Cytokines exert biological functions by activating Janus tyrosine kinases (JAKs), and JAK inhibitors JAB (also referred to as SOCS1 and SS1) and CIS3 (SOCS3) play an essential role in the negative regulation of cytokine signaling. We have found that transgenic (Tg) mice expressing a mutant JAB (F59D-JAB) exhibited a more potent STAT3 activation and a more severe colitis than did wild-type littermates after treatment with dextran sulfate sodium. We now find that there is a prolonged activation of JAKs and STATs in response to a number of cytokines in T cells from Tg mice with the lck promoter-driven F59D-JAB. Overexpression of F59D-JAB also sustained activation of JAK2 in Ba/F3 cells. These data suggested that F59D-JAB up-regulated STAT activity by sustaining JAK activation. To elucidate molecular mechanisms related to F59D-JAB, we analyzed the effects of F59D-JAB on the JAK/STAT pathway using the 293 cell transient expression system. We found that the C-terminal SOCS-box played an essential role in augmenting cytokine signaling by F59D-JAB. The SOCS-box interacted with the Elongin BC complex, and this interaction stabilized JAB. F59D-JAB induced destabilization of wild-type JAB, whereas overexpression of Elongin BC canceled this effect. Levels of endogenous JAB and CIS3 in T cells from F59D-JAB Tg-mouse were lower than in wild-type mice. We propose that F59D-JAB destabilizes wild-type, endogenous JAB and CIS3 by chemically inhibiting the Elongin BC complex, thereby sustaining JAK activation.

Immune and inflammatory systems are controlled by multiple cytokines, including interleukins (ILs) and interferons (IFNs). These molecules exert biological functions through Janus tyrosine kinases (JAKs) and STAT transcription factors. We and others reported a family of cytokine-inducible SH2 proteins (CISs) (also referred to as suppressor of cytokine signaling (SOCS) or STAT-induced STAT inhibitor (SSI)) involved in the negative regulation of cytokine signals, especially JAKs and STATs (see review, Ref. 2). The first identified CIS gene, CIS1, is a negative feedback regulator of the STAT5 pathway (3, 4). We recently cloned other CIS family members, JAB (SOCS1) and CIS3 (SOCS3), which directly bind to the JAK2 tyrosine kinase domain and inhibit JAK tyrosine kinase activity (5, 6). Overexpression of JAB and CIS3 resulted in suppression of cytokine signaling by utilizing JAKs, including IL-6 and IFNγ (7). Gene disruption studies have demonstrated that JAB, CIS2, and CIS3 negatively regulate IFNγ signaling and T cell activation (8–11), growth hormone or insulin-like growth factor signaling (12), and fetal liver hematopoiesis (13), respectively. The physiological and immunological functions of JAB and CIS3 in particular tissues have remained to be determined. JAB knockout mice died within the first three postnatal weeks (11), and CIS3 knockout mice were embryonic lethal (13). We have reported that JAB and CIS3 were highly expressed in tissues in case of inflammatory disease such as rheumatoid arthritis, ulcerative colitis, and Crohn’s disease (14). To define the role of JAK/STAT signaling and their inhibitors, development of a dominant negative form of these inhibitors is quite important. Previously, we have shown that JAB specifically binds to the tyrosine residue (Yyr-1007) in the activation loop of JAK2, the phosphorylation of which is required for activation of kinase activity. An additional N-terminal 12-amino acid region (kinase inhibitory region) of JAB also contributes to high affinity binding to the JAK2 tyrosine kinase domain and is required to inhibit JAK2 signaling and kinase activity (5). We also reported that a JAB mutant (F59D-JAB) containing a point mutation in the KIR region overcame the inhibitory effect of signal transducers and activators of transcription; JAB, JAK-binding protein; SOCS, suppressor of cytokine signaling; SS1, STAT-induced STAT inhibitor; WT, wild-type; Tg, transgenic; EGF, enhanced green fluorescent protein; PMH, porobil 12-myradist 13-acetate; DSS, dextran sulfate sodium; CS, calf serum; LIF, leukemia inhibitory factor; EPO, erythropoietin; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; LLNL, N-acetyl-l-leucinyl-l-leucinyl-l-norleucine; CHX, cycloheximide; VHL, von Hippel-Lindau disease. 2 T. Shouda, T. Yoshida, and Yoshimura, A., unpublished data.
both JAB and CIS3 (14). We developed transgenic (Tg) mice using /H9252-actin promoter and showed that dextran sulfate sodium (DSS) induced a more potent STAT3 activation and a more severe colitis in F59D-JAB-transgenic mice (Tg) than in their wild-type littermates (14). In the present work, we focused on how F59D-JAB modulates the JAK/STAT pathway in molecular mechanisms. F59D-JAB strongly augmented cytokine-induced JAK/STAT activation and proliferation when expressed in T cells. Using the 293 transient expression system, we found that wild-type JAB was destabilized by F59D-JAB overexpression, in a SOCS-box-dependent manner, and levels of endogenous JAB and CIS3 were reduced in T cells from Tg mice. These data suggest that the C-terminal SOCS-box plays an important role in stabilizing endogenous CIS proteins and that the SOCS-box will be a new target for regulating intensity of cytokine actions.

