Both type I and II IFN induce insulin resistance by inducing different isoforms of SOCS expression in 3T3-L1 adipocytes

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Wada T, Hoshino M, Kimura Y, Ojima M, Nakano T, Koya D, Tsuneki H, Sasaoka T. Both type I and II IFN induce insulin resistance by inducing different isoforms of SOCS expression in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab 300: E1112–E1123, 2011. First published March 8, 2011; doi:10.1152/ajpendo.00370.2010.—Although elevation of the blood glucose level is a causal adverse effect of treatment with interferon (IFN), the precise underlying molecular mechanism is largely unknown. We examined the effects of type I and type II IFN (IFN-β and IFN-γ) on insulin-induced metabolic signaling leading to glucose uptake in 3T3-L1 adipocytes. IFN-β suppressed insulin-induced tyrosine phosphorylation of IRS-1 without affecting its expression, whereas IFN-γ reduced both the protein level and tyrosine phosphorylation. Although both IFNs stimulated phosphorylation of STAT1 (at Tyr701) and STAT3 (at Tyr705) after treatment for 30 min, subsequent properties of induction of the SOCS isoform were different. IFN-β preferentially induced SOCS1 rather than SOCS3, whereas IFN-γ strongly induced SOCS3 expression alone. In addition, adenovirus-mediated overexpression of either SOCS1 or SOCS3 inhibited insulin-induced tyrosine phosphorylation of IRS-1, whereas the reduction of IRS-1 protein was observed only in SOCS3-expressed cells. Notably, IFN-β-induced SOCS1 expression and suppression of insulin-induced tyrosine phosphorylation of IRS-1 were attenuated by siRNA-mediated knockdown of STAT1. In contrast, adenovirus-mediated expression of a dominant-negative STAT3 (F-STAT3) attenuated IFN-γ-induced SOCS3 expression, reduction of IRS-1 protein, and suppression of insulin-induced glucose uptake but did not have any effect on the IFN-β-mediated SOCS1 expression and inhibition of insulin-induced glucose uptake. Interestingly, pretreatment of IFN-γ with IL-6 synergistically suppressed insulin signaling, even when IL-6 alone had no significant effect. These results indicate that type I and type II IFN induce insulin resistance by inducing distinct SOCS isoforms, and IL-6 synergistically augments IFN-γ-induced insulin resistance by potentiating STAT3-mediated SOCS3 induction in 3T3-L1 adipocytes.

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relative difference between type I and type II IFN-induced SOCS expressions and their inhibitory effects on insulin signaling has not yet been characterized.

Therefore, in the present study, we investigated the function of STATs (in particular STAT3) in the development of IFN-induced insulin resistance. We directly compared the impact on IFN-β and IFN-γ in insulin signaling in 3T3-L1 adipocytes and investigated the molecular mechanism by which these IFNs cause insulin resistance by interacting with different isoforms of SOCS. In addition, we sought to identify which STAT/SOCS pathway is relevant to insulin resistance induced by each type of IFN.

MATERIALS AND METHODS

Materials. Human recombinant insulin was provided by Novo Nordisk Pharmaceutical (Copenhagen, Denmark). Mouse IFN-β and mouse IFN-γ were provided by Toray Industries (Tokyo, Japan) and Hayashibara Biochemical Laboratories (Okayama, Japan), respectively. Mouse IL-6 was purchased from Sigma-Aldrich Japan (Tokyo, Japan). A polyclonal anti-insulin receptor (IR)β antibody, polyclonal anti-Tyr612 phosphospecific IRS-1 antibody, monoclonal anti-Akt antibody, monoclonal anti-phosphotyrosine (PY) antibody, polyclonal anti-SOCS3 antibody, polyclonal anti-p2 antibody, polyclonal anti-CCAAT/enhancer binding protein (CEBP)α antibody, polyclonal anti-peroxisome proliferator-activated receptor (PPAR)γ antibody, polyclonal anti-sterol regulatory element-binding protein (SREBP)-1 antibody, polyclonal anti-STAT1 antibody, polyclonal anti-Tyr701 phosphospecific antibody, polyclonal anti-IRS-1 antibody, monoclonal anti-Akt antibody, monoclonal anti-phosphotyrosine (PY) antibody, polyclonal anti-SOCS3 antibody, polyclonal anti-p2 antibody, polyclonal anti-CCAAT/enhancer binding protein (CEBP)α antibody, polyclonal anti-peroxisome proliferator-activated receptor (PPAR)γ antibody, polyclonal anti-sterol regulatory element-binding protein (SREBP)-1 antibody, and polyclonal anti-GLUT1 were from Santa Cruz Biotechnology (Santa Cruz, CA). A polyclonal anti-IRS-1 antibody was from Millipore (Billerica, MA). The polyclonal anti-Ser707 phosphospecific IRS-1 antibody, polyclonal anti-Tyr612 phosphospecific IRS-1 antibody, polyclonal anti-Ser673 phosphospecific Akt antibody, polyclonal anti-STAT1 antibody, polyclonal anti-Tyr701 phosphospecific STAT1 antibody, polyclonal anti-STAT3 antibody, polyclonal anti-Tyr705 phosphospecific STAT3 antibody, polyclonal anti-Ser727 phosphospecific STAT3 antibody, and polyclonal anti-β-actin antibody were from Cell Signaling Technology (Beverly, MA). A polyclonal anti-SOCS1 antibody was from Immuno Biological Laboratory (Fujioka, Japan). A monoclonal anti-GLUT4 antibody was from Abcam (Cambridge, UK). Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Life Technologies (Carlsbad, CA). Trypan blue solution (0.4%) was from Wako Pure Chemical Industries, Osaka, Japan. All other reagents were of analytical grade and purchased from Sigma-Aldrich Japan or Wako Pure Chemical Industries.

