SOCS-1 regulates IL-15–driven homeostatic proliferation of antigen-naive CD8 T cells, limiting their autoimmune potential

Gayle M. Davey,[1] Robyn Starr,[1] Ann L. Cornish,[1] J. Theodore Burghardt,[2] Warren S. Alexander,[1] Francis R. Carbone,[3] Charles D. Surh,[2] and William R. Heath[1]

[1]The Walter and Eliza Hall Institute of Medical Research, Parkville, 3050 Victoria, Australia
[2]Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037
[3]Department of Microbiology and Immunology, University of Melbourne, Parkville, 3052 Victoria, Australia

Mice that are deficient in suppressor of cytokine signaling–1 (SOCS-1) succumb to neonatal mortality that is associated with extensive cellular infiltration of many tissues. T cells seem to be necessary for disease, which can be alleviated largely by neutralizing interferon-γ. Examining T cell receptor (TCR) specificity shows that even monospecific T cells can mediate disease in SOCS-1–deficient mice, although disease onset is substantially faster with a polyclonal T cell repertoire. A major phenotype of SOCS-1$^{-/-}$ mice is the accumulation of CD44^{high}CD8^{+} peripheral T cells. We show that SOCS-1–deficient CD8, but not CD4, T cells proliferate when transferred into normal (T cell–sufficient) mice, and that this is dependent on two signals: interleukin (IL)-15 and self-ligands that are usually only capable of stimulating homeostatic expansion in T cell–deficient mice. Our findings reveal that SOCS-1 normally down-regulates the capacity of IL-15 to drive activation and proliferation of naive CD8 T cells receiving TCR survival signals from self-ligands. We show that such dysregulated proliferation impairs the deletion of a highly autoreactive subset of CD8 T cells, and increases their potential for autoimmunity. Therefore, impaired deletion of highly autoreactive CD8 T cells, together with uncontrolled activation of naive CD8 T cells by homeostatic survival ligands, may provide a basis for the T cell–mediated disease of SOCS-1$^{-/-}$ mice.

CORRESPONDENCE
William R. Heath:
heath@wehi.edu.au

Abbreviations used: CFSE, carboxy fluorescein diacetate succinimidyl ester; poly I:C, polyinosine-polycytidylic acid; SOCS-1, suppressor of cytokine signaling–1

Suppressor of cytokine signaling–1 (SOCS-1) is a negative feedback regulator of cytokine signaling (1–3) that acts on JAKs by directly binding to, and inhibiting, their tyrosine-kinase activity (4) and by facilitating ubiquitination, which targets these kinases for proteosomal degradation (5, 6). SOCS-1 expression is induced in vitro by many cytokines, including IL-2, -4, -6, -7, -9, -10, -13, and -15; TNFα; types I and II IFN, as well as by several colony-stimulating factors, growth factors, and hormones (7–10). Its ability to inhibit the activity of several of these cytokines and other factors in vitro also was reported (7–9, 11).

To better understand the role of SOCS-1 in vivo, various SOCS-1–deficient mice have been generated (12–14). Mice with targeted disruption of SOCS-1 throughout the whole animal show extensive pathology that leads to mortality by 3 wk of age (12, 14). Disease consists of lymphocytopenia, growth retardation, fatty liver degeneration, and severe inflammation in multiple organs. A major underlying cause of this disease seems to be dysregulated IFN-γ signaling, because SOCS-1–deficient mice that are crossed to IFN-γ$^{-/-}$ mice survive as neonates and appear relatively healthy up to ∼6 mo of age (15–17). After this time, double-deficient mice begin to show susceptibility to IFN-γ–independent disease, with evidence of polycystic kidneys and inflammation (18). This indicates that pathology is not driven entirely by IFN-γ sensitivity, but may relate to other signaling pathways. Consistent with this, targeted disruption of Stat 6 or Stat 4, transcription factors downstream of IL-4 and IL-12 signaling, respectively, also can limit mortality of SOCS-1–deficient mice in the neonatal period (19, 20). As might be predicted, the aforementioned

R. Starr's present address is St. Vincent's Institute, Fitzroy, 3065 Victoria, Australia.

A.L. Cornish's present address is Department of Microbiology and Immunology, University of Melbourne, Parkville, 3052 Victoria, Australia.

The Journal of Experimental Medicine

findings implicate dysregulated cytokine signaling as the basis for the severe pathology that is associated with SOCS-1 deficiency. However, there is limited understanding of the mechanisms that drive production of these cytokines.

The role of T lymphocytes in the disease that is associated with SOCS-1 deficiency is unclear. Crossing SOCS-1–deficient mice to RAG-2–deficient mice, which do not make lymphocytes, reduces serum levels of IFN-γ and prevents neonatal mortality (17); this suggests that lymphocytes have a role in IFN-γ production and are essential for disease. Reconstitution of RAG-$2^{-/-}$ mice (WT for SOCS-1) with SOCS-$1^{-/-}$ hematopoietic progenitors resulted in death within 7 wk (19), which indicated that deficiency of SOCS-1 in bone marrow–derived cells is sufficient to cause disease. To examine specifically whether lack of expression of SOCS-1 in T cells can lead to pathology, Chong et al. (13) engineered targeted disruption of SOCS-1 in T cells. This led to typical phenotypic changes in T cells that are associated with SOCS-1 deficiency, but mice showed no overt signs of disease and were protected from neonatal mortality. This indicated that SOCS-1 deficiency within T cells alone was insufficient to cause pathology of SOCS-1–deficient mice.

Although Chong et al. (13) did not observe disease when T cells alone lacked SOCS-1, expression of this molecule within T cells was important for the normal homeostasis of this population (13, 21). Mice with SOCS-1–deficient T cells showed an enhanced ratio of mature CD8/CD4 cells in the thymus, and an increase in the number and proportion of memory phenotype $CD44^{hi}$CD8 T cells in the periphery. The increase in $CD44^{hi}$CD8 T cells did not depend on recognition of foreign ligand, because a similar increase was seen when SOCS-1–deficient mice were crossed to transgenic OT-I mice that express a class I–restricted OVA-specific TCR (21). This even was true when these mice were crossed additionally to a RAG-$1^{-/-}$ background to prevent endogenous receptor rearrangement. Although the phenotypic T cell changes did not seem to depend on foreign antigen, and SOCS-1 deficiency in T cells alone failed to precipitate overt pathology, the disease phenotype that is associated with SOCS-1 deficiency showed dependence on TCR specificity. Survival of SOCS-$1^{-/-}$ mice was extended greatly by introduction of the OVA-specific TCR transgene; survival increased from 12 d for SOCS-$1^{-/-}$ mice to 75 d for SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice expressing the OT-I TCR. Because SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice that lacked T cells survived indefinitely, but similar mice that expressed the OT-I TCR died within a mean of 75 d, TCR-expressing cells were implicated in the slow-onset disease progression. Together, the aforementioned studies supported the conclusions that (a) T cells were necessary for disease in SOCS-$1^{-/-}$ mice, (b) their TCR specificity affected the rate of disease onset, and (c) SOCS-1 deficiency within non-T cells was essential.

Demonstration that SOCS-$1^{-/-}$ mice that were manipulated additionally to transgenically express SOCS-1 only in B and T lymphocytes, survived for >6 mo (compared with only 3 wk for unmanipulated SOCS-$1^{-/-}$ mice [22]), suggests that SOCS-1 deficiency in T cells is central to severe disease onset. Although the slower onset of disease in OT-I. SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice, compared with SOCS-$1^{-/-}$ mice, suggests that TCR specificity contributes to severity, it is unclear whether any level of specificity is required for slow disease progression. Clearly, the antigenic ligand OVA is not expressed in OT-I.SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice, but specificity for self-ligands associated with, for example, homeostatic proliferation (23–25) may contribute.