EXPERIMENTAL PROCEDURES

Cells—293 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% calf serum (CS). A mouse pro-B cell line Ba/F3 was maintained in RPMI 1640 medium containing 10% fetal calf serum and 1 ng/ml of murine IL-3. Stable Ba/F3 transformants were obtained by electroporation with pcDNA3 carrying Myc-tagged full-length F59D-JAB and were selected with 1.0 mg/ml G418, and three positive clones were maintained in 0.5 mg/ml G418.

Luciferase Assay—LIF- and EPO-dependent STAT3 and STAT5 reporter activity in 293 cells transfected with Myc- or FLAG-tagged wild-type or mutant JAB cDNA was assayed as described previously (5, 7, 15). Ras-MAPK activity was measured in an Elk-1 reporter assay (PathDetect in vivo signal transduction pathway trans-reporting system, Stratagene) according to the manufacturer’s instructions. In all reporter assays, 1 × 10^5 of 293 cells grown in 6-well dishes were used for transfection with the calcium phosphate method.

Mutant cDNA Construction—Two substitution mutants of JAB, F59D (mutation in the kinase inhibitory region) and R105E (mutation in the SH2 domain), were derived from murine JAB and generated using standard polymerase chain reaction methods. cDNA fragments were subcloned into the EcoRI/XhoI site of pcDNA3-Myc or pCMV2-FLAG as described previously (16).

Immunoprecipitation and Western Blot Analysis—Cells were lysed in a lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1 mM EDTA, 1 mM vanadate, 50 mM NaF, 1 mM dithiothreitol, 0.1 mM (P-Amidinophenyl)-methanesulfonyl fluoride hydrochloride and centrifuged at 12,000 g for 10 min. The supernatants were incubated with indicated antibodies at 4 °C for 1 h. Immune-complexes were precipitated with protein A-Sepharose (Amersham Pharmacia Biotech). After washing three times with lysis buffer, the immunoprecipitates were resolved on SDS-polyacrylamide gel electrophoresis and immunoblotted with the indicated antibodies (17). Anti-JAK2 antibody was purchased from Upstate Biotechnology Inc.; anti-tyrosine-phosphorylated STAT1, anti-tyrosine-phosphorylated STAT3, anti-tyrosine-phosphorylated STAT5, and anti-tyrosine-phosphorylated STAT6 antibodies were from New England BioLabs; anti-active ERK1/2 antibody was from Promega; anti-tyrosine-phosphorylated STAT4 antibody was from Zymed Laboratories Inc.; anti-STAT1, anti-STAT3, anti-STAT4, anti-STAT5, anti-STAT6, and anti-ERK2 antibodies were from Santa Cruz Biotechnology. Anti-phosphotyrosine (4G10), anti-Myc-tag (9E10), and anti-FLAG antibodies were as described previously (5). Treatment of cells with a proteasome inhibitor, N-acetyl-L-leucinyl-l-leucinyl-l-norleucinal (LLnL, Peptide Institute Inc., Osaka, Japan) and cycloheximide (CHX)
cells were preincubated with 2.5 μg/ml antibody. Thymocytes and splenic T cells were obtained as described (17). Ba/F3 cells were infected with IL-3. Then, after being washed three times with phosphate-buffered saline, cells were resuspended in RPMI medium with 10% fetal calf serum containing various concentrations of IL-3 and incubated for a further 7 days. Infected cells expressing EGFP were analyzed with a fluorescence flow cytometer.