Culture and differentiation of 3T3-L1 adipocytes. 3T3-L1 preadipocytes were grown and passaged in DMEM supplemented with 10% newborn calf serum. Cells 2–3 days postconfluence were used for differentiation. The differentiation medium contained 10% fetal calf serum (FCS), 250 nM dexamethasone, 0.5 mM isobutylmethylxanthine, and 500 nM insulin. After 3 days of treatment, the differentiation medium was replaced with postdifferentiation medium containing 10% FCS and 500 nM insulin. After 3 days of incubation, postdifferentiation medium was replaced with DMEM supplemented with 10% FCS (32, 41, 42).

Oil red O staining. The adipocyte differentiation was evaluated by lipid staining with Oil Red O. 3T3-L1 cells were washed with PBS, fixed with 10% formaldehyde, and incubated with Oil Red O solution (0.3% Oil Red O in isopropanol). After washing with PBS, the cells were observed under a microscope.

Expression of wild-type STAT3, dominant-negative STAT3, SOCS1, and SOCS3 in 3T3-L1 adipocytes. The cDNAs encoding wild-type (WT)-STAT3 and a dominant-negative STAT3 (F-STAT3), in which Tyr705 is replaced by phenylalanine, were provided by Dr. W. Ogawa (Kobe University) (7). The cDNAs encoding SOCS1 and SOCS3 were provided by Dr. K. Ueki (University of Tokyo) (37). A control LacZ virus encoding β-galactosidase was constructed as described previously (32, 41, 42). WT-STAT3 or F-STAT3 was transiently expressed in differentiated 3T3-L1 adipocytes by means of adenovirus-mediated gene transfer (42). In brief, a multiplicity of infection of 40 plaque-forming units (pfu)/cell was used to infect 3T3-L1 adipocytes, with the virus being left on the cells for 16 h prior to removal. Subsequent experiments were conducted 24–48 h after initial addition of the virus. The efficiency of adenovirus-mediated gene transfer was ~95%.

Immunoprecipitation and Western blotting. 3T3-L1 adipocytes grown in six-well plates were serum starved for 16 h without or with 200 or 1,000 IU/ml IFN-β, 200 or 1,000 IU/ml IFN-γ, 10 ng/ml IL-6, or IFN-γ plus IL-6 and stimulated with 1.7 nM insulin for 5 min. The cells were lysed in a buffer containing 20 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium deoxycholate, 1 mM β-glycerophosphate, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 50 mM sodium fluoride, 10 μg/ml aprotinin, and 10 μM leupeptin, pH 7.4, for 15 min at 4°C. Lysates obtained from the same number of cells were centrifuged to remove insoluble materials. The supernatants (100 μg of protein) were immunoprecipitated with the indicated antibodies or precipitated with gluthatione sepharose beads for 2 h at 4°C. The precipitates or whole cell lysates were then separated by 7.5% SDS-PAGE and transferred onto polyvinylidene difluoride membranes using Bio-Rad Transblot equipment. The membranes were blocked in a buffer containing 50 mM Tris, 150 mM NaCl, 0.1% Tween 20, and 2.5% bovine serum albumin or 5% non-fat milk, pH 7.5, for 2 h at 20°C. The membranes were then probed with the specified antibodies (1:500 dilutions for anti-SOCS1, SOCS3, and phospho-Ser707 IRS-1 antibodies and 1:1,000 dilutions for other antibodies) for 2 h at 20°C or for 16 h at 4°C. After being washed in a buffer containing 50 mM Tris, 150 mM NaCl, and 0.1% Tween 20, pH 7.5, the membranes were incubated with horseradish peroxidase-linked second antibody followed by enhanced chemiluminescence detection using ECL reagent according to the manufacturer’s instructions (GE Healthcare Bioscience, Uppsala, Sweden). Densitometric analysis was conducted directly using the blotted membrane by utilizing an LAS-4000 lumino-image analyzer system (Fujifilm, Tokyo, Japan) (40, 41).