To understand the basis of the T cell phenotype in SOCS-1–deficient mice, their responsiveness to cytokines that signal through the common γ chain receptor subunit was examined (10, 11). IL-2 and -15, but not IL-7, induced proliferation of SOCS-$1^{-/-}$ $CD8^+$ T cells in vitro. In particular, $CD44^{hi}$CD8 T cells showed a fivefold greater sensitivity to these cytokines when compared with WT cells. This same population demonstrated an increased capacity for bystander proliferation in response to polyinosine-polycytidylic acid (poly I:C) administration in vivo, and proliferated more vigorously when transferred into T cell–depleted recipients—referred to as homeostatic proliferation. This led to the conclusion that the peripheral phenotype of SOCS-1–deficient CD8 T cells related to an increased sensitivity of memory CD8 T cells to IL-15 receptor signaling (11). However, because naive T cells adopt a memory phenotype after they are induced to proliferate homeostatically, these studies failed to exclude IL-15–driven expansion of naive CD8 T cells. Consistent with this possibility, studies using TCR transgenic SOCS-1–deficient mice showed TCR signaling by foreign antigen was not required for up-regulation of CD44 (21).

The aforementioned studies suggest that T cells are vital for disease in SOCS-$1^{-/-}$ mice, that their specificity is important, and that the phenotypic changes to this population may be related to increased sensitivity to cytokines that signal through the common γ chain receptor subunit. Our studies aimed to determine (a) the precise cytokine(s) that is responsible for phenotypic changes in vivo, (b) whether antigen-naive T cells could be affected, (c) the basis for TCR specificity requirements, and (d) whether SOCS-1 deficiency in T cells could affect their autoimmune potential. Here, we show that SOCS-1–deficient CD8, but not CD4, T cells proliferate when transferred into normal T cell–sufficient mice; that this is related to their specificity for self-ligands (that normally drive homeostatic proliferation in T cell-deficient mice); and that it is mediated by IL-15 hypersensitivity in vivo. Finally, we provide compelling evidence that the CD8 T cell repertoire of SOCS-1–deficient mice contains a greater proportion of autoreactive T cells and that, as a population, these cells have an increased autoimmune potential.

RESULTS

Dissecting the T cell phenotype of SOCS-1–deficient mice

To determine whether the phenotypic changes to CD8 T cells in SOCS-$1^{-/-}$ mice were due to their SOCS-1–deficient environment or an innate property of the cells them-

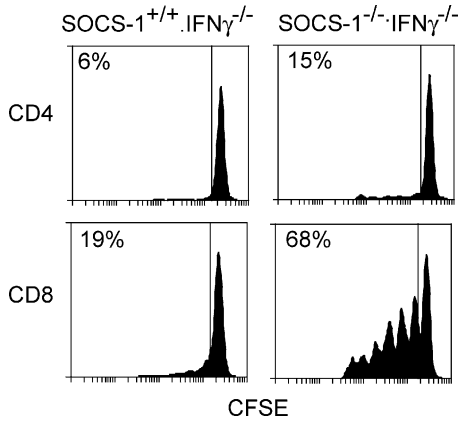

Figure 1. CD8+ SOCS-1–deficient T cells proliferate in C57BL/6 mice. SOCS-1.IFN-$\gamma^{-/-}$ T cells were labeled with CFSE and adoptively transferred into C57BL/6 mice. 6 d later, splenocytes were stained for CD4 and CD8 and analyzed by flow cytometry. Histograms are gated for CD4+ or CD8+ cells; the percentages shown are the proportion of cells that have reduced CFSE intensity.

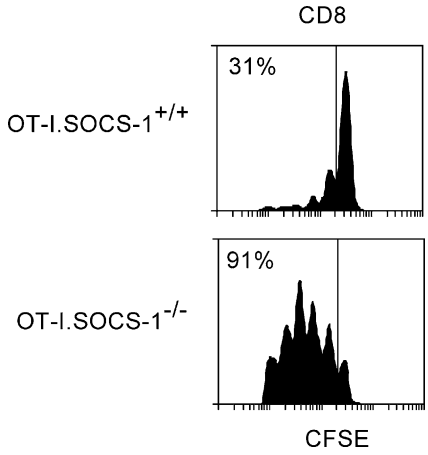

Figure 2. OT-I.SOCS-1–deficient CD8 T cells proliferate in C57BL/6 mice. OT-I.SOCS-1.RAG-$1^{-/-}$ T cells from bone marrow chimeras were labeled with CFSE and adoptively transferred into C57BL/6 mice. 6 d later, lymph node cells were stained for CD8 and analyzed by flow cytometry. Histograms are gated for CD8+ cells; the percentages shown are the proportion of cells that have reduced CFSE intensity.

selves, we examined the fate of SOCS-$1^{-/-}$ T cells after adoptive transfer into normal C57BL/6 (B6) hosts. To obtain sufficient T cells, it was necessary to cross the SOCS-$1^{-/-}$ mice to IFN-$\gamma^{-/-}$ mice, which do not suffer neonatal lethality (15–17). SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ T cells were labeled with carboxy fluorescein diacetate succinimidyl ester (CFSE) and then transferred into B6 mice (containing a full complement of T cells). 6 d later, spleen cells were recovered, stained for CD4 and CD8, and analyzed by flow cytometry. As shown in Fig. 1, CD8, but not CD4, T cells from SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ mice proliferated upon adoptive transfer into normal mice, whereas no such proliferation was seen when SOCS-1–expressing T cells were transferred. Although previous studies had observed enhanced proliferation of SOCS-$1^{-/-}$ CD8 T cells when adoptively transferred into T cell–deficient mice or when examined in intact SOCS-$1^{-/-}$ mice given poly I:C (or untreated), our data provided the first evidence that SOCS-$1^{-/-}$CD8 T cells, but not CD4 T cells, proliferated in a normal T cell–sufficient environment.

Although the proliferation that was seen for SOCS-$1^{-/-}$ CD8 T cells likely was cytokine driven, it was unclear whether there also were TCR signaling requirements. If so, these could have related to several forms of antigenic stimulation, including heightened responses to microbial or food antigens, inappropriate responses to self-antigens, or responses induced by low-level TCR signaling that was not associated with antigenic ligation. To distinguish between some of these possibilities, we examined the influence of TCR specificity on this response by crossing the SOCS-$1^{-/-}$ mice to OT-I transgenic mice that express a class I–restricted OVA-specific TCR. These mice, like the SOCS-$1^{-/-}$ mice, died at a relatively young age (21); to avoid this problem we made irradiated bone marrow chimeric mice by transfer of bone marrow from young OT-I.SOCS-$1^{-/-}$ mice into irradiated B6 recipients. Such OT-I.SOCS-$1^{-/-}$→B6 chimeric mice survived for ≥1 yr (unpublished data). To exclude further the influence of endogenously rearranged TCRs, OT-I.SOCS-1.RAG-$1^{-/-}$ mice were generated. Unless otherwise stated, OT-I.SOCS-1 T cells were obtained from the spleen and/or lymph nodes of OT-I.SOCS-1.RAG-$1^{-/-}$→B6 mice for all subsequent experiments. When OT-I.SOCS-$1^{-/-}$RAG-$1^{-/-}$ CD8 T cells from these chimeras were transferred into WT B6 mice containing a full repertoire of T cells (Fig. 2), these cells proliferated similarly to CD8 T cells from the SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ mice (Fig. 1). The ability of OVA-specific SOCS-$1^{-/-}$ CD8 T cells to proliferate in normal mice in the absence of their specific antigen, OVA, indicated that stimulation by foreign antigen was not necessary for SOCS-1–deficient CD8 T cells to proliferate when transferred into normal mice.

We reported previously that SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice, which do not contain T cells, survive and are healthy in a germ-free environment. In contrast, SOCS-$1^{-/-}$RAG-$1^{-/-}$OT-I mice, which only possess OVA-specific CD8 T cells, have a mean survival time of 72 d (21). This indicates that T cells are essential for disease, and suggests that understanding the basis of dysregulated activation and proliferation of these cells in OT-I.SOCS-$1^{-/-}$ mice is important.