**RESULTS**

**Prolonged Activation of JAK and STATs in T Cells of F59D-JAB Transgenic (Tg) Mice**—We earlier found STAT3 hyperactivation in the colon of β-actin promoter-driven F59D-JAB Tg mice treated with DSS. However, the transgene did not contain a tag for detection, and levels of F59D-JAB in T cells were not so high in these Tg mice. To facilitate further molecular analysis of F59D-JAB-mediated modulation of JAK/STAT pathway in vivo, we developed Tg mice using the lck proximal promoter with the intrinsic enhancer from the immunoglobulin heavy chain locus (Eμ) to express F59D-JAB in lymphocytes. We found that endogenous JAB was highly expressed in thymus and spleen (11). As shown in Fig. 1A, Myc-F59D-JAB expression was confirmed in the thymus and spleen of three independent lines by immunoblotting with anti-Myc antibodies. Northern hybridization indicated that the expression levels of F59D-JAB were 10–50 times higher than that for endogenous JAB (data not shown). The number of thymic T cells and cellularity of the spleen were the same as wild-type littermates (data not shown). Gross and histological analyses of the thymus and spleen from F59D-JAB Tg mice revealed no abnormalities in normal conditions. However, the Tg mice were more sensitive to liver injury induced by concanavalin A (ConA) than were the wild-type littermates, which has been shown to be dependent on IFN-γ and T cells (20–22) (Fig. 1B). These results suggested that F59D-JAB augments inflammatory responses of hepatic T cells to ConA/IFN-γ.

Next, we evaluated effects of F59D-JAB overexpression on the IL-2 or IL-4-induced proliferation of T cells. These cells isolated from the spleen were incubated with various amounts of IL-2 or IL-4. As shown in Fig. 2, proliferative responses of T cells from F59D-JAB mice to both IL-2 and IL-4 were much higher than those from non-Tg littermates. Phorbol 12-myristate 13-acetate (PMA)-induced proliferation of T cells was not affected by F59D-JAB expression in T cells (data not shown). These data indicate that overexpression of F59D-JAB in T cells enhances responses to several cytokines. Similar results were obtained in other two Tg lines.

**Augmentation of Cytokine Signaling by Mutant JAB**

**FIG. 2.** IL-2- and IL-4-dependent proliferative responses of splenocytes from wild-type (WT) and F59D-JAB transgenic (Tg) mice. Naïve splenic T cells from WT or F59D-JAB Tg mice were pre-activated with a plate-bound anti-TCR mAb. After 36 h, the T cells were cultured with various concentrations of IL-2 (A) or IL-4 (B) for 24 h. After pulse labeling with 1 μCi of [3H]thymidine for an additional 8 h, the radioactivity incorporated into cells was measured. Data are presented as the mean ± S.D. of five animals per group. Statistical analysis was made using Student’s t test, and p values of less than 0.05 were considered significant.

**A**

IL-2 (U/ml) | [3H] Thymidine incorporation (cpm)
---|---
0 | 500
1 | 1500
3 | 2000
10 | 1500
30 | 2000
100 | 2000

**B**

IL-4 (U/ml) | [3H] Thymidine incorporation (cpm)
---|---
0 | 500
1 | 1500
3 | 2000
10 | 1500
30 | 2000
100 | 2000

*WT* | Tg

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Augmentation of Cytokine Signaling by Mutant JAB
To define effects of F59D-JAB on the JAK/STAT pathway, we examined STATs phosphorylation in response to several cytokines (Fig. 3). Constitutive activation of STATs was not observed in F59D-JAB Tg mice. However, when thymocytes and splenic T cells were stimulated with several cytokines, phosphorylation of STATs in T cells from F59D-JAB Tg mice was sustained longer than seen with wild-type littermates. The results are listed in Fig. 3A; tyrosine phosphorylation of STAT1 in response to IFNγ, that of STAT3 in response to LIF, that of STAT5 in response to IL-2, that of STAT4 in response to IL-12, and that of STAT6 in response to IL-4. Similar results were obtained in other two transgenic lines. Because F59D-JAB augmented various cytokines and STATs, F59D-JAB probably up-regulates JAK kinase activity. Thus, we examined IL-2-induced JAK1 phosphorylation in T cells. As shown in Fig. 3B, IL-2-induced JAK1 phosphorylation was more extensive and was sustained for a longer period in T cells from Tg mice than from wild-type littermates. However, F59D-JAB did not augment ERK activation in response to PMA, which activates the protein kinase C pathway (23) (Fig. 3C). These data suggest that the effect of F59D-JAB is cytokine/JAK-specific.

