Genetic reduction of embryonic leukemia-inhibitory factor production rescues placentation in SOCS3-null embryos but does not prevent inflammatory disease

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The suppressor of cytokine-signaling (SOCS) proteins act as negative-feedback inhibitors of cytokine and growth-factor-induced signal transduction. *In vivo* studies have implicated SOCS3 as a negative regulator of signaling downstream of gp130, the receptor subunit shared by IL-6-like cytokines. Mice lacking SOCS3 die at midgestation because of placental failure, and SOCS3 ablation in a cell-type-specific manner results in changes in the functional outcome of gp130 signaling in response to IL-6. In this study, we show that genetic reduction of leukemia-inhibitory factor (LIF) production by embryo-derived tissues is sufficient to prevent the placental defect. This establishes LIF signaling as a major physiological regulator of trophoblast differentiation *in vivo*. Mice deficient in both SOCS3 and LIF are born in predicted numbers and appear normal at birth but exhibit failure to thrive and high neonatal mortality. Adult SOCS3-null mice on a LIF-null background succumb to a spontaneous fatal inflammatory disease characterized by neutrophilia and inflammatory-cell tissue infiltrates. The disease spectrum mimics that seen in mice with a conditional deletion of SOCS3 in hematopoietic and endothelial cells, extending the evidence for a major role for SOCS3 in the homeostatic regulation of the inflammatory response and indicates that LIF is not required for this process.

placenta | inflammation | neutrophilia | trophoblast

Inflammation is a critical response to tissue injury and is required for tissue repair and clearance of infection. Cytokines play key roles in modulating inflammation, acting through receptors of the cytokine-receptor superfamily, which phosphorylate downstream Janus kinases (JAKs) that, in turn, activate the signal transduction and activators of transcription (STATs). Homeostasis requires negative regulation of cytokine signaling to limit inflammation and continued tissue injury. In recent years, the suppressor of cytokine signaling (SOCS) proteins have emerged as important negative regulators of cytokine signaling (1, 2). The expression of SOCS genes is controlled by STAT transcription factors, thereby creating a negative-feedback loop to suppress cytokine signaling (3–5). SOCS proteins are characterized by an SH2 domain and a C-terminal SOCS box. The SH2 domain interacts with sites of phosphorylation on receptors or JAKs, thereby competing with other proteins and blocking catalytic activity. In addition, SOCS proteins may target other proteins for proteasomal degradation through recruitment of ubiquitination complexes via the SOCS box (6, 7).

Mice with a null mutation of SOCS3 ($SOCS3^{-/-}$) die at midgestation because of placental failure (8–10). It was shown that genetic reduction of leukemia-inhibitory factor (LIF receptor) signaling rescues the placental defect and enables SOCS3 null mice to survive to term (10). The ligands known to signal through the murine LIF receptor/gp130 complex are LIF, ciliary neurotrophic factor, cardiotrophin (CT-1), CT-1-like cytokine, and neuropoietin (11, 12). However, it was not clear which of these ligands was responsible for the abnormalities observed in SOCS3-null placentas. LIF was a likely candidate because it can regulate trophoblast differentiation *in vitro* (10). To test this hypothesis, we intercrossed $SOCS3^{+/-}$ mice with $LIF^{-/-}$ mice (13).

A role for SOCS3 in inflammation was demonstrated by the generation of mice with a conditional deletion of SOCS3 in hematopoietic cells. SOCS3-null hematopoietic progenitor cells exhibit enhanced responsiveness to G-CSF, and the mice develop neutrophilia and inflammatory disease (14). Experimental autoimmune arthritis requires G-CSF signaling, and it has been shown that adenoviral overexpression of SOCS3 in joints can ameliorate this disease (15). Recently, intracellular delivery of SOCS3 protein was shown to protect mice from the lethal effects of staphylococcal enterotoxin B and lipopolysaccharide by reducing the production of inflammatory cytokines (16).

In this study, we show that genetic reduction of embryonic LIF production is sufficient to rescue the lethal placental abnormalities seen in $SOCS3^{-/-}$ mice, demonstrating that LIF signals in the embryonic component of the developing placenta are finely tuned to regulate trophoblast maturation *in vivo*. In addition, we demonstrate that mice with global deletion of SOCS3 on a LIF-null background develop a fatal inflammatory disease similar to that seen in mice with a conditional deletion of SOCS3 in hematopoietic and endothelial cells.

