Early-onset autoimmunity associated with SOCS1 haploinsufficiency

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Autoimmunity can occur when a checkpoint of self-tolerance fails. The study of familial autoimmune diseases can reveal pathophysiological mechanisms involved in more common autoimmune diseases. Here, by whole-exome/genome sequencing we identify heterozygous, autosomal-dominant, germline loss-of-function mutations in the SOCS1 gene in ten patients from five unrelated families with early onset autoimmune manifestations. The intracellular protein SOCS1 is known to downregulate cytokine signaling by inhibiting the JAK-STAT pathway. Accordingly, patient-derived lymphocytes exhibit increased STAT activation in vitro in response to interferon-γ, IL-2 and IL-4 that is reverted by the JAK1/JAK2 inhibitor ruxolitinib. This effect is associated with a series of in vitro and in vivo immune abnormalities consistent with lymphocyte hyperactivity. Hence, SOCS1 haploinsufficiency causes a dominantly inherited predisposition to early onset autoimmune diseases related to cytokine hypersensitivity of immune cells.

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The immune system is tightly regulated by many central and peripheral checkpoints, in order to prevent reactivity to self-antigens. Their defects can lead to the expansion of self-reactive B and T lymphocytes, immune system over-activation, and thus autoimmune diseases. Several monogenic defects leading to the breakdown of immune tolerance and autoimmunity have been identified, showing that a number of proteins are involved in non-redundant inhibitory checkpoints. Moreover, the study of familial autoimmune diseases can reveal pathophysiological mechanisms also involved in more common autoimmune diseases.

Cytokines, including interleukins and interferons (IFNs), participate in the development, differentiation, and effector functions of lymphoid and myeloid cells. To do so, cytokines activate the Janus kinase (JAK)-STAT pathway that drives the transcription of cytokine-inducible genes. Prolonged signaling (particularly by proinflammatory cytokines) can be harmful, requiring tight regulation of these pathways. The intracellular, inducible suppressors of cytokine signaling (SOCS) family of proteins is part of this negative-feedback system. The SOCS family has eight members, of which four are cytokine-inducible genes.

Results

Clinical features and immunological findings. We assessed ten patients from five unrelated kindreds presenting early onset autoimmune manifestations and lymphoproliferation resulting in impaired SOCS1 function, increased STAT activation, and cytokine hypersensitivity of immune cells.

Fig. 1 Pedigrees and genetics of families with SOCS1 mutations. a Pedigrees of families with SOCS1 mutations. Squares: males; circles: females; black: affected mutation carriers; gray: unaffected mutation carriers. WT: wild-type SOCS1 allele. b Clinical manifestations in patients with SOCS1 mutations. Discoid lupus erythematosus (D1, upper left), active lupus nephritis (E1, lower left) with segmental cellular crescent associated with mesangial C1q deposition by immunofluorescence microscopy, abdominal MRI showing splenomegaly (C1, middle), and plaque, intertrigous and guttata psoriasis (E4, right). (upper panel, black arrows). The kinase inhibitory region (KIR domain) functions as a pseudosubstrate that can inhibit the tyrosine kinase activity of Janus kinase (JAK) proteins. The SOCS box recruits the ubiquitin-ligase system and initiates the proteasomal degradation of captured substrates. SOCS1 is the most potent member of the SOCS family, and is the primary downregulator of cytokines involved in immune responses—particularly those that signal through the yc-chain (the IL-2 family) and interferons. In addition to exerting ubiquitin ligase activity, SOCS1 directly inhibits JAK kinase activity through its kinase inhibitory region (KIR domain).