**Forced Expression of F59D-JAB Conferred a Higher Sensitivity to IL-3 Stimulation in Ba/F3 Cells—**To determine molecular mechanisms of augmentation of cytokine signaling by F59D-JAB, F59D-JAB cDNA was introduced into IL-3-dependent Ba/F3 cells using enhanced green fluorescent protein (EGFP) with a bicistronic retrovirus vector pMX-IRES-EGFP (24). Because the infected cells expressed both EGFP and F59D-JAB, the percentage of infected cells was determined as the EGFP-positive rate using flow cytometry. As seen previously, WT-JAB induced apoptosis of Ba/F3 cells in the presence of IL-3, thus indicating that WT-JAB blocked IL-3/JAK2 signaling. On the other hand, as shown in Fig. 4A, the population of F59D-JAB-infected Ba/F3 cells increased with low concentrations of IL-3 (10 and 50 pg/ml IL-3). At high levels of IL-3 (100 and 1000 pg/ml), the growth rate of F59D-JAB-infected cells was equal to that seen in non-infected cells, which is probably due to full activation of JAK2 in response to high concentrations of IL-3.

To explore the possibility that the F59D-JAB mutant suppressed the down-regulation of JAK kinase activity, we prepared two lines of stable Ba/F3 transformants expressing F59D-JAB. These Ba/F3 transformants survived longer than did the parental Ba/F3 cells when IL-3 was depleted (data not shown). Then we examined the time course of dephosphorylation of JAK2, STAT5, and MAPK (Fig. 4B). Ba/F3-F59D-JAB transformants and parental Ba/F3 cells were stimulated with IL-3 for 30 min, then the cells were cultured without IL-3 for various periods. Dephosphorylation of JAK2, STAT5, and MAPK (ERK1/2) was slower in Ba/F3-F59D-JAB cells than in parental Ba/F3 cells. These data suggest that F59D-JAB augmented IL-3 signaling by suppressing dephosphorylation or degradation of the activated JAK2. Because both STAT5 and MAPK are downstream of JAK2, prolonged activation of STAT5 and MAPK is probably due to the sustained activation of JAK2.

**Augmentation of STAT Transcriptional Activity by F59D-JAB in a JAK-dependent Manner—**As noted earlier, LIF-dependent STAT3 and EPO-dependent STAT5 reporter gene activation in 293 cells were completely inhibited by expression of wild-type (WT) JAB or CIS3 (7). As illustrated in Fig. 5A, JAB mutants of F59D and R105E did not suppress STAT3 and STAT5 activity, rather enhanced their transcriptional activity in a dose-dependent manner. At 0.1-μg plasmid transfection, LIF-dependent STAT3 activation was about 3-fold higher than

**Fig. 3. Effect of F59D-JAB on cytokine-induced STATs activation of thymocytes.** Time course of IL-2-induced STAT5 activation, IFNγ-induced STAT1 activation, IL-4-induced STAT6 activation, and IL-12-induced STAT4 activation in thymocytes and LIF-induced STAT3 activation in splenocytes from wild-type and F59D-JAB Tg mice (A). IL-2-induced JAK1 phosphorylation was also measured by immunoblotting with anti-phosphorylated JAK1 antibody (B). Thymocytes were pre-activated with 2.5 μg/ml ConA for 36 h and stimulated with 100 units/ml IL-2, 50 ng/ml IL-4, or 50 ng/ml IL-12 for indicated periods. Freshly isolated thymocyte and splenocytes were cultured with medium alone for 24 h. These cells were then stimulated with 3000 IU/ml IFNγ or 10 ng/ml LIF for indicated periods, respectively. Total cell lysates were analyzed by immunoblotting with an antibody against the corresponding anti-phosphorylated STATs or anti-phosphorylated JAK1 antibodies. Each membrane was reprobed with the indicated antibodies. C, freshly isolated thymocytes were stimulated with 10 ng/ml PMA for the indicated times. Total cell lysates were analyzed by immunoblotting with anti-phospho-ERK1/2 antibody. The same membrane was reprobed with anti-ERK2 and anti-Myc antibodies.
without mutant JAB constructs. This effect was stimulation-dependent, because basal transcriptional activity without cytokines was marginally affected. To determine if this effect is cytokine-dependent, EGF- and v-src-induced STAT3 activation was measured. As shown in Fig. 5, F59D-JAB did not affect the STAT3 activation induced by EGF and v-src. F59D-JAB also did not augment MAPK activation in response to EGF (Fig. 5D). Therefore, the effect of F59D-JAB is likely to be cytokine- and JAK-specific.