Materials and Methods

Mice. Mice with genetic deletion of the LIF gene were obtained from Phillipe Brûlet (Institut Pasteur, Paris) and were crossed seven generations onto C57BL6 (13). Because $LIF^{-/-}$ females are infertile, the colony was maintained by breeding $LIF^{+/-}$ females with $LIF^{-/-}$ males. The generation of SOCS3-null mice, which are on a C57BL/6 background, is described in ref. 9. $SOCS3^{+/-}$ females were bred to $LIF^{+/-}$ or $LIF^{-/-}$ males and offspring typed by PCR. To generate mice for analysis, $SOCS3^{+/-}LIF^{+/-}$ females were bred with $SOCS3^{+/-}LIF^{+/-}$ or $SOCS3^{+/-}LIF^{-/-}$ males. The mice were kept under a controlled photoperiod of 12 h light/12 h dark, with food and water supplied ad libitum. For timed matings, female mice were placed overnight with males, and pregnancy was determined by detection of a vaginal plug (day 0 of pregnancy). Experiments were approved by the Melbourne Health Animal Ethics Committee.

Histology and *in Situ* Hybridization. For histological analysis, whole embryos, neonates, or adult mice organs were fixed in 10% buffered formalin, blocked in paraffin, sectioned, and stained

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Abbreviations: E, embryonic day; LIF, leukemia-inhibitory factor; SOCS, suppressor of cytokine-signaling; CSF, colony-stimulating factor; G-CSF, granulocyte CSF.

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Table 1. Genotypes of 132 E19 embryos from intercrosses of SOCS3$^{+/-}$LIF$^{+/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ males

		Total (n = 132)	
SOCS3	LIF	Expected	Observed
+/+	−/−	16.5	18
−/−	−/−	16.5	17
+/−	−/−	33	34
+/+	+/−	16.5	12
−/−	+/−	16.5	19
+/−	+/−	33	32

with hematoxylin and eosin. Embryos were serially sectioned. Placental tissues were fixed with 4% paraformaldehyde, positioned by embedding in agarose before processing, and serially sectioned. *In situ* hybridization was performed with digoxigenin-labeled riboprobes on paraffin-embedded sections that were first dewaxed and digested with Proteinase K (Roche Diagnostics, Mannheim, Germany) and then postfixed, acetylated, and dehydrated.

Hematologic Analysis. Analysis of orbital-plexus blood was performed by using an ADVIA 120 blood analyzer equipped with a mouse analysis software module (Bayer, Tarrytown, NY). Neonatal white cell and platelet counts were determined manually. Differential counts were performed manually on May–Grünwald–Giemsa-stained blood smears and cytocentrifuge preparations of femoral bone-marrow, spleen, and peritoneal cells. Clonal cultures of hematopoietic cells were performed as described in ref. 17. Briefly, cultures of 2.5×10^4 bone-marrow cells or 5×10^4 spleen cells in DMEM with a final concentration of 20% selected newborn-calf serum and 0.3% agar were stimulated by various cytokines and, after gelling, were incubated for 7 days at 37°C in a fully humidified atmosphere of 10% CO_2 in air. The final concentrations of growth factors used were 10 ng/ml granulocyte/macrophage CSF, 10 ng/ml G-CSF, 10 ng/ml macrophage CSF, 10 ng/ml IL-3, 100 ng/ml IL-6, 2 units/ml erythropoietin, or 100 ng/ml stem cell factor.