Here, using whole-exome/genome sequencing, we identify heterozygous germline SOCS1 mutations in ten patients from five unrelated families with early onset autoimmune manifestations and SOCS1 mutations (see below) (Fig. 1a). The patients’ main characteristics are summarized in Table 1 and cases are described in more detail in the Supplementary Notes. With the exception of one (E5), the first symptoms appeared during childhood (median range) age: 7.5 years.
(2–44)) in all patients. Five patients (A1, A2, B1, C1, and E2) had autoimmune cytopenia, four patients (A2, B2, E4, and E5) had organ-specific autoimmunity (thyroiditis, coeliac disease, psoriasis, spondyloarthritis, and hepatitis), and two patients (D1 and E1) met the diagnostic criteria for systemic lupus erythematosus (SLE) with skin (discoid lupus) and kidney (lupus glomerulonephritis) involvement (Fig. 1b). Two patients (B1, and C1) had benign lymphoproliferation (lymphadenopathy and/or hepatosplenomegaly (Fig. 1b)), whereas one (B2) developed Hodgkin lymphoma. None had infectious complications, with the exception of recurrent bronchopulmonary infections in B1.

Identification of SOCS1 mutations. Whole-exome or genome sequencing was performed on whole blood DNA samples from six patients (A1, A2, B1, C1, D1, and E1). Heterozygous germline SOCS1 mutations were identified in all six patients, including three missense mutations in families A (c.368 C > G, p.P123R), D (c.64 C > T, p.R22W) and E (c.460 T > C, p.Y154H), respectively, and two frameshift mutations in families B (c.24delA, p.A9fs*76) and C (c.476_480dupGCCGC, p.M161fs*46) (Fig. 1a, Supplementary Fig. 2A). All SOCS1 variants were predicted to be deleterious, with a high CADD (Combined Annotation-Dependent Depletion) score above the cutoff score of 11.63 indicating mutational significance6, and were absent or very rare in public gene mutation databases (Supplementary Fig. 2b). Two mutations (in families B and D) were located at the 5′ end of the kinase inhibitory region, two (in families A and E) were located in the SH2 domain, and one (in family C) was located at the 5′ end of the SOCS-box domain (Fig. 1c). Three distinct missense variants (in families A, D, and E) affected phylogenetically conserved amino acids (Supplementary Fig. 2C). It is noteworthy that residues P123 and Y154 are located within the groove (Fig. 1d) that bears phosphorylated JAK peptides. A model of the SOCS1 three-dimensional (3D) protein structure showed that P123 and Y154 are located within the groove (Fig. 1d) that binds phosphorylated JAK peptides with high affinity5. 3D structure information was not available for R22, belonging to a disordered segment. In view of its position (upstream of the kinase inhibitory region), this mutation could perhaps perturb binding to JAK peptides. Details of the SOCS1 mutants’ 3D modeling and
analysis are given in the Supplementary Notes and in Supplementary Fig. 3-4. Mutations were then identified in four additional patients (B2, E2, E4, and E5) as well as five apparently healthy carriers. The familial segregation thus indicated autosomal-dominant inheritance with incomplete clinical penetrance (Fig. 1a).

The impact of SOCS1 mutations on protein expression was analyzed using patient-derived EBV-transformed B cell lines and HEK293T cells transfected with the various SOCS1 alleles. Ectopic expression of the p.A95Ffs*76 mutation (family B) did not yield detectable protein levels (Fig. 1e bottom and Supplementary Fig. 5) while lower expression was detected in the corresponding EBV-B mutant cell line, indicating haploinsufficiency (Fig. 1e top). The frameshift mutation in family C yielded a detectable protein containing a predicted 46-residues neoepitope within the SOCS box domain and a predicted premature stop codon removing the last three amino acid residues (Fig. 1c–e). Other mutants were normally expressed in both EBV-B cells and HEK293 T cells.

