinosinic:polycytidylic acid [poly(I:C)] was obtained from InvivoGen (San Diego, CA). RPMI 1640 cell culture medium and FBS were from Sigma-Aldrich (Oakville, Ontario, Canada). CFSE was purchased from Molecular Probes (Eugene, OR). Antigenic peptides were custom synthesized to >95% purity by GenScript (Scotch Plains, NJ).

Flow cytometry and magnetic cell sorting

Single-cell suspensions in PBS containing 5% FBS and 0.05% sodium azide were preincubated with anti-CD16/CD32 Ab for 10 min to block FcRs. Expression of cell surface markers was estimated by standard two- or three-color staining using FITC-, PE-, and biotin-conjugated primary Abs, followed by streptavidin-spectral red. Data acquisition and analysis were done on a FACSCalibur using CellQuest software (BD Biosciences, San Jose, CA). CD8^{+} T cells were purified using Dynal mouse CD8 negative isolation kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions to 95–98% purity (18).

Induction of T1D

A total of 5×10^{6} unstimulated or 3×10^{6} peptide-stimulated P14 cells were injected i.v. via the tail vein of RIP-GP or *Rag1*$^{-/-}$RIP-GP recipients along with 200 μg poly(I:C), unless indicated otherwise. Blood glucose levels were monitored using Accu-Check strips (Roche Diagnostic Systems, Somerville, NJ). Animals that showed >15 mM glucose consecutively for 3 d were considered diabetic. At sacrifice, pancreatic tissues were frozen in Tissue-Tek OCT for immunohistochemistry. Animals that did not develop diabetes were sacrificed 4 wk after adoptive cell transfer.

Cell proliferation

Total lymph node cells (1×10^{5} cells/well), or purified CD8 T cells (2.5×10^{4} cells/well) were stimulated in 96-well culture plates in 200 μl RPMI 1640 medium with indicated concentrations of cytokines, Dynabeads Mouse T activator CD3/CD28, or antigenic peptides. Irradiated splenocytes (10^{5} cells/well) from C57BL/6 mice were used as APCs to stimulate purified P14 cells with Ag. One microcurie of [methyl-^{3}H]thymidine (NEN Life Sciences, Boston, MA) was added per well during the last 8 h of culture. The cells were harvested onto glass fiber filter mats 2 d after Ag stimulation or 3 d after cytokine stimulation. The incorporated radioactivity was measured in a Top Count microplate scintillation counter (PerkinElmer, Wellesley, MA).

Homeostatic proliferation

Total splenocytes from mice of the indicated genotypes were labeled with CFSE as described elsewhere (24), and 10×10^{6} cells in 200 μl PBS were injected i.v. into 6- to 8-wk-old *Rag1*$^{-/-}$ mice. After 5 d, LN cells from the recipients were stained for CD8, and homeostatic proliferation was examined by dilution of CFSE within the CD8 gate.

LCMV infection

Socs1$^{-/-}$*Ifng*$^{-/-}$, *Ifng*$^{-/-}$, and C57BL/6 mice were infected with 2×10^{4} PFU of LCMV Armstrong strain by the i.p. route. On days 6 and 8, total splenocytes were stained for CD4 and CD8 markers and analyzed by flow cytometry.

ELISA

The amounts of IFN-γ and IL-2 in the culture supernatants were determined by sandwich ELISA using Ab pairs purchased from BD Pharmingen Biosciences (Palo Alto, CA).

CTL assay

EL-4 cells (H-2^{b}) were prepared by incubation with 400 μCi/ml ^{51}Cr (NEN Life Sciences, Boston, MA) and the stimulatory gp33 (KAVYNFATM) or nonstimulatory AV (SGPSNTPPEI) peptide for 2 h at 37°C. These target cells were washed and plated in 96-well round-bottomed microtiter plates with the indicated effector P14 cells at different E:T cell ratios. After incubation for 7 h at 37°C, 100 μl supernatant was counted in a gamma counter (PerkinElmer). Specific lysis was calculated as detailed previously (18).

Histopathology

Serial 5-μm cryosections of the frozen pancreatic tissues were stained with H&E or subjected to immunohistochemical staining. The sections were incubated with Abs against insulin (Abcam, Cambridge, MA), followed by HRP-conjugated secondary Abs. Diaminobenzamidine was used as substrate chromogen to reveal damage to β cells in the islets.

Statistical analysis

The *p* values were determined by Student *t* test.

Results

SOCS1-deficient P14 cells fail to induce diabetes in RIP-GP mice

Similar to *Socs1*$^{-/-}$ mice bearing a polyclonal TCR repertoire (36, 37), *Socs1*$^{-/-}$ P14 pups showed accelerated thymocyte maturation characterized by the depletion of CD4^{+}CD8^{+} double-positive cells (11 versus 58%) with an increased frequency of CD8 single-positive cells (71 versus 27%) in the thymus (Fig. 1*A*). However, the frequency of CD8^{+} cells in the peripheral lymph nodes was comparable between SOCS1-deficient and control mice (Fig. 1*B*). Even though lymph nodes in SOCS1-deficient P14 mice harbored slightly more CD8^{+} cells bearing the transgenic TCR Vα2 TCR than in control mice, this difference was not statistically significant (Fig. 1*C*). We and others have shown that CD8^{+} T cells in SOCS1-deficient mice bearing a polyclonal TCR repertoire expressed higher levels of the memory cell markers CD44, CD122, and Ly6C, without downmodulating the activation marker CD62L (22, 38), a phenotype typical of memory-like cells arising from cytokine-driven homeostatic proliferation (39). Likewise, P14 cells isolated from the lymph nodes of *Socs1*$^{-/-}$ P14 cells displayed a memory-like phenotype (Fig. 1*D*). Consistent with the reports that SOCS1 is a critical regulator of IL-15 signaling in CD8^{+} T cells (17, 22, 38), *Socs1*$^{-/-}$ P14 cells showed increased proliferation in response to IL-15, which was further augmented by IL-21 (Fig. 1*E*).