It has been shown that TCR specificity can determine whether T cells proliferate homeostatically when adoptively transferred into T cell–deficient mice (23–25). An example of this is the ability of OVA-specific OT-I CD8 T cells (23), but not H-Y–specific transgenic CD8 T cells (26), to undergo homeostatic proliferation when transferred into sublethally irradiated (T cell–depleted) B6 hosts (Fig. 3 A). In this case, H-Y–specific T cells do not seem to receive sufficient TCR signaling from endogenous self-antigens to induce homeostatic proliferation, whereas OT-I T cells are stimulated

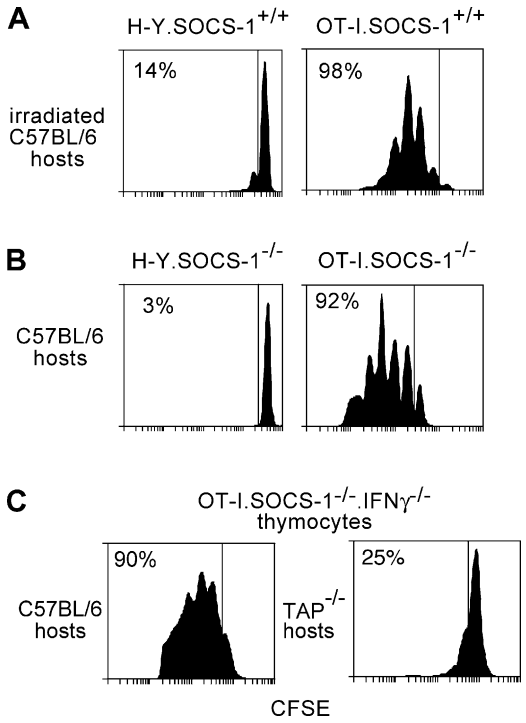

Figure 3. H-Y.SOCS-1–deficient CD8 T cells do not proliferate in C57BL/6 mice. OT-I.SOCS-1.RAG-$1^{-/-}$ or H-Y.SOCS-1.RAG-$1^{-/-}$ T cells from bone marrow chimeras were labeled with CFSE and adoptively transferred into C57BL/6 recipients. SOCS-$1^{+/+}$ cells were transferred into irradiated (700 cGy) C57BL/6 mice (A) and SOCS-$1^{-/-}$ cells were transferred into unmanipulated C57BL/6 mice (B). 5 or 6 d later, splenocytes were stained for CD4 and CD8 and analyzed by flow cytometry. Histograms are gated for $CD8^+$ cells; the percentages shown are the proportion of cells that have reduced CFSE intensity. (C) SOCS-$1^{-/-}$ OT-I T cells do not proliferate in TAP$1^{-/-}$ mice. Thymocytes from OT-I.SOCS-$1^{-/-}$.IFN-$\gamma^{-/-}$ mice were enriched for $CD8^+CD4^-$ cells, labeled with CFSE, and transferred into C57BL/6 or TAP$1^{-/-}$ recipients. Recipient TAP$1^{-/-}$ mice had been treated 15 d earlier with a single i.p. injection of anti-CD8 mAb to remove residual CD8 T cells. 7 d later, splenocytes were labeled with CD8-specific mAb and analyzed by flow cytometry. Line graphs are gated for $CD8^+$ cells; the percentages shown are the proportion of cells that have reduced CFSE intensity. Results from a single experiment, two mice per group.

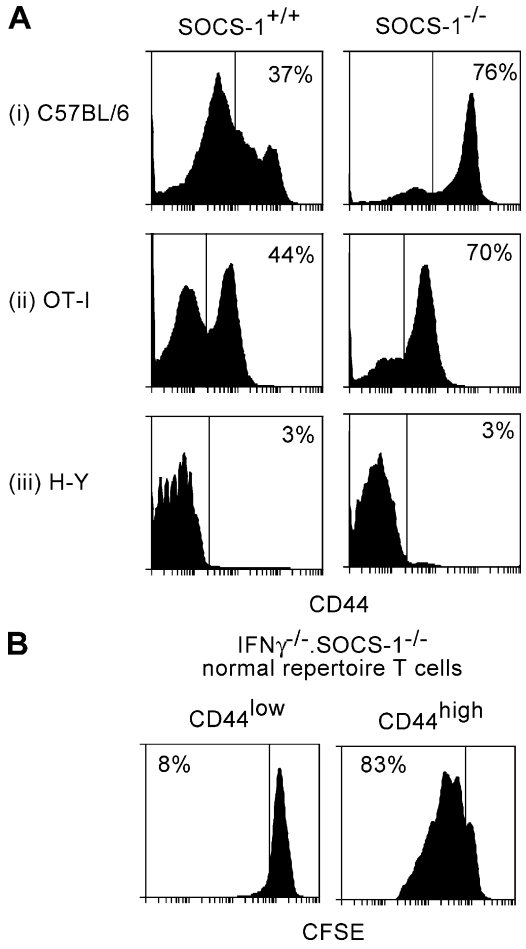

Figure 4. CD44 expression is up-regulated on SOCS-1–deficient CD8 T cells from C57BL/6 or OT-I, but not H-Y mice. (A) CD44 expression of $CD8^+$ splenocytes from SOCS-$1^{+/+}$ (left) and SOCS-$1^{-/-}$ (right) SOCS-1.IFN-$\gamma^{-/-}$ mice (i), OT-I.SOCS-1.RAG-$1^{-/-}$→B6 chimeras (ii), and H-Y.SOCS-1.RAG-$1^{-/-}$→B6 chimeras (iii). (B) SOCS-$1^{-/-}$.IFN-$\gamma^{-/-}$ CD8 T cells were sorted into $CD44^{lo}$ and $CD44^{high}$ populations, labeled with CFSE, and transferred into C57BL/6 recipients. 6 d later splenocytes, were labeled with CD8 and CD44 and analyzed by flow cytometry. Histograms are gated for $CD8^+$ cells from mice that received $CD44^{low}$ cells (left) or $CD44^{high}$ cells (right). The percentages shown are the proportion of cells that have reduced CFSE intensity.

sufficiently. To determine whether such TCR specificity also affected proliferation of SOCS-$1^{-/-}$ CD8 T cells that were transferred into T cell–sufficient mice, we examined proliferation of OT-I and H-Y T cells from their respective SOCS-$1^{-/-}$ crosses after transfer into normal B6 mice (Fig. 3 B). The ability of OT-I.SOCS-$1^{-/-}$, but not H-Y.SOCS-$1^{-/-}$, CD8 T cells to proliferate indicated that proliferation depended on TCR specificity.

One caveat to the aforementioned conclusion is that memory CD8 T cells are generally less reliant on MHC I recognition for homeostatic proliferation than are naive T cells (27, 28). Because most OT-I.SOCS-$1^{-/-}$ cells have a memory phenotype, whereas most HY.SOCS-$1^{-/-}$ cells are naive (see next paragraph), it remained possible that differences in proliferation might relate to their state of activation. To assess further the requirement for a TCR signal in the initiation of OT-I.SOCS-$1^{-/-}$ T cell proliferation, OT-I.SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ $CD8^+CD4^-$ thymocytes were adoptively transferred into B6 or TAP$1^{-/-}$ T cell–sufficient hosts, the latter of which express only very low levels of MHC class I. In this case, we used thymocytes as the source of naive, mature CD8 T cells because OT-I.SOCS-$1^{-/-}$ mice have few $CD44^{low}$ cells in their periphery. Analysis of proliferation on day 7 after transfer (Fig. 3 C) showed that naive OT-I.SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ CD8 T cells failed to proliferate in a TAP$1^{-/-}$ host, but proliferated efficiently in B6 mice. This demonstrated clearly a requirement for MHC class I recognition for proliferation.