Small interfering RNA-mediated knockdown of STAT1, SOCS1, and SOCS3. Gene knockdown by small interfering RNA (siRNA) was performed by Oligonucleotide Research Laboratory (Toray Industries, Tokyo, Japan) (39, 42). The electroporation was conducted according to the manufacturer’s instructions. In brief, differentiated 3T3-L1 adipocytes were scrambled with 0.25% trypsin and 0.5 mg/ml collagenase. After being washed with PBS, cells were mixed with Nucleofector solution and 1 μg siRNA, and electroporation was conducted. The siRNA sense sequence for STAT1 was 5′-GGCAAGAGGAGAU-CUCGGAGAU-3′. The siRNA sense sequence for SOCS1 was 5′-GGCAUAGCGAGGUGUU-3′. The siRNA sense sequence for SOCS3 was 5′-GGCAUAGCGAGGUGUU-3′. Stealth RNAi negative control medium GC duplex (Invitrogen) was used as control scramble siRNA. Cells were harvested and examined 24–48 h postelectroporation.

Measurement of insulin-induced 2-deoxyglucose uptake. 3T3-L1 adipocytes grown in six-well plates were serum starved for 16 h without or with 1,000 IU/ml IFN-β and IFN-γ and further incubated in Krebs-Ringer phosphate-HEPES buffer containing 1% bovine serum albumin for 2 h at 37°C. The cells were subsequently stimulated with various concentrations of insulin. Following 15 min of insulin treatment, 0.1 μg/ml of [3H]2-deoxyglucose (2DG; GE Healthcare Biosciences) was added for 4 min. The reaction was stopped by the addition of 10 μM cytochalasin B. The cells were washed three times with PBS and solubilized with 0.2 mM SDS/0.2 N NaOH (41, 42). The radioactivity incorporated into the cells was measured by liquid scintillation counting.

Statistical analyses. The data are shown as means ± SE. P values were determined by Bonferroni correction for comparison against the control or by Student-Newman-Keuls multiple comparison test to
compare multiple conditions, as appropriate, after ANOVA, and differences among groups were considered significant when $P < 0.05$.

RESULTS

IFN-β and IFN-γ attenuate insulin-induced phosphorylation of IRS-1 by a distinct mechanism. We examined the effect of IFN on the protein amount and insulin-induced tyrosine phosphorylation of IR (Fig. 1). 3T3-L1 adipocytes were incubated with either IFN-β or IFN-γ for 0.5–24 h and stimulated with insulin for 5 min. Neither IFN-β (Fig. 1A) nor IFN-γ (Fig. 1B) affected the protein expression or tyrosine phosphorylation of IR. Concerning the effect on IRS-1, treatment with IFN-β marked suppressed insulin-induced tyrosine phosphorylation of IRS-1 in a time-dependent manner, whereas it did not affect the amount of IRS-1 (Fig. 2, A and B). Following treatment with IFN-β for 24 h, tyrosine phosphorylation of IRS-1, normalized to IRS-1 protein levels, decreased to 50.6 ± 5.9% of the control value. In contrast, treatment with IFN-γ reduced the amount in a time-dependent manner (Fig. 2D). The amount of IRS-1 decreased to 26.3 ± 3.1% of the control value after treatment for 24 h. IFN-γ also apparently reduced the levels of tyrosine phosphorylation of IRS-1 (Fig. 2E). However, the tyrosine phosphorylation of IRS-1, normalized to IRS-1 protein levels, was not affected significantly by the treatment with

Fig. 1. Effect of IFN on insulin-induced tyrosine phosphorylation of insulin receptor (IR). 3T3-L1 adipocytes were treated with IFN-β (A) or IFN-γ (B) for 0.5–24 h and stimulated with 1.7 nM insulin for 5 min. Cell lysates were immunoprecipitated with anti-IRβ antibody, and insulin-induced tyrosine phosphorylation of IR was blotted with anti-phosphotyrosine (PY) antibody. The amount of IRβ was examined by immunoblotting of whole cell lysates with anti-IRβ antibody. The levels of tyrosine-phosphorylated IR were corrected for the loaded amount of IR. Results are shown as the means ± SE of 6 separate experiments. Ins, insulin; ip, intraperitoneal.

Fig. 2. Effect of IFN on the amount, tyrosine phosphorylation, and Ser307 phosphorylation of insulin receptor substrate (IRS-1). 3T3-L1 adipocytes were treated with IFN-β (A–C) or IFN-γ (D–F) for 0.5–24 h and stimulated with 1.7 nM insulin for 5 min. Total cell lysates or immunoprecipitates with anti-IRS-1 antibody were blotted with anti-IRS-1 antibody (A and D), PY antibody (B and E), or anti-phospho-Ser307 IRS-1 antibody (C and F). Results are shown as the mean ± SE of 3 (A–C) or 6 (D–F) separate experiments. *$P < 0.05$ and **$P < 0.01$ vs. IFN-untreated insulin-stimulated cells by the Bonferroni test, as appropriate, after ANOVA.
IFN-γ (Fig. 2E), indicating that the reduction of insulin-induced tyrosine phosphorylation of IRS-1 by IFN-γ is entirely due to the reduction of IRS-1. On the other hand, neither IFN-β nor IFN-γ enhanced phosphorylation of IRS-1 at Ser705 (Fig. 2, C and F) and Ser636/639 (data not shown), indicating that these phosphorylations of IRS-1 at Ser residues are not responsible for the reduction of insulin-induced tyrosine phosphorylation of IRS-1. Although we utilized 1,000 IU/ml of IFN to clarify the distinct signaling property of each IFN, similar inhibitory effects on the insulin signaling were obtained using a lower dose of IFN (200 IU/ml) (Supplemental Fig. S1; Supplemental Material for this article is available online at the AJP-Endocrinology and Metabolism website).