Consequence of SOCS1 mutations on STAT pathway activation. To examine the impact of the SOCS1 mutations on the regulation of the STAT pathways in patients’ lymphocytes, EBV-B cells from A1, B2, and D1 were stimulated with various cytokines and STAT phosphorylation was determined as a surrogate of activation. Of note, constitutive phosphorylation of the STAT1, STAT5 and STAT6 proteins was not detected. However, levels of IFN-γ-induced STAT1 phosphorylation, IL-2-induced STAT5 phosphorylation, and IL-4-induced STAT6 phosphorylation were higher in patients’ cells than in control cells (Fig. 2a, b and Supplementary Fig. 6). Consistently, we observed elevated nuclear translocation of P-STAT1 and P-STAT5 upon stimulation with IFN-γ and IL-2, respectively (Fig. 2c). Furthermore, expression of the IFN-γ inducible CXCL9 and CXCL10 genes and the IL-2-inducible CIS and PIM1 genes was upregulated in patient cells following cytokine stimulation (Fig. 2d). Accordingly, no baseline hyperactivity was detected in patients’ primary monocytes but IFN-γ-induced STAT1 phosphorylation was also moderately but sig-

**Fig. 2 SOCS1 mutations result in uncontrolled STAT pathways activation.** a, b Left: Western blots (WB) of patients (A1, B2, and D1) and healthy controls (CT) derived EBV-B cells stimulated with IFN-γ (103IU/ml for 1 h) (a) or IL-2 (104IU/ml for 2 h) (b). Lysates were incubated with an antibody against tyrosine-phosphorylated STAT (P-STAT) or against total STAT, as indicated. Right: densitometric quantification of the phospho-STAT/α-tubulin or β-actin ratio upon stimulation. a, b Data are representative of n = 3 (patients B2 and D1), n = 4 (A1, IL-2 stimulation), and n = 6 (A1, IFNγ stimulation) independent experiments. Statistics (versus CT2): IFNγ stimulation, A1 p = 0.0008, B1 p = 0.0029, D1 p = 0.0002; IL-2 stimulation, A1 p < 0.0001, B1 p = 0.0118, D1 p < 0.0001. e The nuclear and cytoplasmic fractions of EBV-B cells from a control (CT) and from patient A1 after stimulation with IFN-γ for 1 h (left) or with IL-2 for 2 h (right) were tested by WB for the presence of P-STAT1 and P-STAT5, respectively. Anti-lamin A/C and anti-α-tubulin antibodies were used to normalize the amount of nuclear and cytoplasmic proteins. Data are representative of two independent experiments. d Real-time quantitative RT-PCR assays of CXCL9 and CXCL10 expression 6 h after stimulation with IFN-γ (left), and assays of CIS and PIM1 expression 6 h after stimulation with IL-2 (right) in EBV-B cells from a CT and from patient A1. Results represent the fold-increased expression between stimulated and unstimulated states and are normalized to endogenous GAPDH. Data are representative of n = 3 (IL-2 stimulation) and n = 4 (IFNγ stimulation) independent experiments performed in triplicate. Statistics: IFNγ stimulation, CXCL9 p = 0.0094, CXCL10 p = 0.0063; IL-2 stimulation, PIM1 p = 0.0105, CIS p = 0.0008. e Firefly luciferase activity in HEK293T cells transiently transfected with a gamma-activated sequence-driven IFN-γ reporter plasmid (GAS) and expression plasmids for WT or mutant SOCS1 proteins, and then stimulated with IFN-γ for 24 h. The results correspond to the fold-difference between the stimulated state and the unstimulated state. Results represent at least n = 4 independent experiments. All constructs were compared with WT SOCS1. Protein expression from the transfected plasmids was confirmed by immunoblotting the cell lysates (below, one representative result). Statistics: EV p < 0.0001, P123R p < 0.0001, A95F*76 p < 0.0001, M161F*46 p < 0.0001, R22W p = 0.0011, Y154H p = 0.0009. a, b, d, e Two-tailed p values were determined in an unpaired t test. Data indicate mean with SD. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001; ****P ≤ 0.0001.
Effect of SOCS1 haploinsufficiency on T cells. Given the lymphoproliferation observed in families B and C, we measured the proliferation of the patients’ T cells in response to various stimuli in vitro. The patients’ T cells proliferated more intensely than control T cells in response to stimulation with various concentrations of IL-2 but not in response to T-cell receptor stimulation with anti-CD3 antibodies (Fig. 4a). This hyperproliferation in response to IL-2 was not associated with increased expression of CD25, the alpha chain of the IL-2 receptor and a STAT5 target gene, neither on non-activated primary T cells nor on activated T cells blasts (Supplementary Fig. 10), suggesting an increased receptor internalization and/or shedding of CD25 in the presence of IL-2, or that SOCS1 deficiency may be counterbalanced by another mechanism yet to be determined.