In accordance with the aforementioned finding, like SOCS-1$^{-/-}$ mice, OT-I.SOCS-1$^{-/-}$ mice, but not H-Y. SOCS-1$^{-/-}$ mice, showed an increase in the proportion of CD44^{hi}CD8 T cells (Fig. 4 A). Thus, the increase in CD44^{hi} memory–phenotype CD8 T cells in SOCS-1$^{-/-}$ mice is not an innate property of all CD8 T cells, but depends upon TCR specificity. Only naive CD8 T cells with an appropriate "self"-specificity are able to be transformed into CD44^{hi} cells. The failure of SOCS-1–deficient H-Y, but not OT-I, cells to proliferate and up-regulate CD44 excluded the possibility that responses were induced by low-level TCR signaling in the absence of antigenic ligation.

Further evidence that supported the idea that SOCS-1 deficiency may allow only a proportion of CD8 T cells to proliferate and up-regulate CD44 was shown by sorting a normal repertoire of SOCS-1–deficient CD8 T cells into CD44^{high} and CD44^{low} before adoptive transfer into WT mice. Only the CD44^{high} cells proliferated upon transfer into T cell–sufficient B6 hosts (Fig. 4 B). This indicates that a proportion of CD8 T cells, like the H-Y T cells, never up-regulate CD44 and do not proliferate, even though they are SOCS-1 deficient.

Because the TCR specificity for proliferation of SOCS-1$^{-/-}$ T cells in T cell–sufficient mice paralleled that for homeostatic proliferation of normal T cells in T cell–deficient mice, we considered it likely that the T cell phenotype in SOCS-1$^{-/-}$ mice related to their enhanced sensitivity to homeostatic proliferation signals. For CD8 T cells, IL-7 and -15 were reported to control homeostatic proliferation when T cells were depleted. Naive CD8 cells are highly dependent on IL-7, although they also can respond to IL-15. In contrast, memory CD8 T cells respond mainly to IL-15, with a reduced responsiveness to IL-7 (28, 29). To examine the dependence of OT-I.SOCS-1$^{-/-}$ CD8 T cells on these two cytokines, we adoptively transferred OT-I.SOCS-1$^{-/-}$ cells into mice that were deficient for either of these cytokines. As shown in Fig. 5 A, these cells proliferated in control B6 mice and in IL-7$^{-/-}$ mice, but failed to divide in IL-15$^{-/-}$ mice. This provided the first direct evidence that IL-15 was responsible for driving proliferation in vivo. To confirm the dependence of SOCS-1$^{-/-}$ CD8 T cells on IL-15 for proliferation, purified T cells from SOCS-1$^{-/-}$IFN-$\gamma^{-/-}$ mice were labeled with CFSE and adoptively transferred into B6 or IL-15$^{-/-}$ mice. 6 d later, cells were recovered, stained for CD8, and examined for proliferation by flow cytometry (Fig. 5 B). Consistent with our findings with OT-I.SOCS-1$^{-/-}$ cells, SOCS-1$^{-/-}$IFN-$\gamma^{-/-}$ CD8 T cells from a normal repertoire did not proliferate in IL-15$^{-/-}$ mice, but proliferated well in B6 mice.

Having shown that IL-15 was required to drive the proliferation of CD44^{high} SOCS-1–deficient CD8 T cells in vivo, we were interested in determining whether IL-15 was expanding an existing population of CD44^{high} CD8 T cells or, in fact, contributed to the proliferation of naive CD44^{low} CD8 T cells, which then up-regulated CD44. Because only low numbers of naive OT-I.SOCS-1$^{-/-}$ cells can be found in the periphery of OT-I.SOCS-1$^{-/-}$ chimeric mice (Fig. 4 A), thymocytes were sorted for CD44^{low}CD4^{-}CD8^{+} OT-I.SOCS-1 cells. As shown in Fig. 5 C, naive CD44^{low} CD8 T cells proliferated in B6 mice, but not in IL-15$^{-/-}$ mice. This supports the studies in TAP1$^{-/-}$ mice, which suggest that naive phenotype cells are driven directly to proliferate. Cells that were transferred into IL-15$^{-/-}$ mice did not up-regulate CD44 by day 7 (unpublished data), which supports the view that activation was IL-15 dependent.

These findings are consistent with SOCS-1 deficiency causing enhanced sensitivity to IL-2β receptor signaling (11). Our findings show that this leads to increased responsiveness to IL-15 in vivo, which causes activation and proliferation of the naive CD8 T cells that express TCR specificities that normally are associated with homeostatic proliferation in T cell–deficient environments where IL-7 and IL-15 concentrations are much greater. Once proliferating, these naive CD8 T cells adopt the CD44^{high} memory phenotype, but are not—as previously believed—bona fide memory T cells (11).

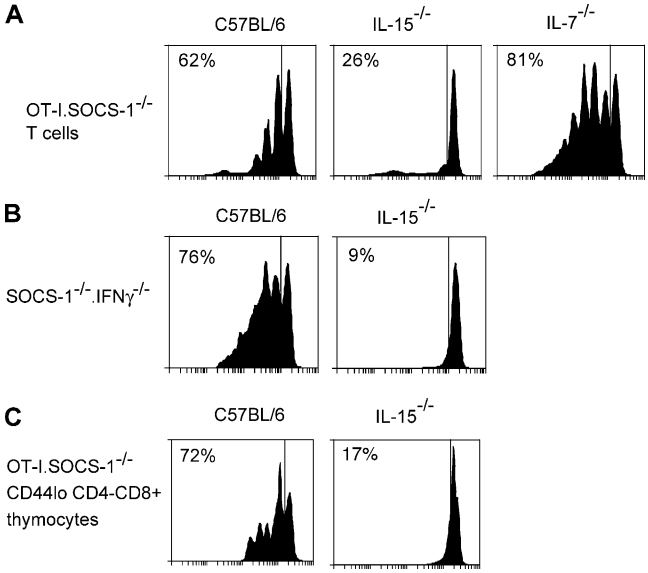

Figure 5. SOCS-1$^{-/-}$ CD8 T cells do not proliferate in IL-15$^{-/-}$ mice. (A) T cells from OT-I.SOCS-1$^{-/-}$.RAG-1$^{-/-}$→B6 mice were labeled with CFSE and adoptively transferred into C57BL/6, IL7$^{-/-}$ or IL-15$^{-/-}$ mice. 7 d later, splenocytes were stained for CD8, Vα2, and Vβ5 and analyzed by flow cytometry. Histograms are gated for CD8^{+}Vα2^{+}Vβ5^{+} cells, and the percentages shown are the proportion of cells that have reduced CFSE intensity. (B) SOCS-1$^{-/-}$.IFN-$\gamma^{-/-}$ T cells were labeled with CFSE and adoptively transferred into C57BL/6 or IL15$^{-/-}$ mice. 6 d later, splenocytes were stained for CD8 and analyzed by flow cytometry. Histograms are gated for CD8^{+} cells; the percentages shown are the proportion of cells that have reduced CFSE intensity. (C) Thymocytes from OT-I.SOCS-1$^{-/-}$.RAG-1$^{-/-}$→B6 mice were sorted for CD44^{low} CD8^{+}CD4^{-} cells, labeled with CFSE, and transferred into C57BL/6 or IL-15$^{-/-}$ recipients. 7 d later, splenocytes were labeled with antibodies for CD8 and analyzed by flow cytometry. Histograms are gated for CD8^{+} cells; the percentages shown are the proportion of cells that have reduced CFSE intensity.