**Both IFN-β and IFN-γ similarly phosphorylate STAT1 and STAT3 but induce a distinct isoform of SOCS.** Numbers of previous studies indicate an important role of STAT3 and SOCS3 in the development of insulin resistance in the adipocyte and liver (11, 33, 37, 38). However, a recent study suggested that IFN-γ activates STAT1 and suppresses adipocyte differentiation (14). Since STAT1 and STAT3 are known to be important for mediating IFN signaling (17, 35), we next examined the effect of IFN-β or IFN-γ on the phosphorylation of STAT1 and STAT3. The protein amount of STAT3 was not altered following 0.5 or 1 h of treatment with both IFN-β and IFN-γ (Fig. 3, A and D), whereas marked phosphorylation of STAT3 at Tyr705 residue was observed at these time points (Fig. 3, B and E). In contrast, slight but significant upregulation of STAT3 was observed following 24 h of treatment with IFN-β or 16–24 h of treatment with IFN-γ (Fig. 3, A and D). The time courses of STAT3 phosphorylation at Ser727 were different between IFN-β and IFN-γ (Fig. 3, C and F), although those at Tyr705 were almost the same (Fig. 3, B and E). IFN-β-induced STAT3 phosphorylation at Ser727 was peaked at 0.5–1 h, whereas IFN-γ-induced STAT3 phosphorylation at Ser727 was observed after 4–24 h of treatment. The augmentation of STAT3 phosphorylation by insulin was observed only at Ser727 and not at Tyr705 (Fig. 3), in accord with the previous study indicating that insulin induced phosphorylation of STAT3 at Ser727 through the MAP kinase pathway (35). These results indicate that the apparent change in phosphorylation of STAT3 at Tyr705 is due to enhanced phosphorylation but not upregulation of STAT3.

Both IFN-β and IFN-γ increased the expression of STAT1 protein after 16 or 24 h of treatment (Supplemental Fig. S2, A and C). The kinetics of the protein induction following IFN treatment was similar between STAT1 and STAT3, consistent with a previous report showing that 24 h of treatment with IFN-γ increased protein amounts of STAT1 and STAT3 (43). Furthermore, both IFNs phosphorylated STAT1 at Tyr701 residue following treatment for 0.5, 1, and 4 h (Supplemental Fig. S2, A, C, S, D, and E), and its time course was similar to that of STAT3 at Tyr705 (Figs. 3, B and E).

IFN-β significantly induced the expression of SOCS1 protein after 4 h of treatment (Fig. 4A), and importantly, this expression was preceded by the phosphorylation of STAT1 at Tyr701 and STAT3 at Tyr705, as described above. IFN-β also stimulated the expression of SOCS3, although the degree of the expression level was lower than that of SOCS1 (Fig. 4B). On the other hand, IFN-γ markedly induced the expression of

Fig. 3. Effect of IFN on expression and phosphorylation of STAT3. 3T3-L1 adipocytes were treated with IFN-β (A–C) or IFN-γ (D–F) for 0.5–24 h and stimulated with 1.7 nM insulin for 5 min. Total cell lysates were blotted with anti-STAT3 antibody (A and D), anti-phospho Tyr705 STAT3 antibody (B and E), or anti-phospho-Ser727 STAT3 antibody (C and F). Results are shown as the mean ± SE of 3 separate experiments. *P < 0.05 and **P < 0.01 vs. IFN-untreated insulin-stimulated cells by the Bonferroni test, as appropriate, after ANOVA.
Involvement of STAT3 in SOCS3 expression by IFN. To investigate the involvement of STAT3 in the induction of SOCS1 and SOCS3 by IFNs, we expressed WT-STAT3 and a dominant-negative mutant F-STAT3, in which Tyr705 residue was replaced with phenylalanine (7), and examined the effect of the expression on IFN-β/H9252 or IFN-γ/H9253-induced expression of SOCS proteins (Fig. 5). Fivefold more WT-STAT3 and F-STAT3 than endogenous STAT3 were expressed by the adenoviral expression at the multiplicity of infection of 40 pfu/cell. IFN-β/H9252-induced expression of SOCS1 was not affected by the expression of either WT-STAT3 or F-STAT3 (Fig. 5A), and SOCS1 expression was not altered in IFN-γ-treated cells expressing either WT- or F-STAT3 (Fig. 5C). On the other hand, IFN-β- and IFN-γ-induced upregulation of SOCS3 was inhibited by the expression of F-STAT3 but was not affected by the expression of WT-STAT3 (Fig. 5, B and D). These results indicate that STAT3 is involved in both IFN-β- and IFN-γ-induced expression of SOCS3 but not SOCS1.