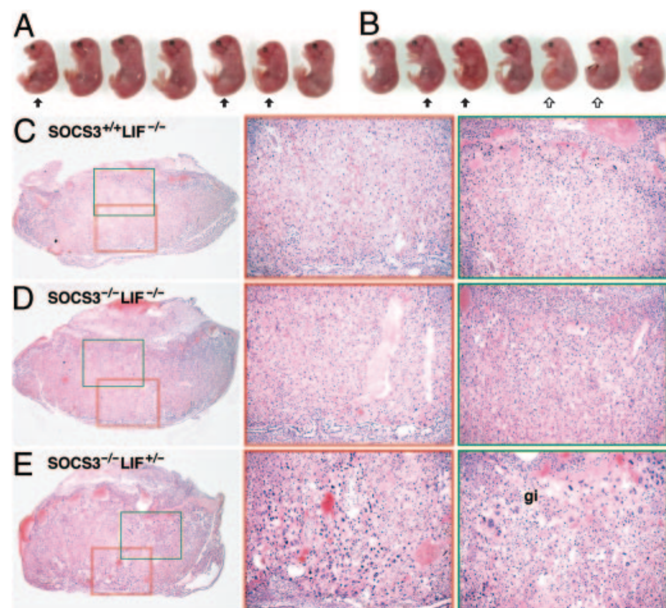

Fig. 1. Genetic reduction of LIF production rescues the placental defect in SOCS3-null embryos and permits normal development of SOCS3-null embryos. (*A* and *B*) E19 pups from matings of SOCS3$^{+/-}$LIF$^{+/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ males. SOCS3$^{-/-}$LIF$^{-/-}$ (filled arrows) or SOCS3$^{-/-}$LIF$^{+/-}$ pups (open arrows) were indistinguishable from littermates. (*C–E*) SOCS$^{+/+}$LIF$^{-/-}$, SOCS3$^{-/-}$LIF$^{-/-}$, and SOCS3$^{-/-}$LIF$^{+/-}$ E17.5 placental histology. On a LIF-null background, the SOCS3-null placenta is indistinguishable from wild-type (data not shown) or LIF$^{-/-}$ placentas. On a LIF-heterozygous background, the labyrinthine layer is disrupted and contains numerous trophoblast giant cells (gi).

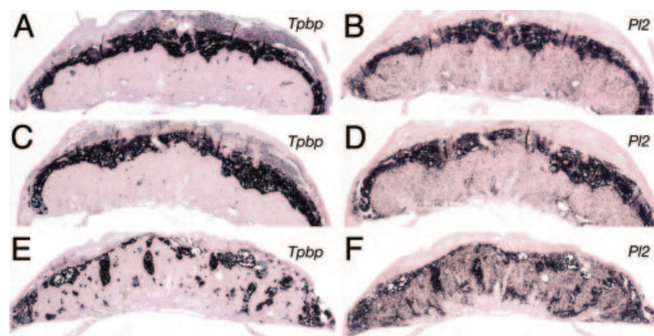

Fig. 2. Expression of trophoblast-specific markers in SOCS3-null placentas on LIF-null and LIF-heterozygous backgrounds. *In situ* hybridization of trophoblast-specific markers in E17.5 wild-type (*A* and *B*), SOCS3$^{-/-}$LIF$^{-/-}$ (*C* and *D*), and SOCS3$^{-/-}$LIF$^{+/-}$ (*E* and *F*) placentas.

Results

Genetic Reduction of LIF Rescues the Placental Abnormalities in SOCS3-Null Embryos. The genotypes of 132 embryonic day (E)19 pups obtained from timed matings of SOCS3$^{+/-}$LIF$^{-/-}$ female mice with SOCS3$^{+/-}$LIF$^{-/-}$ male mice are shown in Table 1. Whereas SOCS3$^{-/-}$ embryos die by E13.5 (8, 9), predicted numbers of SOCS3$^{-/-}$LIF$^{-/-}$ and SOCS3$^{-/-}$LIF$^{+/-}$ pups were present at term. The weight and gross anatomy of the rescued SOCS3$^{-/-}$ embryos did not differ from littermates, and detailed histological examination did not reveal abnormalities in the SOCS3-null embryos (Fig. 1 *A* and *B* and data not shown). Histological examination of the placentas of embryos derived from intercrosses of SOCS3$^{+/-}$LIF$^{+/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ or SOCS3$^{+/-}$LIF$^{+/-}$ males revealed that SOCS3$^{-/-}$LIF$^{-/-}$ placentas were morphologically indistinguishable from wild-type and LIF$^{-/-}$ placentas. In contrast, although adequate to support normal embryonic development, the placentas of SOCS3$^{-/-}$LIF$^{+/-}$ embryos were abnormal, with an increased number of trophoblast giant cells and patchy disruption of the labyrinthine layer by spongiotrophoblast cells and trophoblast giant cells (Fig. 1 *C–E*). *In situ* hybridization demonstrated increased numbers of cells expressing the trophoblast giant-cell marker *Pl2* within the labyrinth of SOCS3$^{-/-}$LIF$^{+/-}$ placentas. In wild-type and SOCS3$^{-/-}$LIF$^{-/-}$ placentas, cells expressing the spongiotrophoblast marker *Tpbp* were largely confined to the spongiotrophoblast layer, but, in SOCS3$^{-/-}$LIF$^{+/-}$ placentas, the spongiotropholast layer was sparse

Table 2. Genotypes of 339 pups from intercrosses of SOCS3$^{+/-}$LIF$^{+/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ males that survived to weaning (3 weeks)