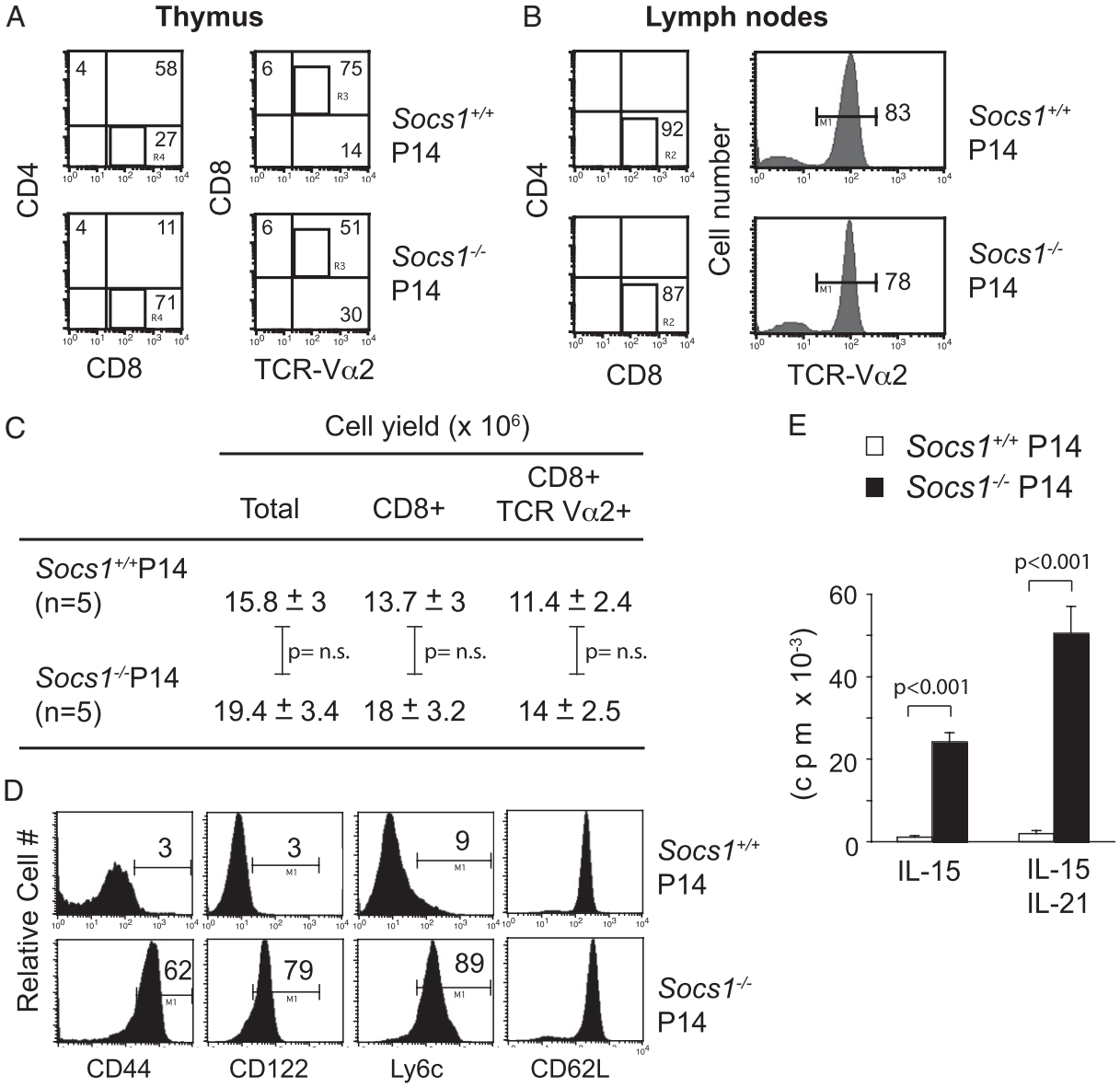

FIGURE 1. SOCS1-deficient P14 cells memory-like phenotype and proliferate robustly to IL-15 stimulation. Total thymocytes (*A*) and lymph node cells (*B*) from 10- to 15-d-old *Socs1*$^{-/-}$ P14 mice and *Socs1*$^{+/+}$ P14 control mice were stained for CD4, CD8, and TCR Vα2 and evaluated by flow cytometry. The frequency of CD4, CD8, and Vα2 TCR^{+} cells are indicated. *C*, Pooled brachial, inguinal, cervical, and mesenteric lymph nodes from individual mice were counted to obtain total cell yield, which was used to calculate the number of CD8^{+} and CD8^{+}TCRVα2^{+} T cells. The cell numbers (mean ± SD) were compared by Student *t* test. *D*, Typical phenotypic profile for the expression of CD44, CD62L, CD122, and Ly-6C on gated CD8^{+} lymph node cells is shown. Numbers indicate the proportion of cells within the marker boundaries. *E*, *Socs1*$^{-/-}$ P14 CD8 T cells respond strongly to cytokine stimulation. Total lymph node cells from 2- to 3-wk-old *Socs1*$^{-/-}$ P14 or *Socs1*$^{+/+}$ P14 mice were stimulated with IL-15 (5 ng/ml), either alone or in the presence of IL-21 (10 ng/ml) for 3 d. Cell proliferation was evaluated by [^{3}H]thymidine incorporation. Unstimulated cells from SOCS1-deficient and control cells incorporated <200 cpm of radioactivity.

Like *Socs1*$^{-/-}$ mice bearing a polyclonal TCR repertoire (20, 21), *Socs1*$^{-/-}$ P14 mice became sick by 3 wk of age and died within 4 wk after birth, which was prevented by ablation of the *Ifng* gene. We generated *Socs1*$^{-/-}$*Ifng*$^{-/-}$ RIP-GP/P14 mice to determine whether SOCS1 deficiency would overcome the "ignorance" of P14 cells as a consequence of increased cytokine stimulation and enable them to recognize the LCMV GP Ag expressed at a low level in the islets. P14 cells from *Socs1*$^{-/-}$*Ifng*$^{-/-}$ RIP-GP/P14 mice also showed a memory-like phenotype and increased cytokine-induced proliferation (data not shown) but did not cause diabetes. These results indicated that increased cytokine stimulation as a result of SOCS1 deficiency was not sufficient to overcome the requirement for IFN-γ to induce disease in the RIP-GP model, although IFN-γ is dispensable in the NOD mouse model (40, 41). To circumvent this limitation, we adoptively transferred *Socs1*$^{-/-}$ P14 cells harboring the wild-type *Ifng* gene into RIP-GP recipients.

Adoptively transferred P14 cells cause diabetes in RIP-GP mice following immunization with gp33 peptide and concomitant administration of poly(I:C) to induce inflammation (30, 31, 33). Instead of peptide immunization, we stimulated P14 cells with gp33 for 2 d in vitro and adoptively transferred these cells to RIP-GP mice with concomitant administration of poly(I:C). This method rapidly induced diabetes in all RIP-GP recipients within 5 d (Fig. 2*A*, 2*B*, Table I, groups A1 and A2). We transferred *Socs1*$^{-/-}$ P14 cells to RIP-GP mice without prior stimulation by gp33 to test whether cytokine priming that might have occurred in the donors would overcome the requirement for ex vivo Ag stimulation. *Socs1*$^{-/-}$ P14 cells did not induce T1D in RIP-GP mice for up to 4 wk after adoptive transfer (Table I, group A3). As control, we stimulated *Socs1*$^{-/-}$ P14 cells with Ag before adoptive transfer. In contrast to cytokine stimulation, expansion of *Socs1*$^{-/-}$P14 cells following Ag stimulation was inefficient (Fig. 3*A*), and these cells failed to cause disease for up to 4 wk after adoptive transfer (Table I, group A4).

SOCS1 deficiency impairs Ag-induced proliferation of P14 cells

To test whether the inability of *Socs1*$^{-/-}$ P14 cells to cause diabetes in RIP-GP mice might have resulted from their impaired Ag responsiveness, we evaluated the proliferation of P14 cells from *Socs1*$^{-/-}$ and control P14 mice following TCR stimulation. Compared with wild-type cells, *Socs1*$^{-/-}$ P14 cells displayed dramatically reduced proliferation to gp33 peptide (Fig. 3*B*) and negligible response to anti-CD3/CD28 beads (Fig. 3*C*). These results showed that SOCS1 deficiency selectively impaired the proliferation of P14 cells in response to TCR stimulation, even though these cells proliferated robustly in response to cytokine stimulation (Fig. 3*A*). P14 cells from *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice also showed significantly reduced proliferation in response to gp33 (Fig. 3*D*), suggesting that the impaired TCR responsiveness of SOCS1-deficient P14 cells was not secondary to the inflammatory milieu caused by deregulated IFN-γ signaling in *Socs1*$^{-/-}$ P14 mice.