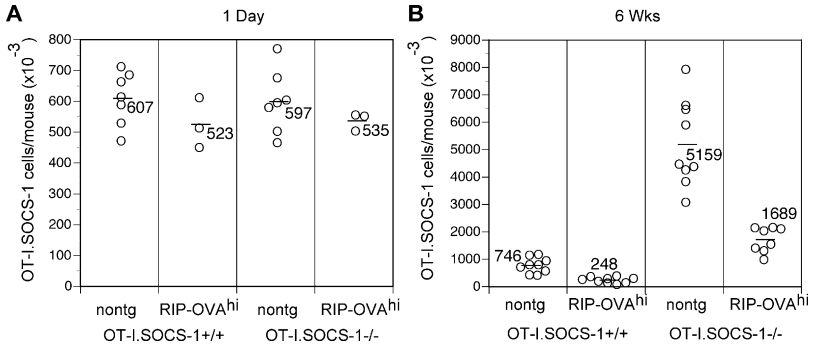

Figure 6. SOCS-1–deficient OT-I CD8 T cells are not deleted by cross-tolerance. 5×10^6 OT-I.SOCS-1.RAG-$1^{-/-}$→B6 cells were adoptively transferred into RIP-OVA^{hi} or C57BL/6 (nontg) mice. After 1 d (A) or 6 wk (B), the number of OT-I cells in the lymph nodes and spleen was determined. Open circles represent individual mice; the bar and number corresponds to the average number of OT-I cells per group. These results are pooled data from two to three independent experiments.

SOCS-1–deficient autoreactive CD8 T cells are not deleted by cross-tolerance

We reported previously that CD8 T cells can be tolerized to self-antigens that are expressed in extrathymic tissues by a mechanism that is called cross-tolerance (30). This requires cross-presentation of tissue antigens on a subset of CD8α^+ dendritic cells that cause bim-dependent deletion of autoreactive CD8 T cells (31–34). Because SOCS-1 deficiency causes a proportion of CD8 T cells to proliferate and increase in number, we questioned whether this might prevent cross-tolerance, and therefore, contribute to the autoimmune phenotype of SOCS-$1^{-/-}$ mice. To determine whether deletion by cross-tolerance was affected by a SOCS-1 deficiency in CD8 T cells, we injected WT B6 mice or those expressing soluble OVA in the pancreas (RIP-OVA^{hi} mice) (35) with SOCS-1–deficient OT-I cells. 1 d after transfer of the cells, equivalent numbers of OT-I or OT-I.SOCS-$1^{-/-}$ cells were present in recipient mice showing a similar "take" of transferred cells (Fig. 6 A). However, by 6 wk after transfer, normal OT-I cells were deleted in RIP-OVA^{hi} mice, but survived in nontransgenic control mice that did not express OVA. Although expression of OVA in the islets reduced the total number of SOCS-1–deficient OT-I cells compared with that seen in nontransgenic mice, the number of these cells increased compared with that seen for WT OT-I cells in nontransgenic mice (Fig. 6 B). Thus, although the cross-tolerance mechanism reduced the total number of SOCS-1–deficient OT-I cells that would have accumulated, it was not efficient enough to reduce this number to less than that originally transferred. In fact, the total number of OT-I cells increased by approximately threefold. These findings indicate that the SOCS-1 deficiency in T cells does not inhibit the bim-dependent deletion mechanism that is associated with cross-tolerance; however, the high rate of proliferation that is associated with lack of SOCS-1 offset the rate of deletion by cross-tolerance. This leads to an overall failure to reduce autoreactive T cell numbers to less than their initial value.

SOCS-1–deficient autoreactive CD8 T cells have greater autoimmune potential

To determine whether SOCS-1 deficiency might enhance the ability of T cells to cause autoimmunity, we examined the induction of diabetes in the RIP-mOVA model (36). RIP-mOVA mice express a membrane-bound form of OVA in the islet β cells of the pancreas, and ectopically in the kidney. Previous studies showed that transfer of 5×10^6 naive OT-I cells usually causes diabetes in these mice; however, transfer of $\leq 2.5 \times 10^5$ OT-I cells never causes disease (37). This is in contrast to the RIP-OVA^{hi} mice that were used for the deletion studies, where only activated OT-I cells, but not naive cells, are able to cause diabetes following adoptive transfer. Functional differences between these RIP-OVA lines probably relates to variations in the amount, nature, and site of antigen expression. To examine autoimmune potential, high and low doses of SOCS-$1^{-/-}$.OT-I T cells were transferred into RIP-mOVA mice. As shown in

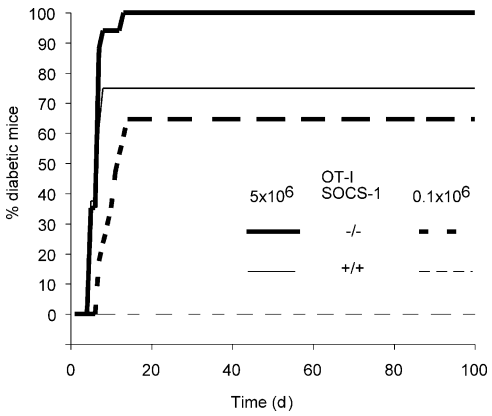

Figure 7. SOCS-1–deficient OT-I CD8 T cells have greater autoimmune potential. 5×10^6 (solid lines) or 0.1×10^6 (dashed lines) T cells from OT-I.SOCS-$1^{+/+,+/-}$ (thin lines) or OT-I.SOCS-$1^{-/-}$ (bold lines) bone marrow chimeric mice were adoptively transferred to RIP-mOVA mice. Mice were monitored for diabetes by urine glucose for 100 d (15–17 mice/group).

Fig. 7, 0.1 × 10^6 OT-I.SOCS-$1^{-/-}$ cells caused diabetes when adoptively transferred into RIP-mOVA mice, whereas SOCS WT OT-I cells failed to do so. This provides the first direct evidence that SOCS-1 deficiency can enhance the capacity of CD8 T cells to cause autoimmunity, even in recipient mice that are WT for SOCS-1 expression.

DISCUSSION

This study aimed to define better the effects of SOCS-1 within T cells. It had been reported that the major phenotypic change in T cells from SOCS-$1^{-/-}$ mice was an increase in the proportion and number of $CD44^{high}$ CD8 T cells (13, 21). Our studies provide an explanation for this phenotype. Homeostatic proliferation of naive CD8 T cells is controlled largely by IL-7 (38, 39), although they can respond weakly to IL-15 (40). Conversely, memory CD8 T cells respond predominantly to IL-15, and respond poorly to IL-7 (28, 29). Our data indicate that SOCS-1–deficient CD8 T cells, which were hyperresponsive in vitro to several cytokines, including IL-2, -4, and -15 (10, 11), require IL-15 in vivo to induce CD44 up-regulation and uncontrolled proliferation (Fig. 5). The uncontrolled proliferation of $CD44^{high}$ CD8 T cells explains the increased number of these cells in SOCS-$1^{-/-}$ mice.