		Total (n = 375)	
SOCS3	LIF	Expected	Observed
+/+	−/−	46.9	44
−/−	−/−	46.9	7
+/−	−/−	93.7	74
+/+	+/−	46.9	81
−/−	+/−	46.9	2
+/−	+/−	93.7	167

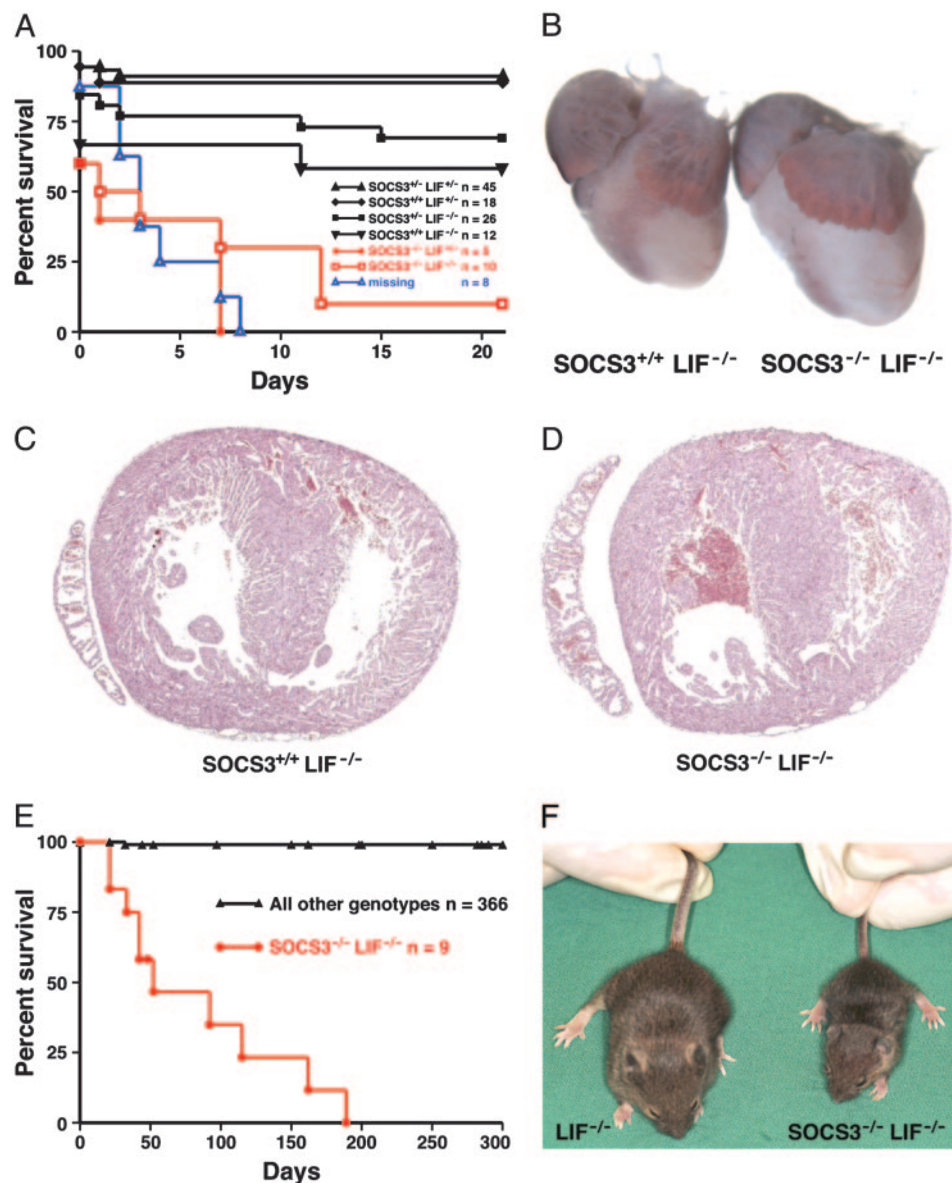

Fig. 3. Survival of SOCS3$^{-/-}$ mice on a LIF-null background. (*A*) Survival curves of pups arising from an intercross of SOCS3$^{+/-}$LIF$^{-/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ males from postnatal day (P)0 to weaning. Pups were obtained by caesarean section and fostered. missing, pups of unknown genotype that were cannibalized; n = number of mice surveyed in each group. (*B–D*) Hearts from SOCS$^{-/-}$LIF$^{-/-}$ and SOCS$^{+/+}$LIF$^{-/-}$ pups found dead on P2. Transverse sections at the level of the ventricles did not reveal enlargement of the SOCS$^{-/-}$LIF$^{-/-}$ heart (note that, at P2, the body weight of SOCS$^{-/-}$LIF$^{-/-}$ and SOCS$^{+/+}$LIF$^{-/-}$ pups does not differ). (*E*) Postweaning survival of mice arising from an intercross of SOCS3$^{+/-}$LIF$^{-/-}$ females with SOCS3$^{+/-}$LIF$^{-/-}$ males. SOCS$^{-/-}$LIF$^{-/-}$ or LIF$^{+/-}$ mice are compared with mice of other genotypes (SOCS$^{+/-}$LIF$^{-/-}$, SOCS$^{+/+}$LIF$^{-/-}$, SOCS$^{+/+}$LIF$^{+/-}$, and SOCS$^{+/-}$LIF$^{+/-}$). Note that there is no excess in the mortality in LIF-null mice after weaning age. (*F*) Adult SOCS$^{-/-}$LIF$^{-/-}$ mice are growth retarded in comparison with SOCS3$^{+/+}$LIF$^{-/-}$ littermates.

and disorganized, and clusters of *Tpbp*-expressing cells were present in the labyrinthine layer (Fig. 2). These results implicate embryonic LIF as the key ligand responsible for the placental pathology observed in SOCS3-null embryos. The presence of abnormalities in SOCS3$^{-/-}$ placentas heterozygous for the LIF gene underscores the sensitivity of the trophoblast lineage to signals generated by the LIF/LIF receptor/gp130 complex.