Effect of SOCS expression on insulin signaling and SOCS knockdown on IFN-induced insulin resistance. To clarify the impact of SOCS1 and SOCS3 on the insulin signaling system, we examined directly the effect of SOCS1 and SOCS3 expressions on insulin-induced tyrosine phosphorylation and the amount of IRS-1 (Fig. 6, A–C). Overexpression of either SOCS1 or SOCS3 significantly suppressed insulin-induced tyrosine phosphorylation of IRS-1 (Fig. 6A). Interestingly, although overexpression of SOCS1 did not affect the amount of IRS-1 protein, SOCS3 expression significantly reduced the protein level of IRS-1 in a time-dependent manner 36 h postinfection (Fig. 6, B and C).

To further study the impact of IFN-induced SOCS expression on the development of insulin resistance, we examined the effect of knockdown of SOCS1 and SOCS3 on the IFN-induced alteration of insulin signaling (Fig. 6, D and E). Specific knockdown of SOCS1 and SOCS3 by siRNA was observed following treatment with both IFNs for 4 h (Fig. 6D), at which the maximal expression of SOCS was obtained in the control cells (Fig. 4). Importantly, insulin-induced tyrosine phosphorylation of IRS-1 blunted by IFN-β was recovered by the knockdown of SOCS1. In contrast, the protein reduction and subsequent attenuation of insulin-induced tyrosine phosphorylation of IRS-1 by IFN-γ were ameliorated by the knockdown of SOCS3 (Fig. 6E). These results further strengthen our view that IFN-β and IFN-γ develop insulin resistance through the distinct SOCS isoform.

Effect of IFN-β and IFN-γ on insulin-induced Akt phosphorylation and glucose uptake. Since Akt is an important downstream molecule of the IRS-phosphatidylinositol 3-kinase pathway implicated in insulin-induced glucose uptake (32), we next examined the effect of IFN-β or IFN-γ on insulin-induced phosphorylation of Akt (Fig. 7, A and B). Both IFN-β and IFN-γ effectively reduced the insulin-induced phosphorylation of Akt in a time-dependent manner. Akt phosphorylation was reduced to 49.1 ± 11.3 and 48.0 ± 2.4% of the control value after treatment for 24 h. The total amount of Akt was unaffected by treatment with both IFNs.
expression and STAT3 in SOCS3 expression has been shown (6), we further examined the role of STAT1 in the development of IFN-induced insulin resistance (Supplemental Fig. S3). Knockdown of STAT1 significantly suppressed the IFN-β-induced SOCS1 induction (Supplemental Fig. S3A). In contrast, the knockdown of STAT1 did not affect SOCS3 induction by both IFN-β and IFN-γ (Supplemental Fig. S3C). In accord with these results, the knockdown of STAT1 recovered only the IFN-β-induced attenuation of IRS-1 phosphorylation and did not have any effect on the IFN-γ-induced insulin resistance (Supplemental Fig. S3, B and D). These results indicate that IFN-β suppresses insulin-induced tyrosine phosphorylation of IRS-1 mainly through the STAT1/SOCS1 pathway, and IFN-γ suppresses insulin signaling independently of STAT1/SOCS1 pathway in 3T3-L1 adipocytes.

Moreover, we measured directly the effect of IFN-β and IFN-γ on insulin-induced 2DG uptake (Fig. 8). Treatment with both IFN-β and IFN-γ significantly suppressed insulin-induced glucose uptake, and the inhibitory effect was greater with treatment with IFN-γ than it was with IFN-β. Interestingly, the expression of F-STAT3 effectively ameliorated the reduction of the glucose uptake in IFN-γ-treated cells but not in IFN-β-treated cells.

IFN-γ and IL-6 synergistically inhibit insulin signaling. Proinflammatory cytokines cooperate with each other in eliciting their activity. IL-6 is known to induce insulin resistance by reducing IRS-1- and GLUT4 in adipose tissue (28). Since IL-6 is also a potent activator of the JAK2-STAT3 pathway and induces SOCS3 more preferentially than SOCS1 (34), we finally examined the cooperative effect of IFN-γ with low-dose IL-6 treatment, in which IL-6 itself does not suppress insulin signals (Fig. 9). Treatment with low-dose IL-6 alone for 1 or 24 h had no significant effect on the expression level of IRS-1 (Fig. 9A), insulin-induced tyrosine phosphorylation of IRS-1 (Fig. 9B), or Akt phosphorylation (Fig. 9C); however, compared with the effect of IFN-γ alone, these insulin signals were profoundly suppressed when cells were coincubated with IL-6 and IFN-γ. After cotreatment for 24 h, the amounts of IRS-1, IRS-1 tyrosine phosphorylation, and Akt phosphorylation were reduced to 14.8 ± 7.2, 7.7 ± 6.9, and 15.8 ± 9.1%, respectively, of the control value. We further examined the protein amounts and phosphorylation levels of STAT3 (Fig. 9, D and E) and protein levels of SOCS3 (Fig. 9F) by cotreatment with IFN-γ and IL-6. The total amount of STAT3 was unaffected, but phosphorylation levels of STAT3 tended to be enhanced by the cotreatment. Moreover, interestingly, a significant additive effect on the expression of SOCS3 was observed. In contrast, the synergistic effect of IFN-β and IL-6 was not observed (data not shown), possibly because of the low levels of SOCS3 induction by IFN-β compared with IFN-γ (Fig. 4).