Generation of the $CD44^{high}$ phenotype of CD8 T cells was shown to be highly dependent on their TCR specificity; SOCS-$1^{-/-}$ HY CD8 T cells showed no up-regulation of CD44 and no capacity to proliferate upon adoptive transfer into unmanipulated B6 mice. This contrasts with SOCS-$1^{-/-}$ OT-I cells, which were largely $CD44^{high}$ and proliferated extensively upon transfer into normal hosts. The dependence on TCR signaling for initiation of this proliferation was shown by the failure of naive SOCS-$1^{-/-}$ OT-I cells from the thymus to proliferate in mice that were deficient in TAP. A similar division of T cells in the normal repertoire into those that proliferate and are $CD44^{high}$, and those that do not proliferate and are $CD44^{low}$, was implicated by comparing the capacity of $CD44^{high}$ and $CD44^{low}$ SOCS-$1^{-/-}$IFN-$\gamma^{-/-}$ cells to proliferate upon transfer into normal hosts. $CD44^{low}$ cells largely remained $CD44^{low}$ and did not proliferate, whereas $CD44^{high}$ cells remained $CD44^{high}$ (not depicted) and proliferated vigorously (Fig. 4 B). There are two possible explanations for how naive SOCS-$1^{-/-}$ CD8 T cells initially up-regulate CD44. They have increased sensitivity to IL-15 and this, together with a homeostatic TCR/self-ligand signal, stimulates this effect. Alternatively, this latter TCR interaction, together with increased IL-7 sensitivity, drives naive CD8 T cells into memory phenotype cells that lose their sensitivity to IL-7 and gain hypersensitivity to IL-15. Although there is some evidence that SOCS-$1^{-/-}$ $CD4^+CD8^+$ thymocytes may have increased sensitivity to IL-7 (13), we favor the view that naive CD8 T cells are driven by IL-15 hypersensitivity for the following reasons. First, exposure of SOCS-$1^{-/-}$ T cells to cytokines in vitro shows proliferation and increased sensitivity to IL-2, -4, and -15, but not IL-7 (10, 11); second, our data, with the transfer of mature naive $CD8^+CD4^-$ SOCS-$1^{-/-}$ OT-I thymocytes into IL-$7^{-/-}$ or IL-$15^{-/-}$ mice showed efficient proliferation in the absence of IL-7 (not depicted), but not IL-15 (Fig. 5 C). However, a caveat to the experiments with IL-$7^{-/-}$ hosts is that these mice are lymphopenic, which may alter the cytokine environment and mask an IL-7 dependency. In a preliminary experiment, we found that OT-I.SOCS-$1^{-/-}$ cells did not proliferate in IL-$7^{-/-}$IL-$15^{-/-}$ (lymphopenic) mice (unpublished data), which suggests that alternative cytokines could not substitute for IL-15. However, thus far, it has not been possible to assess the role of IL-7 in driving SOCS-$1^{-/-}$ T cell proliferation in a T cell–sufficient environment. Thus, our studies are consistent with the simplest model where SOCS-$1^{-/-}$ CD8 T cells are hypersensitive to IL-15, and this, together with TCR signaling by self-ligands, drives their proliferation and conversion to memory phenotype cells.

In addition to providing an explanation for the phenotype of SOCS-$1^{-/-}$ T cells, it was important to address their autoimmune potential and susceptibility to tolerance induction. By using well-defined models of peripheral cross-tolerance induction (31–35), we were able to show that SOCS-$1^{-/-}$ CD8 T cells are deleted inefficiently by cross-tolerance. This mechanism relies on the presentation of tissue-derived OVA by a subset of $CD8\alpha^+$ dendritic cells (34) that stimulate proliferation, and ultimately, the deletion of OVA-specific "self-reactive" CD8 T cells (31, 32). When such CD8 T cells were SOCS-1 deficient they were not deleted from the repertoire (Fig. 6), although when compared with similar cells that were transferred into mice that did not express OVA, their numbers were reduced. This suggests that SOCS-1 does not block cross-tolerance–mediated deletion specifically, but simply that the rate of deletion by cross-tolerance is slower than the rate of expansion that is driven by the IL-15 hypersensitivity of SOCS-1–deficient cells. The consequence of this imbalance is that SOCS-$1^{-/-}$ mice slowly accumulate, rather than delete, CD8 T cells with autoreactivity. These studies predict that within the normal repertoire of a SOCS-$1^{-/-}$ mouse there will be more surviving autoreactive T cells than in WT mice. Just how many more is not predictable because there is no means to measure the contribution of cross-tolerance to the control of a normal peripheral T cell repertoire.

In addition to showing that autoreactive T cells may accumulate, we were able to examine the consequence of their presence. The ability of 10^5 SOCS-$1^{-/-}$, but not WT OT-I, cells to cause autoimmune diabetes in RIP-mOVA mice indicated that as a population, these cells are more autoaggressive than are their WT counterparts. In other words, there is no reason to invoke increased autoaggression on a per cell basis (though this is not excluded), because autoimmunity may be potentiated simply by an ever increasing number of autoreactive cells, which are unable to be deleted. These findings contradict the studies of Chong et al. (13), who found no signs of autoimmunity in mice bearing SOCS-1

deficiency only in T cells. However, two important points need to be considered. First, although our data indicate that self-reactive T cells may accumulate and that, as a population, these cells may cause autoimmunity, we cannot determine how many such cells accumulate for a normal TCR repertoire. Perhaps this number is rather low and the resultant increase in autoaggression is insufficient to cause disease when SOCS-1 is only deficient within the T cells. Second, with respect to this last point, in a mouse where SOCS-1 is deficient in all tissues, the combination of innate immune cells (such as macrophages and NK cells) and the tissues themselves being hypersensitive to cytokine signaling, together with the increased autoaggression of the undeleted autoreactive CD8 T cell population, might be sufficient to trigger overt autoimmunity. Future experiments will assess the increase in autoimmune potential when other bone marrow–derived cells or the islet β cells themselves also are deficient in SOCS-1.

In addition to the aforementioned contributions to SOCS-$1^{-/-}$ autoimmunity, these findings raise the possibility that another set of autoreactive T cells may participate in disease. There is evidence that some self-reactive cells ignore their autoantigen, and remain as sessile naive cells in the periphery of normal animals (31, 41, 42). Such cells have autoimmune potential if activated by cross-reactive environmental ligands. However, if cells of this type could receive homeostatic TCR signals when SOCS-1 deficient, they may become autoaggressive. Future studies will examine this possibility.

In summary, our studies provide an explanation for T cell–dependent destructive autoimmune disease that is seen in SOCS-$1^{-/-}$ mice. SOCS-$1^{-/-}$ naive CD8 T cells are triggered by self-ligands and hypersensitivity to IL-15 to upregulate CD44 and proliferate. These cells are poorly susceptible to peripheral tolerance induction, and in combination with the increased cytokine sensitivity of target tissues, have strong potential to cause autoimmunity.

MATERIALS AND METHODS

Mice. All mice were bred and maintained at the Walter and Eliza Hall Institute for Medical Research, except for the IL-7– and IL-15–deficient mouse lines that were maintained at The Scripps Research Institute. Transgenic, OT-I (43), RIP-mOVA (36), RIP-OVA^{hi} (35), and H-Y (44) mice and knockout SOCS-1 (12), SOCS-1.IFN-$\gamma^{-/-}$ (15), RAG-1 (45), IL-15 (46), IL-7 (47), and TAP1 (48) mice have been described previously. All studies were performed according to protocols approved by the Melbourne Health Animal Ethics Committee.

Bone marrow chimeras. OT-I.SOCS-1→B6 or OT-I.SOCS-1.RAG-$1^{-/-}$→B6 bone marrow chimeras were generated as follows. Adult C57BL/6 mice were irradiated lethally with two doses of 550 cGy 3 h apart, and were reconstituted with 5×10^6 T cell–depleted OT-I.SOCS-1 bone marrow cells. T cell depletion was performed by incubating with anti-CD4 (RL172), anti-CD8 (3.168), and anti-Thy1 (J1j) mAbs and treatment with rabbit complement. On day 1 after reconstitution, all mice were injected i.p. with 100 μg anti-Thy1 mAb (T24) to eliminate radioresistant host T cells. The mice were left for 8–10 wk before use.

Female H-Y.SOCS-$1^{-/-}$.RAG-$1^{-/-}$ mice were moribund at 2–3 wk of age, before bones were processed easily for bone marrow harvesting. Therefore, H-Y.SOCS-1→B6 chimeras were prepared using whole spleen cells from 2–3-wk-old H-Y.SOCS-1 neonates. This approach was not necessary for OT-I-SOCS-$1^{-/-}$RAG-$1^{-/-}$ mice, which live for 3–6 wk and can provide sufficient bone marrow for chimera generation.