In mice, Socs1 was previously shown to be involved in regulatory T cell (Treg) integrity and function, by maintaining Foxp3 expression and by suppressing Stat1 and Stat3 activation9. Moreover, socs1 deletion in Foxp3+ cells in the mouse is sufficient to induce lymph node enlargement and splenomegaly10, prompting us to analyze Tregs in patients. The mean CD4+ CD25+FOXP3+ Tregs frequency was lower in patients with SOCS1 haploinsufficiency (Fig. 4b). This reduction in Tregs frequency was not due to the expansion of memory CD4 T cells (Fig. 4c). With the exception of CTLA-4, the expression of several key Treg markers (including FOXP3, HELIOS, and, to a lesser extent, CD25) was lower in patients’ Tregs (Fig. 4d and Supplementary Fig. 11). Furthermore, the Tregs suppressive activity was moderately but significantly reduced in the patients’ Tregs cells (A2, D1, E1) as compared with control cells (Fig. 4e and Supplementary Fig. 12). Thus, SOCS1 deficiency leads to a defective Treg compartment. Despite SOCS1’s putative role in CD4+ T cell differentiation11, we did not observe an obvious imbalance in Th1, Th2, Th17, and T follicular helper cells (Supplementary Fig. 13). No impact of SOCS1 deficiency on T cell apoptosis was observed (Supplementary Fig. 14).

Immunological features in healthy mutation carriers. We identified five unaffected family members carrying the same SOCS1 mutation as their affected relatives (Fig. 1a). At the time of

Fig. 3 Inflammatory cytokine signature in serum from patients with SOCS1 mutations. Cytokine array analysis of serum from patients with SOCS1 insufficiency, controls, patients with STAT1 GOF mutations, and patients with STAT3 GOF mutations. Gray: symptomatic patients; white: asymptomatic carriers. Decimal logarithms of the values determined in a multiplex bead assay were color-coded as follows: for each individual cytokine, median values obtained in the 17 HCs were de

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the genetic analysis, these healthy carriers (B3, C2, D2, D3, E3) were 10, 54, 23, 52, and 62 years old, respectively. Four (C2, D2, D3, E3) were considered to be completely asymptomatic. One carrier (B3) developed severe asthma at 10 with an elevated serum IgE. Immunologic phenotyping performed in four carriers (B3, C2, D2, and E3) revealed similarities with the patients including low marginal zone and/or switch memory B-cell counts in three of them and elevated CD21low CD38low B-cell count in two (Supplementary Table 1 and Supplementary Fig. 1). One (E3) of the two healthy carriers tested had autoantibodies (antinuclear and anti-SSA autoantibodies). Serum analysis from four of the carriers showed an inflammatory cytokine signature similar to patients (Fig. 3). Finally, IFN-γ-induced STAT1 phosphorylation in primary monocytes was higher than controls in two of the four tested healthy carriers (Supplementary Fig. 7). Taken together these data suggest that the immunological and functional phenotype is relatively penetrant even though the clinical phenotype is not.

**Discussion**

We found that SOCS1 haploinsufficiency is associated with the hyperactivation of JAK kinases through SOCS1-sensitive cytokine receptors—a finding thus with potential therapeutic value.
germline mutations leading to haploinsufficiency result in (i) higher JAK-STAT pathway activation in response to different cytokines, (ii) T cell hyperproliferation in response to IL-2, and (iii) a defective Treg compartment. Our observation of multiple missense and frameshift mutations in patients with autoimmune features strongly suggests that these mutations are pathogenic. Furthermore, our present results in humans are in line with murine studies. In particular, Socs1−/− mice exhibited neonatal lethality due to excessive IFN-γ signaling and massive inflammatory infiltration. Moreover, haploinsufficient mice survive into adulthood but develop a systemic autoimmune disease with age, reminiscent of human SLE. Our findings are also in agreement with a recent study identifying, by whole-genome sequencing in a large primary immunodeficiencies cohort, three patients with autoimmune manifestations and SOCS1 haploinsufficiency.