Reduced Survival of SOCS3$^{-/-}$LIF$^{-/-}$ Mice. It is possible to overcome the SOCS3-null placental defect by tetraploid embryo complementation. This technique circumvents the defect in the extraembryonic tissues (18). In a previous study, a small number of SOCS3-null pups derived in this way were studied (10). All were growth retarded, exhibited marked cardiac hypertrophy, and died by 3 weeks of age. In this study, we observed that whereas, on a LIF$^{+/-}$ or LIF$^{-/-}$ background, expected numbers of viable SOCS3$^{-/-}$ embryos were observed at E19, very few SOCS3-null pups were present at weaning (Table 2). To ascertain when the SOCS3$^{-/-}$ pups were dying, dams were killed at E19 and the pups fostered and observed closely. The newborn pups were accepted by the foster mothers and suckled normally. However, by the end of the postnatal day 0, 40% of SOCS3-null pups were dead. Survival of SOCS3$^{+/-}$LIF$^{-/-}$ pups was also reduced and was comparable with that of LIF$^{-/-}$ pups (Fig. 3*A* and data not shown). Analysis of peripheral-blood from SOCS3$^{-/-}$LIF$^{-/-}$ pups was unremarkable. Dead pups were autopsied and sectioned. No specific pathology was observed that might have accounted for the increased perinatal death. No structural abnormalities were seen, and cardiac hypertrophy was not observed (Fig. 3 *B–D*).

Table 3. Colony formation by bone-marrow cells from SOCS3$^{-/-}$LIF$^{-/-}$ mice in response to cytokine stimulation