To determine whether the impaired Ag responsiveness of *Socs1*$^{-/-}$ P14 cells is a cell intrinsic defect or a sequel to the functional alterations of other cell types in vivo, we purified CD8 T cells from *Socs1*$^{-/-}$ P14 mice and evaluated their proliferation in response to gp33 peptide presented by irradiated C57BL/6 splenocytes or soluble anti-CD3ε Ab cross-linked by normal APCs. *Socs1*$^{-/-}$ P14 cells failed to proliferate under these conditions as well (Fig. 4*A*). In parallel, we expanded *Socs1*$^{-/-}$ P14 cells using IL-15 and IL-21 before stimulation with gp33 peptide presented by normal APCs. Again, proliferation of cytokine-expanded *Socs1*$^{-/-}$ P14 cells remained low at all concentrations of the antigenic peptide (Fig. 4*B*). These results showed that SOCS1 deficiency in P14 cells caused a stable, cell-intrinsic functional alteration that selectively impaired cell proliferation in response to TCR stimulation.

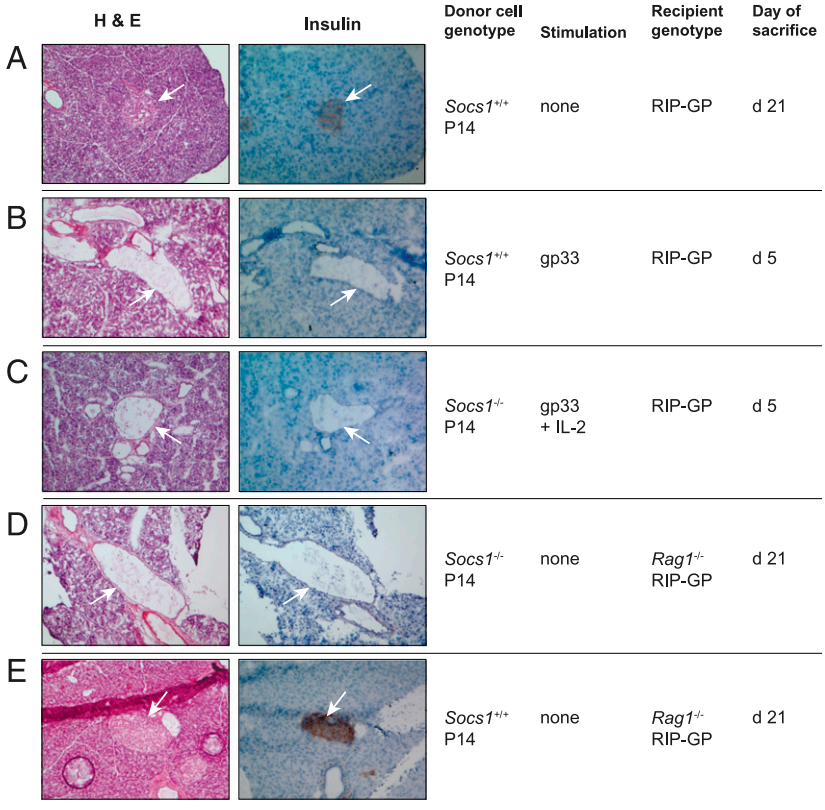

FIGURE 2. Induction of diabetes by SOCS1-deficient P14 cells in RIP-GP recipients. Serial 5-μm sections of the frozen pancreatic tissues from RIP-GP recipients that were adoptively transferred with unstimulated (*A*) or gp33-stimulated (*B*) P14 cells were stained with H&E or anti-insulin Ab as indicated. Islets were damaged in *B* with loss of insulin staining but not in *A*. Arrows point to the intact or damaged islets (original magnification ×16). RIP-GP recipients that received *Socs1*$^{-/-}$ P14 cells activated with gp33 in the presence of IL-2 lost the insulin-producing cells in the islets (*C*). *Socs1*$^{-/-}$ P14 cells adoptively transferred to *Rag1*$^{-/-}$ RIP-GP recipients destroyed the insulin-producing cells after 3 wk (*D*), whereas control cells did not damage the islets in the same genotype recipients (*E*).

Table I. *Induction of diabetes in RIP-GP recipients by SOCS1-deficient P14 cells*

Expt. Group	Genotype of Donor Cells	In Vitro Stimulation (2 days)	Genotype of Recipient Mice	Poly(I:C)	Disease Onset (d)	Diabetes Incidence (*n*)	Observation Period (d)
A							
1	P14 TCR Tg	None	RIP-GP	Yes	n.a.	0/15	28
2	P14 TCR Tg	gp33	RIP-GP	Yes	5 ± 0	24/24	—
3	*Socs1*$^{-/-}$ P14 TCR Tg	None	RIP-GP	Yes	n.a.	0/6	28
4	*Socs1*$^{-/-}$ P14 TCR Tg	gp33	RIP-GP	Yes	n.a.	0/6	28
B							
1	*Socs1*$^{-/-}$ P14 TCR Tg	gp33 + IL-2	RIP-GP	Yes	5 ± 0	6/6	—
2	*Socs1*$^{-/-}$ P14 TCR Tg	IL-15 + IL-21	RIP-GP	Yes	n.a.	0/6	28
C							
1	*Socs1*$^{-/-}$ P14 TCR Tg	None	*Rag1*$^{-/-}$ RIP-GP	Yes	21 ± 2	7/7	—
2	P14 TCR Tg	None	*Rag1*$^{-/-}$ RIP-GP	Yes	n.a.	0/6	28
3	P14 TCR Tg	gp33	*Rag1*$^{-/-}$ RIP-GP	Yes	5 ± 0	12/12	—
4	*Socs1*$^{-/-}$ P14 TCR Tg	None	*Rag1*$^{-/-}$ RIP-GP	No	21 ± 2	4/4	—

P14 cells of the indicated genotype, either freshly isolated (5×10^6 cells) or following in vitro stimulation with 0.1 μg/ml gp33 peptide for 2 d (3×10^6 cells), were injected into T cell-replete or lymphopenic RIP-GP recipients. All mice received 200 μg poly(I:C) i.v. at the time of cell transfer, except in one experiment (C4), as indicated. The recipient mice were followed for the onset of diabetes by testing the blood glucose level twice a week for up to 4 wk. Animals that showed >15 mM blood glucose were tested consecutively for 3 d before euthanasia. Mean ± SD of disease onset is given. Total number of animals for each data set was pooled from two to six independent experiments.
n.a., not applicable.