The assessment of the molecular consequences of single-gene mutations has provided a better understanding of the mechanisms underlying autoimmunity in humans. Multiple defects can lead to a breakdown in immune tolerance, which includes the impairment of negative T-cell selection, defective lymphocyte apoptosis, a loss of B cell tolerance, abnormal Treg function, uncontrolled activation of T cells, and accentuated immune effector cell functions. Our observations in SOCS1 insufficiency provide mechanistic insight into the development of autoimmunity induced by cytokine hypersensitivity in immune cells following the loss of a downregulatory element. STAT1 and STAT3 GOF mutations could also be included in this category, given that they enhance cytokine signaling and function. Interestingly, the clinical phenotypes of SOCS1 insufficiency and STAT3 GOF mutations have some common features (including lymphadenopathy and autoimmune cytopenia) but also possibly differences (SLE in SOCS1 insufficiency vs. enteropathy and diabetes with STAT3 GOF mutations). This may reflect the scope of the various cytokine signaling pathways involved. We have not seen increased infection susceptibility in the SOCS1 mutant patients, but this has to be confirmed in larger cohorts.

Several mechanisms can be proposed to explain autoimmunity in the context of SOCS1 insufficiency. In mice, deletion of Socs1 in T cells is both necessary and sufficient to promote autoimmunity. Mechanistically, this might be due to defective Treg development and function in the absence of SOCS1, as previously shown in the mouse and here in humans. However, mice with a conditional knock-out of Socs1 in Treg exhibit a milder autoimmune phenotype than mice in which Socs1 has been deleted in all T cells—suggesting that the cytokine hypersensitivity of conventional T cells also contributes to autoimmunity. A role for SOCS1 in cytokine regulation of B cells is also plausible and may account, at least in part, for the abnormal B cell phenotype. Myeloid cells are plausibly also involved, since the autoimmune phenotype is more severe when Socs1 is knocked out in both T cells and macrophages. Moreover, SOCS1 deficiency in dendritic cells alone leads to the production of high levels of BAFF, the aberrant expansion of B cells, and the production of autoreactive antibody, and systemic autoimmunity. Hence, given the widespread expression of SOCS1 and the cytokines’ orchestrating role in the immune system, several cell types probably contribute to autoimmunity in the context of SOCS1 haploinsufficiency.

Of the 15 individuals with a heterozygous SOCS1 mutation studied here, five (including four adults) were healthy carriers (33%). This incomplete clinical penetrance is frequently observed...
in autosomal-dominant primary immunodeficiencies caused by haploinsufficiency (e.g. CTLA-424,25, IKZF1/IKAROS26 or in TNFRSF6/FAS deficiencies27, which have penetrance of 67%, 65%, and 30%, respectively). The age at onset differs in individuals carrying the same mutation, and the clinical manifestations sometimes appear as late as the sixth decade of life25,26,28. This incomplete penetrance may indicate the requirement for additional genetic28 or epigenetic events and/or the involvement of infectious/environmental events to induce full clinical expression of the disease29.

Several monogenic primary immunodeficiencies have been linked to an increased risk of malignancies30. This could be related to treatments with immunosuppressants and/or the gene product’s role in tumor surveillance27. Somatic loss-of-function mutations in SOCS1 (including 2 mutated residues found in families A and E: P123 and Y15428) are frequently observed in B-cell or Hodgkin lymphoma, and are associated with activation of the JAK-STAT pathway in tumor cells31–33. It is noteworthy that one patient in family B developed Hodgkin lymphoma at the age of 34, whereas benign lymphoproliferation was seen in two other patients with SOCS1 mutation. This observation should prompt the physicians to carefully monitor patients with SOCS1 deficiency and their healthy carrier relatives.