Effect of IFN-β and IFN-γ on the cell viability or differentiation status in adipocytes. Since a recent study indicated that IFN-γ attenuated insulin signaling by suppressing differentiation status in human adipocytes (14), we finally examined the influence of IFN (1,000 IU/ml) on the cell viability and differentiation in 3T3-L1 adipocytes. In the trypan blue staining test, the numbers of living cells treated without or with IFN-β and IFN-γ for 48 h were 69.6 ± 4.2, 64.9 ± 4.8, and 62.7 ± 5.6, respectively, of the control value.

Since treatment with IFN-γ preferentially stimulated the expression of SOCS3 through the STAT3-dependent pathway (Fig. 5), we examined the effect of WT-STAT3 and F-STAT3 expression on the IRS-1 amount, IRS-1 tyrosine phosphorylation, and Akt phosphorylation. IFN-γ-induced reduction of the amount of IRS-1, insulin-stimulated tyrosine phosphorylation of IRS-1, and phosphorylation of Akt were protected by the expression of F-STAT3 (Fig. 7, C–E). These results reinforce our view that STAT3-mediated SOCS3 expression is a crucial mechanism for IFN-γ-induced insulin resistance. In contrast, the expression of F-STAT3 had only a slight impact on IFN-β-induced alteration of the phosphorylation of IRS-1 and Akt (data not shown).

IFN-β preferentially induced SOCS1 expression and caused insulin resistance independently of the expression of F-STAT3 (Figs. 4, 5, and 7). Since the principal role of STAT1 in SOCS1
accumulation evaluated by Oil Red O staining in fully differentiated 3T3-L1 adipocytes (Supplemental Fig. S4B). The protein amounts of adipocyte differentiation markers C/EBP-1, PPAR-2, and SREBP-1c, in addition to GLUT1 and GLUT4, were not affected by the treatment for 24 h (Supplemental Fig. S4C). These results indicate that treatment with 1,000 IU/ml of IFN for 24 h did not affect the differentiation status in 3T3-L1 adipocytes in the present experimental condition.

**DISCUSSION**

IFNs are known to cause glucose dysregulation and increase the risk of diabetes mellitus (5). Type I and II IFN bind to the cognate receptor IFN-αR1/2 and IFN-γR1/2, followed by the activation of receptor-coupled kinase JAK1/2 and JAK1/ JAK2, respectively (30, 31). Activated JAK1 and Tyk2 by type I IFN phosphorylate and activate STAT proteins (30, 31). Based on studies of STAT1-deficient mice, STAT1 plays a major role in either the IFN-α- or IFN-γ-mediated biological response in immune cells (15). STAT3 is also important for the immune response mediated by IFNs (24, 31). In addition, IFN-induced STAT signaling is known to induce mainly SOCS1 and SOCS3 proteins (27, 30, 34); however, the relative importance of different STAT/SOCS signaling pathways in both type I and type II IFN-mediated insulin resistance in adipocytes is largely unknown. Therefore, in the present study, we provided a direct comparison of the signaling properties of IFN-α and IFN-γ to inhibit the insulin action in 3T3-L1 adipocytes. We found that IFN-α and IFN-γ differentially modulate insulin signaling by inducing different types of SOCS proteins.

Previous studies have shown that SOCS proteins are implicated in insulin resistance (27, 29, 37). Overexpression of SOCS1 and SOCS3 inhibited insulin-induced 2DG uptake in 3T3-L1 adipocytes (37). Liver-specific overexpression of SOCS1 in mice resulted in impaired glucose tolerance via the degradation of IRS-1 and IRS-2 in the liver (29). In addition, the expression of SOCS1 and SOCS3...
proteins was elevated in the liver and muscle of LPS-
injected mice and diabetic db/db mice (37). Expression of
SOCS3 was also increased in the liver of patients with
metabolic syndrome (22). In the present study, we demon-
strated that treatment with both IFN-β and IFN-γ effectiv-
ely suppressed insulin signaling, leading to the reduction of
glucose uptake via the induction of SOCS expression in
3T3-L1 adipocytes (Figs. 2–4, 7, and 8). Interestingly,
IFN-β preferentially induced the expression of SOCS1
rather than SOCS3 and suppressed insulin-induced tyrosine

![Fig. 7](http://ajpendo.physiology.org/)