Exogenous IL-2 restores the Ag-induced proliferation in SOCS1-deficient P14 cells

Evaluation of IL-2 production following TCR stimulation showed that *Socs1*$^{-/-}$ P14 cells failed to produce significant quantities of IL-2 (Fig. 5*A*). Therefore, we examined whether P14 cells isolated from *Socs1*$^{-/-}$ mice were in a state similar to anergy (42, 43) by adding exogenous IL-2 to *Socs1*$^{-/-}$ and control P14 cells stimulated with gp33. As shown in Fig. 5*B*, addition of IL-2 completely restored the gp33-induced proliferation in *Socs1*$^{-/-}$ P14 cells, with a 10-fold increase in Ag sensitivity (Fig. 5*B*). IL-2 alone did not induce any detectable proliferation

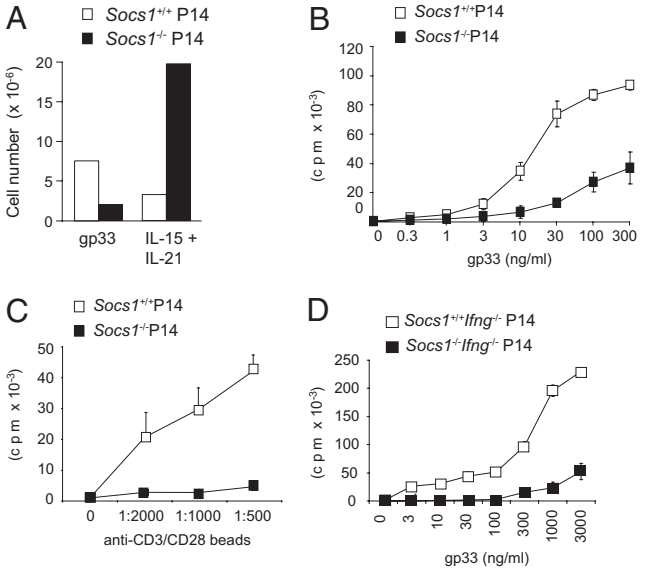

FIGURE 3. SOCS1-deficient P14 cells proliferate poorly in response to TCR stimulation. *A*, Cytokines, but not Ag stimulation, efficiently expand *Socs1*$^{-/-}$ P14 T cells. Lymph node cells from 2- to 3-wk-old *Socs1*$^{-/-}$ P14 and *Socs1*$^{+/+}$ P14 control mice were cultured in the presence of Ag or the cytokines IL-15 and/or IL-21, which were replenished on day 3. On day 5, the cells were counted and stained with Abs against CD8. As shown previously (17), >90% of cells recovered from cultures containing IL-15 and IL-21 was CD8$^+$. *B* and *C*, Lymph node cells from 2- to 3-wk-old *Socs1*$^{-/-}$ and control P14 mice were stimulated with the indicated concentrations of gp33 antigenic peptide (*C*) or Dynabeads coated with anti-CD3ε and anti-CD28 mAb (*D*) for 2 d. Cell proliferation was evaluated by [^{3}H]thymidine incorporation. *D*, Impaired TCR responsiveness of *Socs1*$^{-/-}$ P14 cells does not result from dysregulated IFN-γ signaling. Lymph node cells from *Socs1*$^{-/-}$*Ifng*$^{-/-}$ P14 mice were stimulated with gp33 peptide, and proliferation was measured after 2 d. Representative data from at least three separate experiments are shown for each assay.

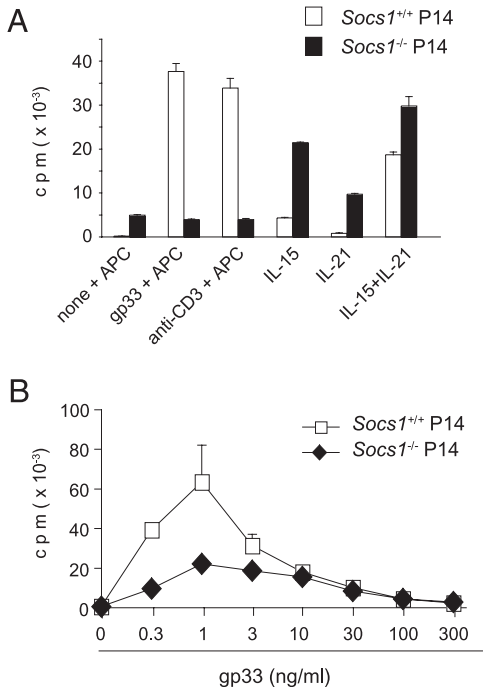

FIGURE 4. Impaired TCR responsiveness of *Socs1*$^{-/-}$ P14 cells is a T cell intrinsic defect. *A*, APCs from *Socs1*$^{+/+}$ mice did not reverse the unresponsiveness of *Socs1*$^{-/-}$ P14 cells to TCR stimulation. CD8 T cells purified from *Socs1*$^{-/-}$ P14 and control mice by negative magnetic selection were stimulated with gp33 peptide or soluble anti-CD3ε in the presence of irradiated splenocytes from C57BL/6 mice as APC for 2 d. For comparison, the P14 cells were stimulated with cytokines alone. Cell proliferation was measured by [^{3}H]thymidine incorporation. *B*, Prior expansion by cytokines does not reverse the unresponsiveness of SOCS1-deficient P14 cells to Ag. SOCS1-deficient and control P14 T cells, expanded in vitro using IL-15 and IL-21 as indicated in Fig. 3*A*, were purified and stimulated with the indicated concentrations of gp33 peptide in the presence of irradiated C57BL/6 splenocytes. Cell proliferation was evaluated on day 2. Representative data from three separate experiments are shown.