SOCS1-insufficient patients have been treated with various nonspecific immunosuppressive agents, some of which are associated with toxic effects. Given the molecular etiology of the autoimmune and allergic disorders35,36.

Whole-genome sequencing was performed by Edinburgh Genomics. Genomic DNA was prepared from whole blood samples from probands and parents, using the Quagen Mini Kit according to the manufacturer’s instructions. Genomic DNA (gDNA) samples were evaluated for quantity using Quant-IT PicoGreen reagent, Lambda Standard DNA, and a Spectramax XPS Gemini plate reader. Quality was Enriched using AATI Fragment analyzer and the Standard Sensitivity DNA Analysis Kit. Samples with >100 ng and a quality score of >5 passed the sample quality control. Sequencing libraries were prepared using a specific Illumina SeqLab TruSeq Nano High Throughput kit. A total of 200 ng of gDNA was normalized and sheared to 450 bp mean insert size. TruSeq adapters were ligated onto the ends of each fragment prior to PCR amplification. Caliper GX Touch with an HT DNA HI SENSE Reagent Kit was used to produce mean fragment sizes of 530–950 bp. Libraries were normalized to 1.5 nM and sequenced at 300 pM. Demultiplexing was performed by the Illumina29.

The raw sequencing data were quality-checked using FastQC and MultiQC, and subsequently aligned with the GRCh37 hs375s reference using the Edico Dragen V2 single-sample workflow. Indels and SNPs were called using the same workflow on an FPGA AWS instance. The resulting variant call format files were then uploaded to the Congenica platform via the latter’s Upload Client (v1.7.4) for annotation and causal variant prioritization. Variants were annotated using the Exome Aggregator, Variant Effect Predictor, and SnpEff to assess their functional impact.

For each variant in the proband, we determined the mode of inheritance in the family trio (de novo, autosomal recessive homozgyous, autosomal recessive compound heterozygous, or X-linked recessive). DoNovoGear was used for the joint analysis of samples with unclassified variants in order to detect de novo mutations. Variants with qualifying genotypes under each mode of inheritance were ranked using the Exomiser combined score and evaluated for rarity (MAF in ExAC and UK10K), pathogenicity (Polyphen2 and SIFT scores), genic intolerance to functional variation (RVIS and pLI scores) and biological relevance in the context of the proband’s phenotype (using a Phenotype Consensus Analysis and a literature search). A rule-based scheme was used to identify candidate genes.

The pathogenicity of the identified variants was defined according to the minor allele frequency in the general population according to the GnomAD database (https://gnomad.broadinstitute.org/); and the Combined Annotation-Dependent Depletion (CADD) score37.

Sanger sequencing was performed to confirm the next-generation sequencing results and to analyze each mutation’s familial segregation. The primers used for PCR are reported in Supplementary Table 1. Purified PCR products were directly sequenced using BigDye Terminators (version 1.1) and a 3500xl Genetic Analyzer (Applied Biosystems).

Three-dimensional structure modeling. The model of the human SOCS1 3D structure was built on the basis of the experimental 3D structure of Chicken SOCS1 (71 % sequence identity, pdb 6C7Y), according to the alignment given in Supplementary Figure 2. The possible path of a phosphorytoseptide, represented in purple in Figure 1d (bottom view), with the side chain of the phosphophorysine shown, was taken from the experimental 3D structure of the SOCS3/p113 complex (Protein Data Bank 2HM4). The model of the whole JAK1/SOCS1/elongin B/elongin C complex shown in Figure 1d (top) is based on the chicken SOCS1/JAK1 (Protein Data Bank 6C7Y) and X. laevis SOCS1/Elongin B/Elongin C (Protein Data Bank 6CSX) 3D structures. Modeling was made using Modeller v9.228 and 3D visualisation using Chimera29.