Genotype	Stimulus	No. of colonies formed					
		Blast	G	GM	M	EO	MEG
SOCS3$^{-/-}$LIF$^{-/-}$ (n = 4)	GM-CSF	0.5 ± 1.0	26 ± 6	11 ± 4	32 ± 8	4 ± 3	
	G-CSF		26 ± 9*	0.5 ± 0.6	2 ± 2		
	M-CSF		5 ± 2	7 ± 1	67 ± 20		
	IL-3	9 ± 5	22 ± 4	18 ± 5	24 ± 7	2 ± 2	10 ± 6
	SCF	5 ± 1	22 ± 1	1 ± 0	2 ± 1		
	IL-6	1 ± 2	11 ± 3	12 ± 2†	2 ± 2		
	SCF IL-3 EPO	12 ± 6	29 ± 5	21 ± 6	20 ± 7	2 ± 1	25 ± 2
SOCS3$^{+/+}$LIF$^{-/-}$ (n = 4)	GM-CSF		11 ± 4	3 ± 1	25 ± 0	0 ± 0	
	G-CSF		6 ± 0	0 ± 0	0 ± 0		
	M-CSF		3 ± 3	3 ± 1	48 ± 21		
	IL-3	5 ± 0	13 ± 2	8 ± 3	14 ± 1	1 ± 1	2 ± 1
	SCF	4 ± 1	10 ± 2	0 ± 0	0 ± 0		
	IL-6		8 ± 1	1 ± 1	0 ± 0		
	SCF IL-3 EPO	8 ± 6	13 ± 7	10 ± 2	15 ± 3	2 ± 1	15 ± 3
SOCS3$^{-/\Delta}$ (n = 6)	GM-CSF	2 ± 1	25 ± 10	13 ± 4	28 ± 13	4 ± 3	
	G-CSF	0.5 ± 0.8	23 ± 10†	2 ± 1	3 ± 1		
	M-CSF		7 ± 5	6 ± 3	59 ± 22		
	IL-3	10 ± 4	28 ± 8	20 ± 8	18 ± 12	4 ± 2	9 ± 3
	SCF	8 ± 4	17 ± 3	3 ± 1	3 ± 2		
	IL-6	0.5 ± 0.8	11 ± 7	10 ± 6‡	2 ± 2		
	SCF IL-3 EPO	14 ± 4	24 ± 9	18 ± 6	18 ± 6	4 ± 1	29 ± 9
SOCS3$^{-/fl}$ (n = 5)	GM-CSF	0.3 ± 0.6	13 ± 4	7 ± 2	22 ± 6	2 ± 2	
	G-CSF		9 ± 3	0.6 ± 0.9	0.6 ± 0.9		
	M-CSF		3 ± 2	3 ± 2	45 ± 8		
	IL-3	7 ± 2	16 ± 7	10 ± 3	11 ± 1	1 ± 0	6 ± 2
	SCF	6 ± 3	12 ± 3	2 ± 1	2 ± 2		
	IL-6		7 ± 3	2 ± 2	2 ± 2		
	SCF IL-3 EPO	10 ± 2	18 ± 5	10 ± 6	13 ± 5	1 ± 1	27 ± 6

A total of 25,000 cells in agar culture were stimulated by the cytokines listed, and colonies were enumerated after 7 days. Colonies were fixed, stained, and identified as blast (B), granulocyte (G), granulocyte/macrophage (GM), macrophage (M), eosinophil (EO), or megakaryocyte (MEG). SCF, stem cell factor; EPO, erythropoietin.

*$P < 0.05$ vs. SOCS3$^{+/+}$LIF$^{-/-}$.

†$P < 0.05$ vs. SOCS3$^{-/fl}$.

Some SOCS3$^{-/-}$ mice on a LIF-null background survived for several months after birth, although all were dead by 190 days (Fig. 3*E*). As had been previously observed, the adult LIF$^{-/-}$ mice were 10–15% smaller than wild-type mice. SOCS3$^{-/-}$LIF$^{-/-}$ mice were even smaller, weighing 35.7 ± 6.7% that of age- and sex-matched LIF-null littermates (Fig. 3*F*). In contrast to the previous study, SOCS3$^{-/-}$LIF$^{-/-}$ mice did not exhibit cardiac hypertrophy. In the neonatal period, the mean body weight and heart size of SOCS3$^{-/-}$LIF$^{-/-}$ mice did not differ from littermates. After postnatal day 5, the SOCS3$^{-/-}$LIF$^{-/-}$ mice were obviously smaller than littermates. However, the heart size of the older, growth-retarded SOCS3$^{-/-}$LIF$^{-/-}$ mice, expressed as weight as a percentage of total body weight, was not statistically different from that of SOCS3$^{+/+}$LIF$^{-/-}$ littermates (SOCS3$^{-/-}$LIF$^{-/-}$ 0.86 ± 0.1%, n = 7; SOCS3$^{+/+}$LIF$^{-/-}$ 0.77 ± 0.2%, n = 7). The cardiac hypertrophy observed in tetraploid complementation-derived SOCS3-null pups may have resulted from failure to down-regulate cardiotropin or LIF signaling, both of which have been implicated in induction of cardiac hypertrophy (19, 20). Absence of this pathology in the SOCS3$^{-/-}$LIF$^{-/-}$ pups implicates LIF as the likely mediator of this process. Alternatively, other differences in the genetic background of the mice used in the two studies may have contributed to the absence of a cardiac phenotype.