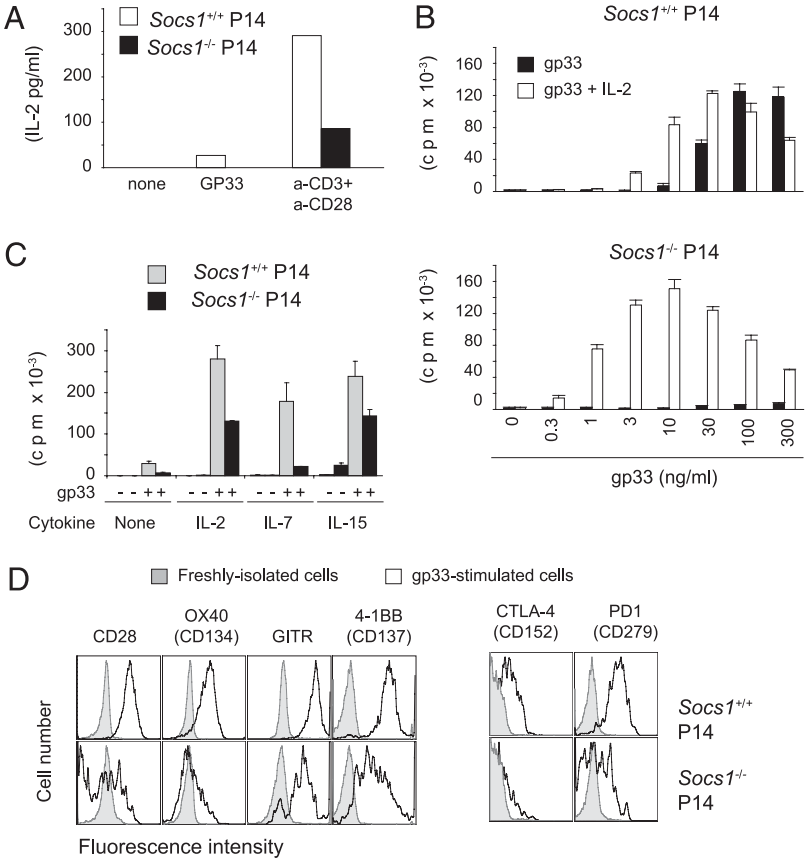

FIGURE 5. Impaired IL-2 production underlies the defective proliferation of SOCS1-deficient P14 cells in response TCR stimulation. *A*, *Socs1*$^{-/-}$ P14 cells are defective in IL-2 production following TCR stimulation. Lymph node cells from *Socs1*$^{-/-}$ P14 and control mice were stimulated with gp33 peptide or Dynabeads coated with anti-CD3 and anti-CD28 mAb. IL-2 was measured in the culture supernatants after 2 d. *B*, Exogenous IL-2 restores the responsiveness of *Socs1*$^{-/-}$ P14 cells to gp33 peptide and lowers the Ag threshold for activation. Lymph node cells from control (*upper panel*) and *Socs1*$^{-/-}$ (*lower panel*) P14 mice were stimulated with the indicated concentrations of gp33 peptide in the absence or presence of 5 ng/ml IL-2. Cell proliferation was measured after 2 d. *C*, IL-15 can substitute for IL-2 in reversing the TCR unresponsiveness of *Socs1*$^{-/-}$ P14 cells. CD8 T cells purified from *Socs1*$^{-/-}$ and control P14 mice were stimulated with 300 ng/ml gp33 peptide presented by irradiated C57BL/6 splenocytes, in the absence or presence of the indicated cytokines for 2 d, and cell proliferation was measured. *D*, SOCS1 deficiency does not modulate the expression of costimulatory or inhibitory receptors. Lymph node cells from *Socs1*$^{-/-}$ and control P14 mice were stained for the indicated costimulatory molecules and inhibitory receptors, and their expression levels on gated CD8 T cells are shown. At the same time, lymph node cells from *Socs1*$^{-/-}$ and control P14 mice stimulated for 3 d with gp33 peptide Ag were evaluated. Data shown are representative of at least three independent experiments.

of *Socs1*$^{-/-}$ P14 cells (Fig. 5*C*). We also observed that IL-15, but not IL-7, was able to substitute for IL-2 in reversing the Ag-specific proliferation defect in *Socs1*$^{-/-}$ P14 cells (Fig. 5*C*). These results demonstrate that SOCS1 deficiency compromises the ability of P14 cells to produce IL-2 following TCR stimulation and impairs their proliferation, a defect that can be reversed by exogenous IL-2 or IL-15.

The Ag-specific proliferation defect of SOCS1-deficient P14 cells to TCR stimulation did not result from the lack of costimulatory receptors (CD28, OX40, GITR, and 4-1BB) or from the induction of inhibitory receptors (CTLA-4 and programmed death-1) (44–46) (Fig. 5*D*). However, upregulation of these costimulatory and inhibitory receptors following Ag stimulation was impaired to variable extent in *Socs1*$^{-/-}$ P14 cells, presumably as a consequence of defective Ag responsiveness.

SOCS1-deficiency compromises Ag-induced proliferation of CD8 T cells independently of the TCR specificity

The above experiments suggested that naive CD8 T cells require SOCS1 to preserve their ability to proliferate in response to cognate Ags, which was supported by our observation on CD8 T cells with a different TCR specificity. *Socs1*$^{-/-}$ CD8 T cells expressing the pmel-1 transgenic TCR specific to the melanoma-specific gp100 Ag (35) also showed defective proliferation to peptide stimulation despite showing robust cytokine-induced proliferation (Fig. 6*A*). By comparing the proliferation of adoptively transferred CD8 T cells in congenitally T cell-deficient mice raised in conventional or germ-free facility, Kieper et al. (47, 48) defined two types of homeostatic proliferation. Whereas proliferation driven by self-Ags and homeostatic cytokines occurred slowly in both recipients, only the congenitally T cell-deficient mice raised in conventional facility supported a faster rate of proliferation. The faster homeostatic proliferation was presumably driven by gut-derived Ags, which were absent in germ-free animals. To determine how SOCS1 deficiency influenced the Ag-dependent homeostatic proliferation in vivo, we labeled P14 cells from *Socs1*$^{-/-}$ or control mice with CFSE and adoptively transferred the cells to *Rag1*$^{-/-}$ RIP-GP recipients. Five days after transfer, we evaluated homeostatic proliferation of *Socs1*$^{-/-}$ and control P14 cells in mesenteric or pooled inguinal and brachial lymph nodes of *Rag1*$^{-/-}$ RIP-GP mice. We observed that the Ag-driven rapid homeostatic proliferation was severely impaired in *Socs1*$^{-/-}$ P14 cells (Fig. 6*B*, peaks on the left side of the dotted line). In contrast, these cells displayed strong cytokine-dependent homeostatic proliferation compared with control cells in inguinal and brachial lymph nodes, although this increase was less pronounced in mesenteric lymph nodes (Fig. 6*B*, peaks on the right side of the dotted line). These results showed that SOCS1 deficiency compromised the proliferation of CD8 T cells induced by cross-reactive foreign Ags in vivo.

To examine whether SOCS1 was also needed to preserve the Ag-induced proliferation of CD8 T cells with a polyclonal TCR specificity, we infected *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice and the control animals with LCMV and evaluated the expansion of the CD8 T cell pool after 6 and 8 d. In C57BL/6 and *Ifng*$^{-/-}$ control mice, LCMV infection elicited a 3-fold increase in expansion of the CD8 T cell compartment with a negligible change in the CD4 compartment on day 8 following infection (Fig. 6*C*). In contrast, the frequency of CD8 T cells decreased in *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice following LCMV infection, and this decrease was not due to accelerated kinetics of expansion (Fig. 6*C*). In agreement with the requirement for IFN-γ signaling for efficient expansion of CD8 T cells following LCMV infection (49), spleens from *Ifng*$^{-/-}$ mice harbored significantly less number of CD8 T cells compared with C57BL/6 controls 8 d postinfection. LCMV-induced expansion of