The SOCS-box and Elongin BC Complex Are Essential for the Effect of F59D-JAB—Among the CIS family, both JAB and CIS3 can directly interact with JAKs (5, 6). We reported that F59D-JAB could overcome the negative effect of wild-type JAB and CIS3 (14). Thus, we proposed that F59D-JAB augmented cytokine-dependent STAT activation by suppressing the effect of endogenous wild-type JAB and CIS3. We hypothesized that F59D-JAB prevents the binding of endogenous JAB and CIS3 to the JAK catalytic pocket, because this mutant lacks one of the two JAK binding regions (SH2 domain and KIR). However, we found that the N-terminal truncation mutant (dN75), which lacks both functional KIR and SH2 domain could augment STAT3 activity (Fig. 6A). This event could not be explained by the simple competition model. In contrast, the C-terminal SOCS-box deletion mutant (F59D-dC40) did not increase STAT3 activity (Fig. 6A). We also found that R105E-dC40 did not enhance STAT3 activity (data not shown). Therefore, the SOCS-box seems to be important for augmentation of STAT activation.

It was reported that the SOCS-box interacts with the Elongin BC complex and that the binding of Elongin BC complex stabilizes wild-type JAB (25). We next examined the effect of Elongin BC overexpression. As shown in Fig. 6B, overexpression of Elongin B or Elongin C alone had little effect on F59D-JAB; however, overexpression of Elongin B and Elongin C together overcame the effects of F59D-JAB. Overexpression of the Elongin BC complex alone did not directly affect LIF-
induced STAT3 activation. Therefore, the SOCS-box and Elongin BC complex are closely involved in the hyperactivation of JAK/STATs by F59D-JAB.

F59D-JAB Destabilized Wild-type JAB and CIS3 by Chelating Elongin BC—Because it has been shown that the Elongin BC complex stabilizes wild-type JAB (25), we considered that F59D-JAB destabilizes endogenous, wild-type JAB and CIS3 by chelating the Elongin BC complex. Thus, we examined effects of F59D-JAB on the stability of wild-type JAB. F59D-JAB did not affect JAK2 and STAT5 stability (Fig. 7A). However, as shown in Fig. 7B, half-life of wild-type JAB shortened by co-expression of F59D-JAB. In contrast, the C-terminal SOCS-box deletion mutant (F59D-dC40) did not influence the stability of wild-type JAB. The acceleration of degradation of wild-type JAB by F59D-JAB was blocked by overexpression of Elongin BC as did treating the cells with proteasome inhibitors (Fig. 7C). These data support our hypothesis.

For verification in Tg mice, we detected the levels of endogenous JAB and CIS3 in F59D-JAB transgenetic mice. As noted earlier (11), T cells expressed high levels of endogenous JAB prior to cytokine stimulation, and CIS3 was induced in response to LIF in wild-type littermates (WT in Fig. 8). As shown in Fig. 8 (right panels), the levels of endogenous JAB and CIS3 in thymocytes from F59D-JAB transgenic mice were significantly reduced compared with those in wild-type littermates. These data strongly supported our proposal that F59D-JAB induced degradation of endogenous JAB and CIS3 by occupying the Elongin BC complex, thereby sustaining the activation of JAKs.

DISCUSSION

We demonstrated that F59D-JAB augmented cytokine-dependent signaling both in vivo and in vitro. The effect of F59D-JAB was selective to cytokine-induced STAT activation, because F59D-JAB did not affect EGF- or v-src-induced STAT activation in 293 cells. We also confirmed that PMA- and ConA-induced MAPK activation in T cells from mice were not affected by F59D-JAB (data not shown). We further demonstrated that F59D-JAB overexpression resulted in hyperactivation of JAK1 in response to IL-2 in T cells as well as sustained JAK2 activation in Ba/F3 cells. These data suggest that F59D-JAB hyperactivates JAKs, thereby augmenting STAT activity. However, the molecular mechanism of augmentation of JAKs by F59D-JAB was not clarified.

We first hypothesized that F59D-JAB blocked binding of endogenous, wild-type JAB and CIS3, because F59D-JAB lacks functional kinase inhibitory region (KIR) but still interacts with JAK activation loop through the SH2 domain. However, this is not the case, because the N-terminal deletion mutant (dN75), which does not bind to JAKs at all, could augment...
LIF-induced STAT3 activation. In contrast, we found that C-terminal SOCS-box played an essential role in this augmentation. Kamura et al. (25) found that the Elongin BC complex interacts with the SOCS-box and stabilizes wild-type JAB. Therefore, we then considered that F59D-JAB up-regulates JAKs by destabilizing endogenous JAB and CIS3 by chelating endogenous Elongin BC complex.