Bone Marrow Cells from Adult SOCS3$^{-/-}$LIF$^{-/-}$ Mice Show Enhanced G-CSF-Induced Colony Formation. Growth of SOCS3$^{-/-}$LIF$^{-/-}$ bone-marrow cells in semisolid agar cultures in response to a range of cytokines was compared with bone marrow from littermate controls and from mice with a conditional deletion of SOCS3 in hematopoietic cells (SOCS3$^{-/\Delta}$) and their littermates (SOCS3$^{-/fl}$) (14). As had been observed, there were distinct abnormalities in cultures of SOCS3$^{-/\Delta}$ bone-marrow cells: a selective increase in the number of granulocyte-colony-forming cells in cultures stimulated with G-CSF, an increase in the number of granulocyte-macrophage-colony-forming cells in cultures stimulated with IL-6, and an increased number of macrophages in colonies arising in G-CSF- and IL-6-stimulated cultures (Table 3). These abnormalities were also seen in cultures of SOCS3$^{-/-}$LIF$^{-/-}$ bone marrow, indicating that LIF signaling does not contribute to this phenotype.

Table 4. Peripheral-blood profiles

Parameter	SOCS3$^{-/-}$LIF$^{-/-}$*	SOCS3$^{+/+}$LIF$^{-/-}$†
Peripheral blood		
Platelets, $\times 10^6$/ml	1,162 ± 230	1,558 ± 480
Hemoglobin, g/liter	14.1 ± 1.4	12.0 ± 1.6
White blood cell count, $\times 10^6$/ml‡	28.1 ± 17.5	10.0 ± 2.1
Neutrophils‡	18.2 ± 0.9	2.8 ± 0.8
Lymphocytes	8.8 ± 4.5	7.4 ± 1.0
Monocytes	1.0 ± 0.5	0.3 ± 0.1
Eosinophils	0.1 ± 0.1	0.1 ± 0.1

*, n = 9; †, n = 6; ‡, $P < 0.001$.

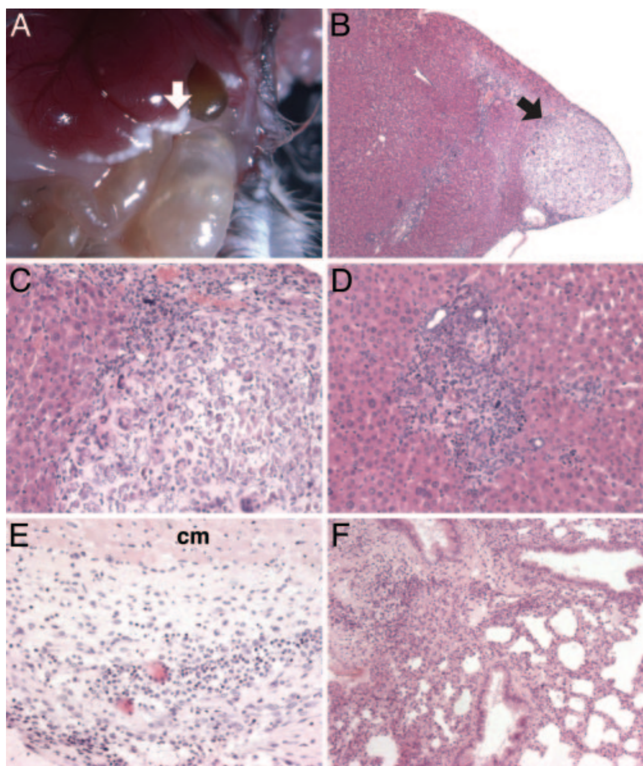

Fig. 4. Adult SOCS$^{-/-}$LIF$^{-/-}$ or LIF$^{+/-}$ mice succumb to inflammatory disease. (*A*) Inflammatory lesions (arrows) along the liver edge in a SOCS$^{-/-}$LIF$^{-/-}$ mouse. (*B* and *C*) Section through the lesion shown in *A* reveals granuloma formation. (*D*) Microabscesses in liver parenchyma. (*E*) Massive pericardial thickening due to infiltration by inflammatory cells. cm, cardiac muscle. (*F*) Peribronchial inflammation and thickening of alveolar walls.

SOCS3$^{-/-}$LIF$^{-/-}$ Mice Develop Inflammatory Disease. SOCS3$^{-/-}$LIF$^{-/-}$ mice that survived the neonatal period lived for a further 2–6 months before succumbing to inflammatory disease. Peripheral-blood profiles from SOCS3$^{-/-}$LIF$^{-/-}$ mice showed a marked neutrophilia (Table 4). At autopsy, sick SOCS3$^{-/-}$LIF$^{-/-}$ mice had splenomegaly, and six of the nine mice examined had macroscopic lesions along the liver edge and thoracic pleura (Fig. 4*A*). Histological examination revealed that these lesions were granulomas (Fig. 4*B*). One mouse had a florid pericarditis (Fig. 4*E*). All nine sick SOCS3$^{-/-}$LIF$^{-/-}$ mice examined had focal aggregates of neutrophils, lymphocytes, and plasma cells in the liver parenchyma, increased neutrophils in bone marrow, and areas of consolidation in the lung (Fig. 4 *D* and *F*). Sections from age- and sex-matched littermates of other genotypes killed and examined in parallel did not contain inflammatory lesions (data not shown).