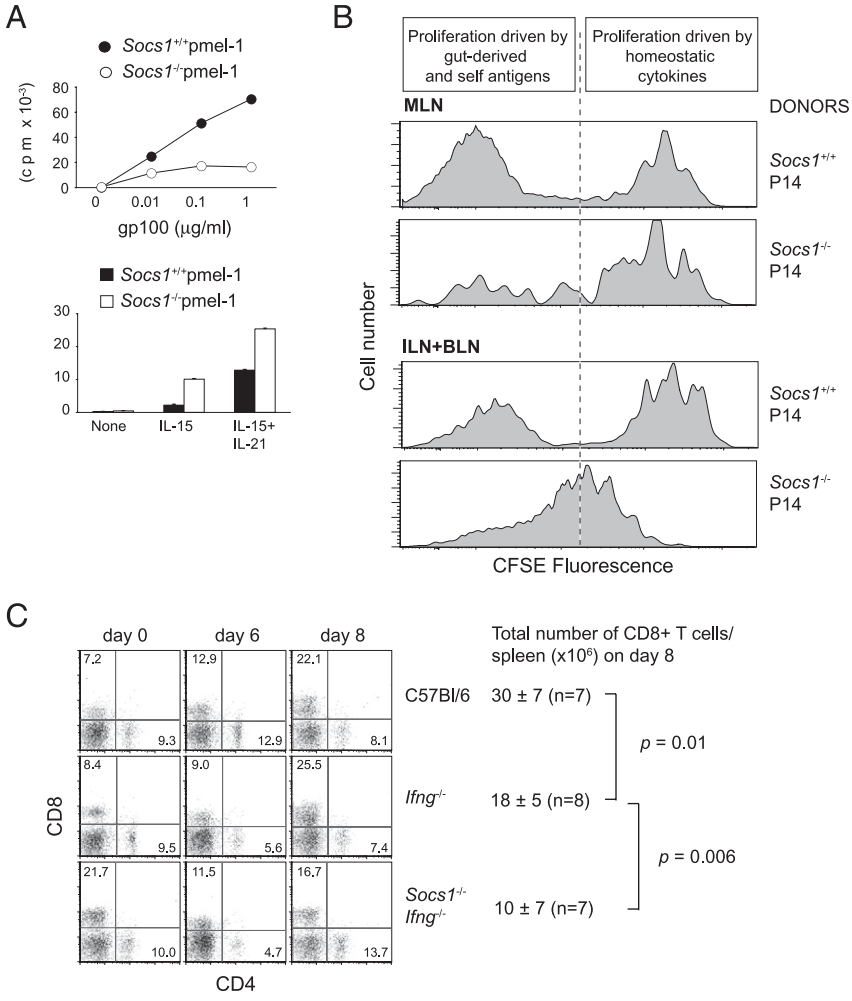

FIGURE 6. SOCS1 deficiency impairs the Ag-induced proliferation of CD8 T cells independently of their TCR specificity. *A*, Splenocytes from *Socs1*$^{-/-}$ or control pmel-1 TCR transgenic mice were stimulated with gp100 peptide for 2 d (*left panel*) or the indicated cytokines for 3 d (*right panel*). Cell proliferation was evaluated by [^{3}H]thymidine incorporation. *B*, SOCS1 deficiency impairs Ag-induced proliferation of P14 cells in vivo. Purified *Socs1*$^{-/-}$ and control P14 cells were labeled with CFSE and 10×10^6 cells were adoptively transferred to *Rag1*$^{-/-}$ RIP-GP mice. After 5 d, proliferation of gated CD8 T cells in MLNs and pooled ILNs and BLNs of the recipient mice was evaluated by flow cytometry. The two types of homeostatic proliferation, one driven by cytokines and another driven by gut-derived environmental Ags (47, 48), are separated by the hypothetical dotted line, based respectively on slower (right side of the dotted line) and faster (left side of the dotted line) rates of CFSE dilution observed in wild-type donor cells. Results from one of the two similar experiments are shown. *C*, Defective expansion of SOCS1-deficient CD8 T cells following LCMV infection. *Socs1*$^{-/-}$*Ifng*$^{-/-}$, *Ifng*$^{-/-}$, and C57BL/6 mice were infected with LCMV. On days 6 and 8, expansion of the CD8 T cell compartment in the spleen was evaluated by flow cytometry. Representative data from three independent experiments are shown. Total number of CD8 T cells was calculated from the frequency, and the mean ± SD values were compared by Student *t* test. BLN, brachial lymph node; ILN, inguinal lymph node; MLN, mesenteric lymph node.

CD8 T cells in *Socs1*$^{-/-}$*Ifng*$^{-/-}$ mice even reduced further and is significantly less when compared with *Ifng*$^{-/-}$ controls (Fig. 6*C*). Taken together, the above results demonstrate that SOCS1 is essential to sustain the proliferative capacity of CD8 T cells following Ag stimulation.

SOCS1 deficiency enhances the Ag-specific effector functions of P14 cells

In a previous report, Davey et al. (23) have shown that SOCS1-deficient OVA-specific OT-I TCR transgenic CD8 T cells induced diabetes rapidly and more efficiently than wild-type OT-I cells in RIP-mOVA recipients, which express OVA in a membrane-bound form in pancreatic β cells. Even though proliferation of *Socs1*$^{-/-}$ OT-I T cells in response to OVA was not evaluated in this study, evidently *Socs1*$^{-/-}$ OT-I T cells had mounted potent Ag-specific cytolytic activity against the pancreatic islets. Therefore, we examined whether gp33 stimulation elicited effector functions in *Socs1*$^{-/-}$ P14 T cells.

Following Ag stimulation, *Socs1*$^{-/-}$ P14 cells produced significantly more IFN-γ than SOCS1-expressing cells (Fig. 7*A*). Even more strikingly, *Socs1*$^{-/-}$ P14 cells efficiently lysed the gp33-pulsed targets even without TCR stimulation (Fig. 7*B*). The spontaneous lytic activity of *Socs1*$^{-/-}$ P14 cells was not a result of nonspecific CTL activity because they failed to kill target cells pulsed with the nonagonist AV peptide. Prior exposure to IL-15 and IL-21 induced the Ag-specific cytolytic activity in wild-type P14 cells, which was further augmented by SOCS1 deficiency, resulting in markedly enhanced cytolytic activity (Fig. 7*C*, *left panel*). Subsequent Ag stimulation with a suboptimal concentration of gp33 peptide further enhanced the Ag-specific CTL activity of cytokine-primed *Socs1*$^{-/-}$ P14 cells (Fig. 7*C*, *right panel*). These results demonstrated that SOCS1-deficient P14 cells, despite their inability to proliferate, deployed potent effector functions upon Ag stimulation.

Homeostatic expansion of SOCS1-deficient P14 cells in RIP-GP mice induces diabetes

Induction of strong effector functions in SOCS1-deficient P14 cells following Ag stimulation suggested that the inability of these cells to induce diabetes in RIP-GP recipients could be attributed to their impaired proliferative capacity. Therefore, we tested whether the diabetogenic potential of SOCS1-deficient cells could be elicited if their proliferative defect was overcome by exogenous IL-2. *Socs1*$^{-/-}$ P14 cells expanded in vitro by gp33 in the presence of IL-2 readily induced disease with the same efficiency as wild-type P14 cells stimulated with gp33 (Fig. 2*C*, Table I, group B1). The above results indicated that SOCS1-deficient P14 cells retained their functional capacity to respond to Ag but must undergo expansion to cause overt disease. However, *Socs1*$^{-/-}$ P14 cells stimulated in vitro with IL-15 and IL-21 alone did not induce diabetes in RIP-GP recipients (Table I, group B2). Next, we used *Rag1*$^{-/-}$ RIP-GP recipients to examine whether greater homeostatic expansion of *Socs1*$^{-/-}$ P14 cells induced by endogenous IL-15 in vivo (23, 24) would elicit their diabetogenic potential. Following adoptive transfer to *Rag1*$^{-/-}$ RIP-GP recipients, *Socs1*$^{-/-}$ P14 cells induced diabetes in all animals, albeit the disease onset was delayed to 3 wk (Fig. 2*D*, Table I, group C1). These results suggested that even though cytokine stimulation induced strong proliferation of