This hypothesis was supported by observations that 1) overexpression of the Elongin BC complex overcame effects of F59D-JAB; 2) the C-terminal SOCS-box-deleted F59D-JAB did not increase STAT3 activation; and 3) F59D-JAB destabilized wild-type JAB and CIS3 in a proteasome-dependent manner. The mechanism of chelating the Elongin BC by F59D-JAB is just simple overexpression (the levels of F59D-JAB was at least 50 times higher than the endogenous JAB in Tg mice). F59D-JAB can bind to Elongins with equal affinity as wild-type JAB, but F59D-JAB does not inhibit JAKs. Thus, overexpressed F59D-JAB occupies Elongins, which reduces the number of endogenous JAB and CIS3 molecules interacting with Elongins. This increases the degradation of endogenous JAB and CIS3, because JAB and CIS3 without Elongins are unstable.

The Elongin BC complex was identified initially to be a positive regulator of RNA polymerase II elongation factor Elongin A (26, 27) and subsequently as a component of the multi-protein von Hippel-Lindau disease (VHL) tumor-suppressor complex (28, 29). The Elongin BC complex has also been shown to interact with SOCS-box-containing proteins, including CIS family proteins as well as a protein family containing either a Ras-like domain, WD40 domains, ankyrin-like repeats, or an SPRY domain at their N terminus (25, 30). Therefore, the effect of 

**Fig. 6.** Augmentation of the JAK/STAT pathway by F59D-JAB is dependent on the SOCS-box. **A**, LIF-dependent STAT3 reporter assay was carried out in 293 cells transfected with indicated amounts of Myc-tagged JAB mutants. After transfection, cells were incubated in the presence or absence of 10 ng/ml LIF for 6 h, and cell extracts were prepared. Data normalized with the β-galactosidase activity from duplicate experiments are shown. Cell lysates were immunoblotted with anti-Myc antibody (9E10). **B**, to measure the effect of Elongin BC on F59D-JAB, an LIF-dependent STAT3 reporter assay was carried out in 293 cells cotransfected with the indicated amounts of FLAG-tagged F59D-JAB and Elongin B (0.3 μg), Elongin C (0.3 μg), or both Elongins B and C. Cell lysates were immunoblotted with anti-JAB and anti-FLAG antibodies.
of F59D-JAB may result from proteins other than endogenous JAB and CIS3. F59D-JAB could potentially affect other Elongin BC complex-interacting proteins such as VHL and Elongin A as well as other SOCS proteins. However, we did not see any gross abnormality other than cytokine responses in the F59D-JAB Tg mice driven by either β-actin or lck promoter. This could be merely due to a low level of expression of F59D-JAB protein in Tg mice using the β-actin promoter or restricted expression of F59D-JAB in Tg mice using the lck promoter, because we could not obtain Tg mice with high levels of F59D-JAB expression using the β-actin promoter (14). The phenotypic effect of F59D-JAB in Tg mice was observed only when cytokine levels were conditionally changed. For example, we observed a more severe inflammation when the Tg mice were treated with DSS or ConA, which induce production of high levels of IL6 and IFNγ, respectively. Therefore, the apparent effect of F59D-JAB may be specific to cytokines in vivo. Moreover, the effect of F59D-JAB may be specific for a class of SOCS-box-containing proteins, such as JAB and CIS3, because we found no changes in the half-life of CIS1 and VHL protein with the overexpression of F59D-JAB in 293 cells (data not shown). The specific effect of F59D-JAB on JAB and CIS3...
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**FIG. S** F59D-JAB reduced the expression level of endogenous JAB and CIS3 in thymocytes. Freshly isolated thymocytes were cultured with medium alone for 24 h, then stimulated with 10 ng/ml LIF for the indicated periods. Each of the total cell lysates was subjected to 13% SDS-polyacrylamide gel electrophoresis and blotted with an anti-CIS3 antibody. The same membrane was reprobed with anti-JAB, anti-Myc, and anti-STAT5 antibodies.
A Mutant Form of JAB/SOCS1 Augments the Cytokine-induced JAK/STAT Pathway by Accelerating Degradation of Wild-type JAB/CIS Family Proteins through the SOCS-box

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