**Fig. 7.** Effect of IFN on insulin-induced Akt phosphorylation and impact of F-STAT3 expression on the IFN-γ-induced inhibition of insulin signaling. 3T3-L1 adipocytes were treated with IFN-β (A) or IFN-γ (B) and stimulated with 1.7 nM insulin for 5 min. The expression and insulin-induced phosphorylation of Akt were examined by anti-Akt and anti-phospho-Ser473 Akt antibody, respectively. *P < 0.05 and **P < 0.01 vs. IFN-untreated insulin-stimulated cells by the Bonferroni test, as appropriate, after ANOVA. C–E: 3T3-L1 adipocytes were transfected with adenovirus encoding LacZ, WT-STAT3, or F-STAT3. Then cells were treated with IFN-γ for 4 h and further stimulated with 1.7 nM insulin for 5 min. Whole cell lysates or anti-IRS-1 immunoprecipitates were blotted with anti-IRS-1 antibody (C), PY antibody (D), anti-Akt antibody, or anti-phospho-Ser473 Akt antibody (E). The levels of Akt phosphorylation were corrected for the loaded amount of Akt. Results are shown as the mean ± SE of 4–6 separate experiments. **P < 0.01 vs. LacZ-transfected insulin-untreated cells (C) or LacZ-transfected insulin-treated cells (D and E) by the Bonferroni test; †P < 0.05 and ††P < 0.01 for comparison between 2 groups, as indicated in the figure, by Student-Newman-Keuls test, as appropriate, after ANOVA.

![Fig. 8](http://ajpendo.physiology.org/)

**Fig. 8.** Effect of F-STAT3 expression on IFN-induced suppression of 2-[3H]deoxyglucose uptake. 3T3-L1 adipocytes were transfected with LacZ or F-STAT3. Cells were treated with IFN-β or IFN-γ and stimulated with various concentrations of insulin for 15 min. 2-[3H]deoxyglucose uptake for 4 min was measured by a liquid scintillation counter. Each measurement was performed in duplicate, and the results are the mean ± SE of 8 separate experiments. *P < 0.05 vs. IFN-untreated insulin-stimulated LacZ cells by the Bonferroni test and †P < 0.05 for comparison between 2 groups, as indicated in the figure, by Student-Newman-Keuls test, as appropriate, after ANOVA.
phosphorylation of IRS-1 without affecting the expression level. On the other hand, IFN-β/H9252 predominantly induced the expression of SOCS3, resulting in the degradation of IRS-1. In fact, knockdown of SOCS1 ameliorated only IFN-β-induced suppression of tyrosine phosphorylation of IRS-1, whereas knockdown of SOCS3 effectively ameliorated IFN-γ-induced reduction of IRS-1 protein (Fig. 6E). Along these lines, adenovirus-mediated overexpression of SOCS3, but not SOCS1, effectively reduced the amount of IRS-1, whereas the expression of SOCS1 and SOCS3 markedly suppressed insulin-induced tyrosine phosphorylation of IRS-1 in both cases (Fig. 6). These results are consistent with the previous report that transgenic mice overexpressing SOCS3 demonstrated the decreased expression level of IRS-1 and subsequent reduction of insulin signaling, leading to the impairment of insulin-induced glucose uptake in adipose tissue (33).

In addition, the expression of a dominant-negative STAT3 (F-STAT3) significantly suppressed IFN-β- and IFN-γ-induced expression of SOCS3 but not SOCS1 (Fig. 5). IFN-γ-induced degradation of IRS-1, reduction of insulin-induced phosphorylation of IRS-1 and Akt, and decrease in glucose uptake were effectively ameliorated by the expression of F-STAT3 (Figs. 7 and 8); therefore, IFN-γ-mediated insulin resistance appears to be caused mainly by the STAT3/SOCS3 pathway. In contrast, knockdown of STAT1 suppressed IFN-β-induced upregulation of SOCS1 (but not SOCS3) and ameliorated subsequent inhibition of insulin-induced tyrosine phosphorylation of IRS-1 (Supplemental Fig. S3); therefore, IFN-β-induced insulin resistance may be mediated predominantly via the STAT1/SOCS1 pathway. These results are consistent with a canonical model proposing the principal role of STAT1 in the SOCS1 expression and STAT3 in the SOCS3 expression (6). The underlying mechanism of type-specific utilization of the STAT1/SOCS1 or STAT3/SOCS3 pathway by different IFNs is unknown. Serine phosphorylation of STAT might be related to the selective induction of SOCS isoform, since the time course of STAT3 phosphorylation at Ser727 by IFN-β and IFN-γ was apparently different, regardless of the similar phosphorylation at Tyr705 residue (Fig. 3). In addition, a posttranscriptional mechanism might be involved, at least in part, in high-level expression of SOCS3 protein under IFN-γ. In this regard, treatment with IFN-γ induced both SOCS1 and SOCS3 mRNA expressions, contrary to the protein levels. On the other hand, IFN-β preferentially induced mRNA expression of SOCS1 rather than SOCS3, consistent with the results of protein levels (data not shown).