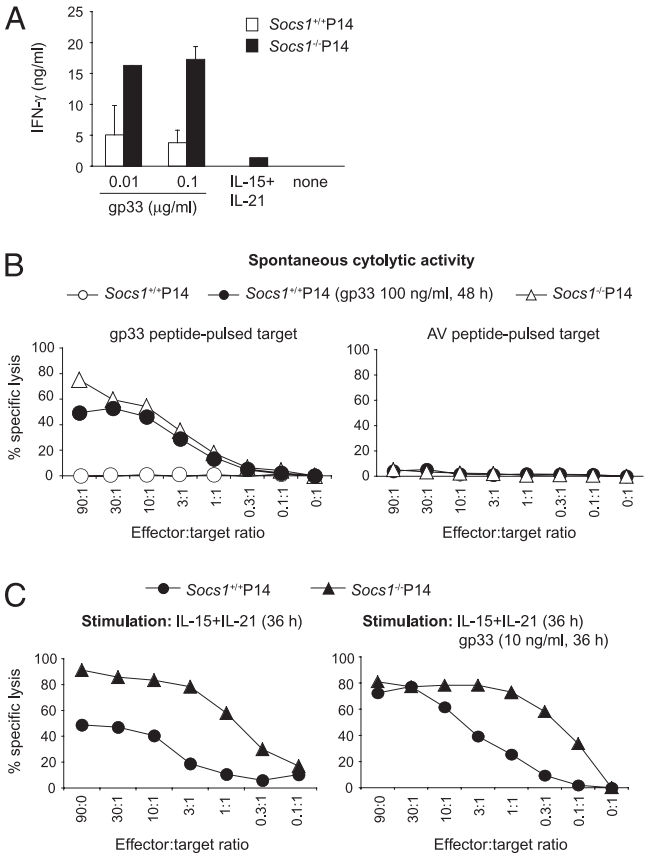

FIGURE 7. SOCS1-deficient P14 cells display potent Ag-specific effector functions. *A*, *Socs1*$^{-/-}$ P14 cells produce abundant IFN-γ following Ag stimulation. Lymph node cells from control and *Socs1*$^{-/-}$ P14 mice were stimulated with gp33 peptide or a combination of IL-15 and IL-21. IFN-γ was measured in the culture supernatant 2 d later. Mean ± SD from three separate experiments are shown. *B*, *Socs1*$^{-/-}$ P14 cells show potent Ag-specific cytolytic activity without prior Ag stimulation. *Socs1*$^{-/-}$ and control P14 cells, freshly purified by negative magnetic selection, were used as effector cells in the CTL assay with EL4 target cells loaded with the agonist peptide of the P14 TCR (gp33, *left panel*) or the nonagonist control peptide (AV, *right panel*) at different E:T cell ratios. As a positive control, SOCS1-sufficient P14 cells stimulated with gp33 peptide for 2 d were used as effector cells (●) in the same experiment. *C*, Cytokine stimulation enhances the Ag-specific cytolytic activity of *Socs1*$^{-/-}$ P14 cells. Lymph node cells from *Socs1*$^{-/-}$ and control P14 mice were stimulated with IL-15 and IL-21 for 36 h, and the CD8 cells were purified to test CTL activity against gp33-loaded EL4 targets (*left panel*). In the *right panel*, the cytokine-treated cells were stimulated with 10 ng/ml gp33 peptide in the presence of irradiated C57BL/6 splenocytes for 36 h and equalized for the number of P14 cells before the CTL assay. In both instances, nonspecific lysis of AV peptide-loaded EL4 targets was negligible (data not shown). Data shown in *B* and *C* are representative of at least three independent experiments, containing a total of five to six mice in each group.

Socs1$^{-/-}$ P14 cells with potent Ag-specific cytolytic activity, further in vivo expansion was required to induce diabetes. However, in vivo expansion of wild-type P14 cells in *Rag1*$^{-/-}$ RIP-GP mice did not induce diabetes (Fig. 2*E*, Table I, group C2), unless the donor cells were prestimulated with gp33 (Table I, group C3). These results suggested that the propensity of SOCS1-deficient P14 cells to develop effector functions upon cytokine stimulation was important to elicit their pathogenic potential. Surprisingly, T1D caused by homeostatic proliferation of *Socs1*$^{-/-}$ P14 cells in *Rag1*$^{-/-}$ RIP-GP mice did not require poly(I:C) (Table I, group C4), suggesting that the homeostatically expanded *Socs1*$^{-/-}$ P14 cells behaved like Ag-stimulated P14 cells following LCMV infection in their ability to cause T1D in lymphopenic recipients (30). These results showed that cytokine-driven homeostatic proliferation facilitates the expansion of *Socs1*$^{-/-}$ P14 cells in vivo and that these cells become cytolytic upon recognition of the gp33 Ag in the pancreatic β cells, leading to islet destruction.

LCMV infection of RIP-GP mice bearing a polyclonal T cell repertoire can activate gp33-specific CD8 T cells, resulting in islet destruction and induction of diabetes (30). The inefficient expansion of SOCS1-deficient CD8 T cells following LCMV infection (Fig. 6*C*) raised the possibility that SOCS1 deficiency would impair the LCMV-induced activation of gp33-specific CD8 T cells from a polyclonal repertoire. However, this issue was difficult to address directly because *Socs1*$^{-/-}$ RIP-GP mice failed to survive for >2 wk. Activation of SOCS1 knockout cells adoptively transferred to RIP-GP mice by LCMV infection or peptide immunization would be confounded by competition from gp33-specific CD8 cells of the recipient mice. Generating a bone marrow chimera with SOCS1-null cells in RIP-GP mice would lead to homeostatic expansion of SOCS1-deficient CD8 T cells leading to associated systemic pathology. Therefore, we addressed this issue indirectly following a published method (30). We infected *Socs1*$^{-/-}$*Ifngra*$^{-/-}$ mice bearing wild-type *Ifng* gene with LCMV to activate gp33-specific CD8 T cells from the polyclonal repertoire. One week postinfection, we adoptively transferred total splenocytes from these mice to *Rag1*$^{-/-}$ RIP-GP recipients. All the recipient mice developed diabetes ($n = 5$ from two experiments). This experiment indicated that even though CD8 T cells in SOCS1 knockout mice failed to undergo robust expansion following LCMV infection (Fig. 6*C*), gp33-specific cells were activated and presumably expanded to a limited extent, which, upon homeostatic expansion in *Rag1*$^{-/-}$ RIP-GP recipients, caused disease.

Discussion

In this study, we demonstrate that SOCS1 prevents the activation of potentially autoreactive CD8 T cells primarily by controlling the Ag-independent, cytokine-driven proliferation, and functional differentiation into effector cells. Our findings also demonstrate a nonredundant function for SOCS1 in preserving the ability of naive CD8 T cells to proliferate in response to Ag stimulation.