Carboxy fluorescein diacetate succinimidyl ester labeling adoptive transfer and FACS analysis. CD8 T cells, from lymph nodes and spleen, were enriched by incubation with anti-CD4 (RL172) and anti-HSA (J11d) mAb followed by rabbit complement. For carboxy fluorescein diacetate succinimidyl ester (CFSE)-labeling, cells were resuspended at 10^7 cells/ml in 0.1% BSA/PBS and incubated with 5 μM CFSE stock solution (5 mM in DMSO; Invitrogen) for 10 min at 37°C. $1–2 \times 10^6$ CFSE-labeled T cells were injected i.v. into unmanipulated C57BL/6 mice or mice that had been exposed to 700 cGy whole body irradiation 3–4 h earlier. 5–7 d later, lymph nodes and/or spleens were harvested and analyzed by flow cytometry on a FACScan or BD-LSR (Becton Dickinson) instrument. Antibodies used for flow cytometry staining were CD44, CD4, CD8, Vα2-TCR (Caltag Labs; BD Biosciences) and H-Y-TCR (T1/70) (provided by A. Strasser, Walter and Eliza Hall Institute of Medical Research, Victoria, Australia).

For the adoptive transfer of sorted CD44 subsets, thymocytes or lymph node and spleen CD8 T cells were enriched by magnetic bead depletion of CD4 T cells and B cells using anti-CD4 (GK1.5) and anti-HSA (J11d) (lymph node and spleen only) antibodies, followed by anti–rat IgG and anti–mouse IgG magnetic beads (QIAGEN). Subsequent CD44 separation was achieved using anti-CD44 antibody and either MACS microbeads and autoMACS columns (Miltenyi Biotec) or MoFlo (DakoCytomation) flow cytometry sorting. Sorted cell populations were labeled with CFSE before transfer to recipient mice.

For the adoptive transfer into TAP$1^{-/-}$ mice, the residual CD8 T cells had to be depleted to prevent rejection of the transferred OT-I cells expressing high MHC class I levels relative to the TAP$1^{-/-}$ hosts (49). TAP1 and C57BL/6 controls were given a "low-dose" injection of 50 μl anti-CD8 mAb ascites, 15 d before adoptive transfer of OT-I.SOCS-1.IFN-$\gamma^{-/-}$ thymocytes. Mice were bled on the day of transfer to determine the remaining CD8 T cell population.

Cross-tolerance deletion experiments. 5×10^6 OT-I.SOCS-1→B6 T cells were enriched for CD8 T cells, by antibody and complement depletion, and injected i.v. into recipient mice. After 1 d or 6 weeks, spleen and lymph nodes were removed and assessed for the number of OT-I T cells present by staining with anti-TCR antibodies or tetramers. For antibody staining, anti–Vβ5-FITC (MR9-40), anti–CD8-Pe (Caltag Labs), and anti–Vα2-biotin (B20.1) revealed with Streptavidin Tricolor (Caltag Labs) were used. For tetramers, OT-I T cells were revealed by staining with K^b-$OVA_{257-264}$ tetramer-PE and anti–CD8-FITC (Caltag Labs). The total number of OT-I T cells was determined from the formula: (% OT-I in the $CD8^+$ cells of adoptively transferred mice − background) × (% $CD8^+$ T cells in total live cells) × total cell number/10,000. Background was the percentage $TCRV\alpha2^+$ or $tetramer^+$ cells in uninjected mice; on average, this was 1.4% for mAb and 0.1% for tetramer-stained cells. Total cell numbers were determined by flow cytometry using a known number of small nonfluorescent Sphero beads (BD Biosciences) added to a known volume of the cell sample. Therefore, cells/ml = number cells collected × (numbers beads in sample/number beads collected) × 1/sample volume.

Diabetes. Lymph node T cells from OT-I.SOCS-1→B6 mice, enriched by antibody and rabbit complement depletion, were adoptively transferred by i.v. injection into the lateral tail vein of RIP-mOVA mice. Two doses of cells were used, either 5×10^6 or 0.1×10^6 $CD8^+$ cells per mouse. Recipient mice were monitored for diabetes, by urine glucose testing, from day 5 after transfer. Animals were monitored for 3 mo and were considered diabetic after two consecutive days with readings ≥55 mmol/L.

We thank L. Coverdale, J. Langley, K. Jordan, L. Inglis and J. Lochland for their technical assistance.

This work was funded by National Institutes of Health grant nos. CA22556 (to

W.S. Alexander), AI41079, AI45809, and AG20186 (to C.D. Surh); the National Health and Medical Research Council of Australia Program grant no. 215402 and project grant nos. 257500 and 208981; and a Howard Hughes Medical Institute International Fellowship (to W.R. Heath).

The authors have no conflicting financial interests.

Submitted: 3 January 2005
Accepted: 29 August 2005

REFERENCES

1. Naka, T., M. Narazaki, M. Hirata, T. Matsumoto, S. Minamoto, A. Aono, N. Nishimoto, T. Kajita, T. Taga, K. Yoshizaki, et al. 1997. Structure and function of a new STAT-induced STAT inhibitor. *Nature*. 387:924–929.
2. Starr, R., T.A. Willson, E.M. Viney, L.J. Murray, J.R. Rayner, B.J. Jenkins, T.J. Gonda, W.S. Alexander, D. Metcalf, N.A. Nicola, and D.J. Hilton. 1997. A family of cytokine-inducible inhibitors of signalling. *Nature*. 387:917–921.
3. Endo, T.A., M. Masuhara, M. Yokouchi, R. Suzuki, H. Sakamoto, K. Mitsui, A. Matsumoto, S. Tanimura, M. Ohtsubo, H. Misawa, et al. 1997. A new protein containing an SH2 domain that inhibits JAK kinases. *Nature*. 387:921–924.
4. 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.* 18:1309–1320.
5. Kamura, T., S. Sato, D. Haque, L. Liu, W.G. Kaelin Jr., R.C. Conaway, and J.W. Conaway. 1998. The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. *Genes Dev.* 12: 3872–3881.
6. Zhang, J.G., A. Farley, S.E. Nicholson, T.A. Willson, L.M. Zugaro, R.J. Simpson, R.L. Moritz, D. Cary, R. Richardson, G. Hausmann, et al. 1999. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proc. Natl. Acad. Sci. USA*. 96:2071–2076.
7. Alexander, W.S. 2002. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat. Rev. Immunol.* 2:410–416.
8. Fujimoto, M., and T. Naka. 2003. Regulation of cytokine signaling by SOCS family molecules. *Trends Immunol.* 24:659–666.
9. 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: 4115–4122.
10. 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 gamma c-dependent cytokines in peripheral T cells. *J. Biol. Chem.* 278:22755–22761.
11. 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: 2435–2445.
12. Starr, R., D. Metcalf, A.G. Elefanty, M. Brysha, T.A. Willson, N.A. Nicola, D.J. Hilton, and W.S. Alexander. 1998. Liver degeneration and lymphoid deficiencies in mice lacking suppressor of cytokine signaling-1. *Proc. Natl. Acad. Sci. USA*. 95:14395–14399.
13. 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*. 18:475–487.
14. Naka, T., T. Matsumoto, M. Narazaki, M. Fujimoto, Y. Morita, Y. Ohsawa, H. Saito, T. Nagasawa, Y. Uchiyama, and T. Kishimoto. 1998. Accelerated apoptosis of lymphocytes by augmented induction of Bax in SSI-1 (STAT-induced STAT inhibitor-1) deficient mice. *Proc. Natl. Acad. Sci. USA*. 95:15577–15582.
15. 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 gamma signaling and prevents the potentially fatal neonatal actions of this cytokine. *Cell*. 98:597–608.
16. Bullen, D.V., R. Darwiche, D. Metcalf, E. Handman, and W.S. Alexander. 2001. Neutralization of interferon-gamma in neonatal $SOCS1^{-/-}$ mice prevents fatty degeneration of the liver but not subsequent fatal inflammatory disease. *Immunology*. 104:92–98.
17. 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*. 98:609–616.
18. Metcalf, D., S. Mifsud, L. Di Rago, N.A. Nicola, D.J. Hilton, and W.S. Alexander. 2002. Polycystic kidneys and chronic inflammatory lesions are the delayed consequences of loss of the suppressor of cytokine signaling-1 (SOCS-1). *Proc. Natl. Acad. Sci. USA*. 99:943–948.
19. 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-gamma and IL-4 signaling in vivo. *Immunity*. 14:535–545.
20. Eyles, J.L., D. Metcalf, M.J. Grusby, D.J. Hilton, and R. Starr. 2002. Negative regulation of interleukin-12 signaling by suppressor of cytokine signaling-1. *J. Biol. Chem.* 277:43735–43740.
21. Cornish, A.L., G.M. Davey, D. Metcalf, J.F. Purton, J.E. Corbin, C.J. Greenhalgh, R. Darwiche, L. Wu, N.A. Nicola, D.I. Godfrey, et al. 2003. Suppressor of cytokine signaling-1 has IFN-gamma-independent actions in T cell homeostasis. *J. Immunol.* 170:878–886.
22. 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*. 19:437–450.
23. Goldrath, A.W., and M.J. Bevan. 1999. Low-affinity ligands for the TCR drive proliferation of mature $CD8^+$ T cells in lymphopenic hosts. *Immunity*. 11:183–190.
24. Ernst, B., D.S. Lee, J.M. Chang, J. Sprent, and C.D. Surh. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity*. 11:173–181.
25. Kieper, W.C., and S.C. Jameson. 1999. Homeostatic expansion and phenotypic conversion of naive T cells in response to self peptide/MHC ligands. *Proc. Natl. Acad. Sci. USA*. 96:13306–13311.
26. Rocha, B., and H. von Boehmer. 1991. Peripheral selection of the T cell repertoire. *Science*. 251:1225–1228.
27. Tanchot, C., F.A. Lemonnier, B. Perarnau, A.A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science*. 276:2057–2062.
28. Tan, J.T., B. Ernst, W.C. Kieper, E. LeRoy, J. Sprent, and C.D. Surh. 2002. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. *J. Exp. Med.* 195:1523–1532.
29. Goldrath, A.W., P.V. Sivakumar, M. Glaccum, M.K. Kennedy, M.J. Bevan, C. Benoist, D. Mathis, and E.A. Butz. 2002. Cytokine requirements for acute and basal homeostatic proliferation of naive and memory CD8+ T cells. *J. Exp. Med.* 195:1515–1522.
30. Heath, W.R., and F.R. Carbone. 2001. Cross-presentation in viral immunity and self-tolerance. *Nat. Rev. Immunol.* 1:126–134.
31. Kurts, C., R.M. Sutherland, G. Davey, M. Li, A.M. Lew, E. Blanas, F.R. Carbone, J.F. Miller, and W.R. Heath. 1999. $CD8^+$ T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc. Natl. Acad. Sci. USA*. 96:12703–12707.
32. Kurts, C., H. Kosaka, F.R. Carbone, J.F. Miller, and W.R. Heath. 1997. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. *J. Exp. Med.* 186: 239–245.
33. Davey, G.M., C. Kurts, J.F. Miller, P. Bouillet, A. Strasser, A.G. Brooks, F.R. Carbone, and W.R. Heath. 2002. Peripheral deletion of autoreactive CD8 T cells by cross presentation of self-antigen occurs by a Bcl-2-inhibitable pathway mediated by Bim. *J. Exp. Med.* 196:947–955.