Cell culture. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll separation (Lonza) and then cultured in Panserin 401 medium (Duchefa) with 5% human AB serum, 100 ng/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific) at 37 °C in a humidified 5% CO2 incubator. Expansion of T cell blasts was obtained by incubating PBMCs for 72 h with CD3/CD28 beads (LifeTechnologies) in complete Panserin. After 3 days, dead cells were removed by Ficoll-Plaque density gradient centrifugation. Complete Panserin supplemented with IL-2 (10 IU/mL). Epstein–Barr virus (EBV)–transformed B lymphoblastoid cell lines (EBV-B cells) were generated from PBMCs obtained from patients (A1, B1, and D1) and HCUs using standard methods and then cultured in RPMI 1640 (LifeTechnologies) supplemented with 10% fetal bovine serum (FBS) and penicillin/ streptomycin.

Human embryonic kidney (HEK) 293T cells were cultured in DMEM (LifeTechnologies) supplemented with 10% FBS and penicillin/streptomycin.
**Flow cytometry analysis.** For surface staining, PBMCs (either freshly isolated or thawed after storage in liquid nitrogen) were washed with cold PBS and then incubated for 30 min at 4°C (in the dark) with a fluorescein-labeled monoclonal antibodies. For intracellular staining, PBMCs were fixed and permeabilized after surface staining using the FoxP3 staining kit (eBioscience) according to the manufacturer’s instructions, and then incubated with anti-FOXP3 and anti-CTLA4 for 30 min on ice. The antibodies used for flow cytometry are listed in Table 2. All samples were acquired using a flow cytometer (Becton Dickinson LSRFortessa X-20) and the data were analyzed using the FlowJo software (TreeStar).

**Expression plasmids, mutagenesis, and transfection experiments.** Complementary DNA from WT human SOCS1 (SOCS1; NM_003745) was subcloned into the mammalian expression vector pCMV-HA-tagged (Clontech). The indicated SOCS1 mutants were generated using the Q5 site-directed mutagenesis kit protocol (NEB). All cDNA sequences were confirmed by Sanger sequencing. Wild-type or mutant SOCS1 plasmids were transfected into HEK293T cells by transient transfection using lipofectamine 2000 (Life Technologies).

**Western blot analysis.** The EBV-B cells were exposed to various stimuli prior to protein extraction. The following optimal stimulation conditions were used: 60 min with 10^3 IU/ml IFN-γ, 120 min with 10^4 IU/ml IL-2, 60 min with 10^5 IU/ml IL-4, 45 min with 10 and 100 ng/ml IL-21, and 60 min with 100 ng/ml IL-6. For blocking experiments, cells were preincubated with ruxolitinib (500 nM) for an hour prior to stimulation. Cell activation was blocked with cold 1X PBS, and then lysed in RIPA lysis buffer supplemented with a protease and phosphatase inhibitor cocktail (ThermoFisher Scientific). The extracted proteins were quantified using a Pierce BCA assay kit (ThermoFisher Scientific), subjected to SDS-PAGE, and transferred to a PVDF membrane. For the extraction of nuclear and cytoplasmic proteins, EBV-B cells were stimulated with INFγ for 1 h or IL-2 for 2 h and extracted with NE-PER Nuclear and Cytoplasmic Extraction Regents (ThermoFisher Scientific), according to the manufacturer’s instructions. After blocking, the membrane was incubated overnight with the following primary antibodies: anti-SOCS1 (Cell Signaling Technology), anti-phosphorylated STAT1 Y701 (p-STAT1; Cell Signaling Technology), anti-STAT1 (Cell Signaling Technology), anti-p-STAT3 Y705 (p-STAT3; Cell Signaling Technology), anti-STAT3 (Cell Signaling Technology), anti-p-STAT5 Y694 (p-STAT5, Cell Signaling Technology), anti-phosphorylated STAT1 Y701 (P-STAT1, ThermoFisher Scientific), anti-GAPDH (Cell Signaling Technology), anti-HA (Cell Signaling Technology), anti-α-tubulin (Cell Signaling Technology), anti-β-actin (ThermoFisher Scientific), anti-GAPDH (Cell Signaling Technology), anti-HA (Cell Signaling Technology), and anti-lamin A/C (Cell Signaling Technology). The results were visualized by chemiluminescence using species-specific HRP-linked secondary antibodies.