Although several studies have addressed the cytokine responses of SOCS1-deficient CD8 T cells in vitro and in vivo (17, 22–24), few studies have examined their response to Ags. Davey et al. (23) attributed the increased diabetogenic potential of *Socs1*$^{-/-}$ OT-I cells in RIP-mOVA mice to increased IL-15–driven proliferation. Because synergistic stimulation by IL-15 and IL-21 enhances the Ag sensitivity of CD8 T cells (18), it is likely that increased cytokine-induced proliferation of *Socs1*$^{-/-}$ CD8 T cells (17) would further enhance their Ag sensitivity and contribute to their pathogenic potential. However, it is not possible to distinguish the contribution of SOCS1 deficiency in boosting the Ag response from the cytokine response in the RIP-mOVA/OT-I model because, unlike the P14 cells in the RIP-GP model, wild-type OT-I cells are pathogenic without prior cytokine or Ag stimulation (23). The use of the RIP-GP/P14 model allowed us to distinguish how SOCS1 influenced the responses of CD8 T cells to Ag compared with those induced by cytokines. In this study, we have shown that *Socs1*$^{-/-}$ P14 cells display heightened Ag-specific cytolytic activity and produce copious amounts of IFN-γ upon Ag stimulation. Yet, these cells failed to induce T1D in RIP-gp33 mice because of their inability to proliferate in response to Ag. *Socs1*$^{-/-}$ P14 cells do not have any defect in cell proliferation per se because they proliferate vigorously to cytokine stimulation. Furthermore, reversal of the Ag-specific proliferation defect by exogenous IL-2 allowed *Socs1*$^{-/-}$ P14 cells to induce diabetes

in RIP-GP mice as efficiently as SOCS1-sufficient P14 cells. Most significantly, IL-15–dependent homeostatic expansion in the lymphopenic environment of Rag1-null RIP-GP recipients was sufficient to elicit the diabetogenic potential of *Socs1*$^{-/-}$ P14 cells. Hence, the RIP-GP/P14 model clearly demonstrates that the protective role of SOCS1 in preventing the activation of autoreactive CD8 T cells relies exclusively on the ability of SOCS1 to control the cytokine-driven differentiation of naive CD8 T cells to effector cells.

Our findings on the RIP-GP/P14 model have unraveled a hitherto unrecognized role of SOCS1 in preserving the ability of naive CD8 T cells to proliferate in response to Ag. The inability of *Socs1*$^{-/-}$ CD8 T cells to proliferate in response to TCR stimulation is not unique to P14 cells as SOCS1 deficiency also impaired the Ag-induced proliferation of pmel-1 TCR transgenic CD8 T cells as well as compromised the acute homeostatic proliferation of P14 cells stimulated by environmental Ags and the polyclonal expansion of CD8 T cells following LCMV infection. The proliferative unresponsiveness of *Socs1*$^{-/-}$ P14 cells to Ag stimulation appears to be tightly linked to the cytokine-driven differentiation toward effector cells. In this context, it is noteworthy that increased availability of IL-15 as a sequel to its lack of utilization caused by IL-2Rβ deficiency promotes massive expansion of CD8 T cells and their differentiation toward IFN-γ–secreting effector cells (50).

The defective Ag-induced proliferation of *Socs1*$^{-/-}$ P14 cells arises from their inability to produce sufficient quantities of IL-2 for autocrine growth stimulation. Nonetheless, these cells display potent Ag-specific cytolytic activity and abundantly secrete IFN-γ in response to Ag stimulation. Because the steady-state expression of costimulatory receptors is not affected in *Socs1*$^{-/-}$ P14 cells, their proliferative unresponsiveness does not seem to result from split anergy (51, 52). Even after stimulation via the TCR and the costimulatory receptors, CD8 T cells can develop a transient defect in proliferation called Ag-induced nonresponsiveness (AINR) that can be reversed by IL-2 or by help from CD4 T cells (53). Following rescue by IL-2, the AINR CD8 T cells regain the ability to proliferate in response to Ag (53). Compared with AINR, the Ag-specific proliferation defect of *Socs1*$^{-/-}$ P14 cells was not reversible following proliferation stimulated by IL-15 (Fig. 4*B*). These results suggested that the signal rewiring, which was proposed to occur upon reversal of AINR (54), did not occur in the absence of SOCS1. An intriguing question is, how does SOCS1 deficiency induce the Ag-specific proliferation defect in P14 cells? Although split anergy and AINR are induced by TCR signaling, the Ag-specific proliferation defect of *Socs1*$^{-/-}$ P14 cells occurs in the absence of cognate Ag. Nonetheless, although increased cytokine-induced proliferation is certainly an important factor, it is likely that basal level of TCR signaling stimulated by self- and environmental Ags might also have contributed to the Ag-specific proliferation defect of *Socs1*$^{-/-}$ P14 cells. This possibility is supported by the inability of *Socs1*$^{-/-}$ OT-I cells to undergo homeostatic proliferation in TAP1-deficient mice (23).

Potential contribution of cytokines, such as IL-7, IL-21, and IL-6, in autoimmune diseases has been well documented in mouse models (14, 15, 55, 56). These homeostatic and inflammatory cytokines would be abundantly available during lymphopenia and inflammatory conditions. It has long been suggested that cytokine-driven homeostatic proliferation may favor expansion of autoreactive CD8 T cells (5, 6, 13, 57, 58). Cytokine-driven bystander activation of autoreactive CD8 T cells may occur in normal animals during microbial infections and chronic inflammation, and this may also contribute to the triggering of potentially autoreactive CD8 T cells (59). Ehl et al. (60) have investigated the contribution of bystander activation of autoreactive CD8 T cells by infecting the RIP-GP/P14 TCR double-transgenic mice with vaccinia virus that does not express any cross-reactive Ags. Even though this study showed that bystander activation of P14 cells could lead to insulitis, the infected animals did not develop diabetes. A limitation of this experiment could be the competition for cytokine resources by T cells reactive to vaccinia Ags and the limited duration of activation compared with homeostatic proliferation. Even in the absence of T cells competing for foreign Ags in lymphopenic RIP-GP mice, SOCS1-deficent P14 cells required 3–4 wk to cause overt disease (Table I, group C). It is noteworthy that progression from insulitis to clinical diabetes takes 1–3 mo in the NOD mouse and in the BB-DP rat models of autoimmune diabetes (7, 61). Hence, as the "fertile-field hypothesis of autoimmunity" (59) predicts, Ag nonspecific activation of autoreactive CD8 T cells by heterologous infectious agents, presumably via induction of the priming cytokines (19), will require successive episodes of cytokine-driven expansion over a long period and escape from the peripheral tolerance mechanisms before the manifestation of clinical disease.

The two functions of SOCS1 in CD8 T cells described in this paper, namely prevention of Ag-independent cytokine-driven functional differentiation and preservation of Ag-induced proliferation, have important implications for the higher incidence of autoimmunity as well as impaired immune response to infectious agents during aging. For instance, accumulation of oligoclonal CD8 T cell population in aged mice and humans, which arises from cytokine-driven expansion (62), may also be associated with their functional differentiation that might underlie the higher incidence of autoimmune disorders and less efficient immune responses toward pathogens upon aging (63, 64). It is noteworthy that the *Socs1* gene is highly susceptible to epigenetic repression by CpG methylation (65) and regulation by microRNA (66). Further studies will determine whether these regulatory processes that suppress the expression of the *Socs1* gene are amplified in CD8 T cells that accumulate in aged mice and humans.

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Disclosures

The authors have no financial conflicts of interest.

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