Fig. 9. Synergistic effects of IFN-γ and IL-6 on insulin signaling. 3T3-L1 adipocytes were treated with 10 ng/ml IL-6 for 1 or 24 h with or without IFN-γ for 24 h and further stimulated with 1.7 nM insulin for 5 min. Total cell lysates or immunoprecipitates with anti-IRS-1 antibody were blotted with anti-IRS-1 antibody (A), PY antibody (B), anti-phospho-Ser473 Akt antibody (C), anti-STAT3 antibody (D), anti-Tyr705 phosphospecific STAT3 antibody (E), or anti-SOCS3 antibody (F). The levels of Akt phosphorylation were corrected for the loaded amount of Akt. Results are shown as the mean ± SE of 4–6 independent experiments. *P < 0.05 and **P < 0.01 vs. IFN-untreated insulin-stimulated LacZ cells by the Bonferroni test; †P < 0.05 and ††P < 0.01 for comparison between 2 groups, as indicated in the figure, by Student-Newman-Keuls test, as appropriate, after ANOVA.
A recent study showed that treatment with IFN-γ inhibited insulin signaling in the human adipocytes by causing robust STAT1 phosphorylation and SOCS1 mRNA expression and by suppressing the differentiation of preadipocytes into adipocytes (14). Treatment with human IFN-γ for 24 h suppressed insulin-induced glucose uptake via suppressed expression of PPARγ, insulin receptor, IRS-1, and GLUT4 mRNA, possibly because of the promotion of the dedifferentiation in human adipocytes (14). In the present study, we observed that treatment with murine IFN-γ for 24 h inhibited insulin action without affecting differentiation status in 3T3-L1 adipocytes (Supplemental Fig. S4). In contrast, treatment with IFN-γ for longer than 48 h has been shown to suppress glucose uptake by the mechanism of promotion of dedifferentiation in 3T3-L1 adipocytes (43). Thus, it appears that IFN-γ induces insulin resistance directly through the STAT/SOCS pathway and indirectly through suppression of adipocyte differentiation (4, 14, 43). In the present study, we could clarify the signal transduction mechanism underlying IFN-γ-induced insulin resistance independently of adipocyte differentiation status in 3T3-L1 adipocytes. It is of note that the difference of species-specific activation process after IFN treatment (10, 30) may contribute to the discrepancy between our results and previous findings (14).

It is known that IFNs activate alternative pathways in addition to the STAT/SCOS pathway in relation to insulin signaling (23, 25). Thus, IFN-γ is reported to activate phosphatidylinositol 3-kinase-Akt and MAP kinase pathways through receptor-bound JAK2 (23, 25). Since treatment with either IFN-β or IFN-γ did not induce the phosphorylation of Akt (Fig. 7) and MAP kinase (data not shown), at least in our study, we consider that the inhibitory effect of IFNs on insulin signaling does not depend on the alternative pathway in adipocytes.

Serum concentrations of multiple cytokines are known to be elevated in response to various stresses, including virus infection, (17, 33). IL-6 is one of the proinflammatory cytokines implicated in the pathogenesis of insulin resistance (28). Treatment with IL-6 decreased the expression of IRS-1 and insulin-induced glucose uptake in 3T3-L1 adipocytes (28). Insulin-induced expression of SOCS3 is synergistically augmented by cotreatment with IL-6 in 3T3-L1 adipocytes (1). In addition, increased IL-6 was reported in the serum of hepatitis C virus-infected subjects (2) and in the fat tissue of insulin-resistant subjects (28). Since IL-6 plays a crucial role in the state of insulin resistance, we examined the effect of cotreatment with IFN and IL-6 on insulin signaling. Compared with IFN-γ alone, insulin signaling was further suppressed in cells incubated with IFN-γ and IL-6 despite no significant effect with IL-6 alone (Fig. 9). Since proinflammatory cytokines are generally known to enhance the production of other cytokines, we could not exclude the possibility that other factors recruited in the treatment with IFN activate STAT3 and affect the insulin sensitivity. More attention should be paid to clinical use of IFNs to prevent the augmentation of insulin resistance caused by proinflammatory cytokines in viral infections with hepatitis.

Recently, the accumulation of T lymphocytes has been shown in adipose tissues following high-fat feeding (19, 26, 44). Accumulation appears to impair systemic insulin sensitivity by causing macrophage infiltration and inflammation (28). IFN-γ is synthesized in immune cells, including CD4 Th1 cells and CD8 cytotoxic T cells (30). Indeed, the expression of IFN-γ has been shown to be increased in the adipose tissue of obese mice (26). Importantly, improved systemic glucose tolerance is observed with lower expression levels of tumor necrosis factor-α and monocyte chemoattractant protein-1 in adipose tissue in obese IFN-γ-deficient mice (26). On the basis of this, we consider that IFN-γ-mediated insulin resistance in adipose tissue may be one of the fundamental events in obesity-related glucose dysregulation.

In conclusion, the present results indicate first that both type I and type II IFNs induce insulin resistance by the distinct STAT/ SOCS pathway and that IFN-γ binds to cognate IFN receptors (IFN-α receptor 1 and 2 and IFN-γ receptor 1 and 2, respectively) and phosphorylate STAT families of the transcriptional factor. Phosphorylated STATs translocate to the nucleus and stimulate the transcription of SOCSs. IFN-β mainly induces the expression of SOCS1, which inhibits insulin-induced tyrosine phosphorylation of IRS-1. In contrast, IFN-γ preferentially stimulates the transcription of SOCS3, which facilitates the degradation of IRS-1. Notably, IFN-β- and IFN-γ-induced expressions of SOCS1 and SOCS3 are mediated by STAT1 and STAT3, respectively.

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**DISCLOSURES**

The authors have nothing to disclose.
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