**Real-time quantitative RT-PCR.** The EBV-B cells were stimulated for 6 h with 10^3 IU/ml IFN-γ or 10^4 IU/ml IL-2. Total RNA was isolated using the RNeasy Mini Kit (Qiagen), and cDNA was prepared using the Quantitect Reverse Transcription Kit (Qiagen) after depletion of genomic DNA. Real-time quantitative PCR was performed using the LightCycler VIIA7 System (Roche). Gene transcript levels were determined using a luciferase reporter vector under the control of the interferon-γ response element (MFI) in CD14+ cells or CD33+ cells. The EBV-B cell lines were stimulated with INFγ for 1 h or IL-2 for 2 h and stimulated by Staphylococcus enterotoxin E (500 nM) or stimulated by Staphylococcus enterotoxin F (500 nM). The cells were cultured for 4 days, washed with PBS, and stained with anti-CD3, CD4, CD8, CD25, and CD69 antibodies prior to flow cytometry measurement.

**Treg cell suppression assay.** PBMCs were incubated for 30 min at 4°C with specific, labeled monoclonal antibodies, washed, and then sorted using an ARIA II cytometer (BD Biosciences). naive T effector cells (Teff) were defined as CD3+ CD4+ CD25− CD127+ CD45RA+ T cells, Tregs were defined as CD3+ CD4+ CD25+ CD127− T cells, and dendritic cells (DCs) were defined as CD3− CD4− CD14+ CD16+ CD11c+ cells. After cell sorting, Teff was washed and stained with CellTrace CFSE. Next, the cells were incubated with DCs (Teff:DC ratio 1.0:4) and stimulated by Staphylococcus enterotoxin E (0.2 ng/ml). The cells were cultured for 5 days in complete Panserin medium (Dutscher) in 96-well plates and the proportion of proliferating cells was measured with a MACSquant system (Miltenyi). The data were analyzed using FlowJo software. The percentage of suppression was calculated with the following formula: [log_{2}(y) of (Teff cells alone) − log_{2}(y) of (Teff + Treg cells)]/log_{2}(y) of (Teff cells alone) × 100. The y value corresponds to the mean fluorescence intensity of the CFSE of the whole Teff cell population, divided by the mean fluorescence intensity of the CFSE of undivided Teff cells.

**Cytoxins.** Serum levels of the cytokines EGF, eotaxin-1, FGF-2, Fth-3L, fractalkine, G-CSF, GM-CSF, GRO(alpha), IFN-alpha, IFN-gamma, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-12(p40), IL-13, IL-15, IL-17A, IL-18, IP-10, MCP-1, MCP-3, MDC, MIP-1a, MIP-1p, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, TGFα, TNFα, TNFβ, VEGF-A were determined using a multiplex bead assay (Human Cytokine Array/Chromekine Array 42-Plex with IL-18 (HD42), Ewe Technologies; Canada).

**Statistics.** All statistical analyses were performed using GraphPad Prism (version 6). As indicated in the Figures, results were analyzed in a two-tailed, unpaired or paired Student’s t test, or a two-sided Mann–Whitney U test. For all analyses, the threshold for statistical significance was set to p < 0.05.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

SOCS1 variants sequences are deposited in the ClinVar database [https://www.ncbi.nlm.nih.gov/clinvar/variant/603597/ (MMB)] with the following accession codes SCV001430913, SCV001430914, SCV001430915, SCV001430916, SCV001430917. Gnomad database (https://gnomad.broadinstitute.org) was used to calculate the minor allele frequency of all the mutations. PopViz webservice (http://shiva.rockefeller.edu/PopViz/) was used to calculate the CADD score and the mutation significance (MSC) cutoff of all the mutations. The datasets generated and/or analyzed during the current study are available from the corresponding authors on reasonable request. Source data are provided with this paper.

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