34. Belz, G.T., G.M. Behrens, C.M. Smith, J.F. Miller, C. Jones, K. Lejon, C.G. Fathman, S.N. Mueller, K. Shortman, F.R. Carbone, and W.R. Heath. 2002. The CD8alpha(+) dendritic cell is responsible for inducing peripheral self-tolerance to tissue-associated antigens. *J. Exp. Med.* 196:1099–1104.
35. Kurts, C., J.F. Miller, R.M. Subramaniam, F.R. Carbone, and W.R. Heath. 1998. Major histocompatibility complex class I-restricted cross-presentation is biased towards high dose antigens and those released during cellular destruction. *J. Exp. Med.* 188:409–414.
36. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F. Miller, and H. Kosaka. 1996. Constitutive class I-restricted exogenous presentation of self antigens in vivo. *J. Exp. Med.* 184:923–930.
37. Kurts, C., F.R. Carbone, M.F. Krummel, K.M. Koch, J.F. Miller, and W.R. Heath. 1999. Signalling through CD30 protects against autoimmune diabetes mediated by CD8 T cells. *Nature.* 398:341–344.
38. Tan, J.T., E. Dudl, E. LeRoy, R. Murray, J. Sprent, K.I. Weinberg, and C.D. Surh. 2001. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc. Natl. Acad. Sci. USA.* 98:8732–8737.
39. Vivien, L., C. Benoist, and D. Mathis. 2001. T lymphocytes need IL-7 but not IL-4 or IL-6 to survive in vivo. *Int. Immunol.* 13:763–768.
40. Berard, M., K. Brandt, S. Bulfone-Paus, and D.F. Tough. 2003. IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. *J. Immunol.* 170:5018–5026.
41. Oldstone, M.B., M. Nerenberg, P. Southern, J. Price, and H. Lewicki. 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. *Cell.* 65: 319–331.
42. Ohashi, P.S., S. Oehen, K. Buerki, H. Pircher, C.T. Ohashi, B. Odermatt, B. Malissen, R.M. Zinkernagel, and H. Hengartner. 1991. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. *Cell.* 65:305–317.
43. Hogquist, K.A., S.C. Jameson, W.R. Heath, J.L. Howard, M.J. Bevan, and F.R. Carbone. 1994. T cell receptor antagonist peptides induce positive selection. *Cell.* 76:17–27.
44. Bluthmann, H., P. Kisielow, Y. Uematsu, M. Malissen, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz. 1988. T-cell-specific deletion of T-cell receptor transgenes allows functional rearrangement of endogenous alpha- and beta-genes. *Nature.* 334:156–159.
45. Spanopoulou, E., C.A. Roman, L.M. Corcoran, M.S. Schlissel, D.P. Silver, D. Nemazee, M.C. Nussenzweig, S.A. Shinton, R.R. Hardy, and D. Baltimore. 1994. Functional immunoglobulin transgenes guide ordered B-cell differentiation in Rag-1-deficient mice. *Genes Dev.* 8:1030–1042.
46. Kennedy, M.K., M. Glaccum, S.N. Brown, E.A. Butz, J.L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C.R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191:771–780.
47. von Freeden-Jeffry, U., P. Vieira, L.A. Lucian, T. McNeil, S.E. Burdach, and R. Murray. 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J. Exp. Med.* 181:1519–1526.
48. Van Kaer, L., P.G. Ashton-Rickardt, H.L. Ploegh, and S. Tonegawa. 1992. TAP1 mutant mice are deficient in antigen presentation, surface class I molecules, and CD4-8^+ T cells. *Cell.* 71:1205–1214.
49. Aldrich, C.J., H.G. Ljunggren, L. Van Kaer, P.G. Ashton-Rickardt, S. Tonegawa, and J. Forman. 1994. Positive selection of self- and alloreactive CD8+ T cells in Tap-1 mutant mice. *Proc. Natl. Acad. Sci. USA.* 91:6525–6528.

Author/s:
Davey, GM; Starr, R; Cornish, AL; Burghardt, JT; Alexander, WS; Carbone, FR; Surh, CD; Heath, WR

Title:
SOCS-1 regulates IL-15-driven homeostatic proliferation of antigen-naive CD8 T cells, limiting their autoimmune potential

Date:
2005-10-17

Citation:
Davey, G. M., Starr, R., Cornish, A. L., Burghardt, J. T., Alexander, W. S., Carbone, F. R., Surh, C. D. & Heath, W. R. (2005). SOCS-1 regulates IL-15-driven homeostatic proliferation of antigen-naive CD8 T cells, limiting their autoimmune potential. JOURNAL OF EXPERIMENTAL MEDICINE, 202 (8), pp.1099-1108. https://doi.org/10.1084/jem.20050003.

Persistent Link:
http://hdl.handle.net/11343/242534

File Description:
published version