Discussion

Gene-targeting experiments have revealed that SOCS3 is a major physiological regulator of signaling by gp130. When SOCS3 was ablated in a cell-type-specific manner, it resulted in prolonged STAT3 activation and consequent changes in the functional outcome of gp130 signaling in response to IL-6 (21–23). Embryonic stem cells lacking SOCS3 had altered responses to LIF signaling, resulting in diminished self-renewal and increased potential for differentiation (24). In this study, we demonstrate that SOCS3 is required for regulation of embryo-derived LIF signaling during placental development. In the absence of SOCS3, the placental spongiotrophoblast layer was reduced, and excess trophoblast giant cells were present. Genetic reduction of LIF production by embryonic tissues completely reversed this phenotype, providing definitive evidence for the role of LIF in trophoblast differentiation during placental development.

LIF has pleiotropic functions (25, 26). The generation of LIF-null mice revealed that maternal LIF is absolutely required for embryo implantation. Consequently, LIF-deficient female mice are infertile, and this can be reversed by provision of exogenous LIF at the implantation window (27, 28). Studies of LIF-null mice have established that LIF regulates stress-hormone response in the pituitary (29) and is required for recovery of muscle and neuronal tissue from injury (30–33). A role for LIF in support of motor-neuron induction has been uncovered by analysis of mice lacking LIF and ciliary neurotrophic factor (CNTF) or LIF, CNTF, and cardiotrophin (34, 35), and one study found a reduction in the number of trigeminal motoneurons in E18.5 LIF-null embryos (36). Whereas the overall anatomy of the brains of LIF-null mice is normal, glial cells are reduced in number (37). LIF-null mice that survive to weaning are growth retarded but have a normal lifespan and do not exhibit any major hematopoietic abnormalities (ref. 13 and our unpublished observations). LIF can mediate aspects of the peripheral and central nervous systems and hypothalamic–pituitary–adrenal axis responses to inflammation. However, LIF-null mice do not spontaneously develop inflammatory disease, although they are hyperresponsive to LPS-mediated shock (38–41).

LIF-null mouse strains have been generated independently by three groups, all of whom reported reduced numbers of homozygous offspring arising from heterozygous intercrosses (13, 26, 27, 42, 43). It is likely that the decreased survival of LIF-null mice in the neonatal period is a reflection of the interplay between physiological abnormalities and environment and genetic background (43). In this study, we observed that SOCS3 deficiency further increased neonatal mortality. In a study by Takahashi *et al.* (10), SOCS3$^{-/-}$ pups rescued by tetraploid complementation died in the neonatal period and exhibited marked cardiac hypertrophy resembling cardiomyopathy, attributed to dysregulated LIF or cardiotropin signaling. In contrast, we did not observe cardiac enlargement in SOCS3$^{-/-}$LIF$^{-/-}$ or LIF$^{+/-}$ pups dying in the neonatal period or in adult SOCS3$^{-/-}$LIF$^{-/-}$ mice, suggesting that LIF, rather than cardiotropin, was the primary mediator of neonatal cardiac hypertrophy in SOCS3$^{-/-}$ pups and concurring with studies showing that SOCS3 overexpression can suppress cardiac hypertrophy induced by LIF (44).

The neutrophilia and the spectrum of inflammatory pathologies observed in SOCS3$^{-/-}$ mice on a LIF-null background was similar to that observed in mice with a conditional deletion of SOCS3 in hematopoietic cells (14). It is likely that it is due to aberrant responses of multiple hemopoietic cell types to G-CSF and other cytokines, and new mouse models with lineage-specific deletions of SOCS3 will be required to dissect this finding further. SOCS3, like other SOCS proteins, can be induced by a large number of cytokines, and its expression has been linked to inhibition of action of many cytokines (2). However, it is difficult to interpret the biological significance of these data because they are often derived from studies using *in vitro* systems and employing ectopic expression of SOCS proteins. Gene-targeting studies in mice continue to pinpoint essential *in vivo* roles of SOCS proteins and indicate that there is little physiological redundancy. This study demonstrates that SOCS3 is a key regulator of LIF signaling during placental development and, in adult life, plays an essential role in the negative regulation of the inflammatory response.

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