Interferon γ Attenuates Insulin Signaling, Lipid Storage, and Differentiation in Human Adipocytes via Activation of the JAK/STAT Pathway

Received for publication, September 1, 2009 Published, JBC Papers in Press, September 23, 2009, DOI 10.1074/jbc.M109061655

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Recent reports demonstrate T-cell infiltration of adipose tissue in early obesity. We hypothesized that interferon (IFN) γ, a major T-cell inflammatory cytokine, would attenuate adipocyte functions and sought to establish signaling mechanisms. Differentiated human adipocytes were treated with IFNγ ± pharmacological inhibitors prior to insulin stimulation. [3H]Glucose uptake and AKT phosphorylation were assessed as markers of insulin sensitivity. IFNγ induced sustained loss of insulin-stimulated glucose uptake in human adipocytes, coincident with reduced AKT phosphorylation and down-regulation of the insulin receptor, insulin receptor substrate-1, and GLUT4. Loss of adipocyte triglyceride storage was observed with IFNγ, co-incident with reduced expression of peroxisome proliferator-activated receptor γ, adiponectin, perilipin, fatty acid synthase, and lipoprotein lipase. Treatment with IFNγ also blocked differentiation of pre-adipocytes to the mature phenotype. IFNγ-induced robust STAT1 phosphorylation and SOCS1 mRNA expression, with modest, transient STAT3 phosphorylation and SOCS3 induction. Preincubation with a non-selective JAK inhibitor restored glucose uptake and Akt phosphorylation while completely reversing IFNγ suppression of adipogenic mRNAs and adipocyte differentiation. Specific inhibition of JAK2 or JAK3 failed to block IFNγ effects suggesting a predominant role for JAK1-STAT1. We demonstrate that IFNγ attenuates insulin sensitivity and suppresses differentiation in human adipocytes, an effect most likely mediated via sustained JAK-STAT1 pathway activation.

Obesity has emerged as a major pandemic in Western society. Adipose inflammation is a key component of the pathophysiology in obesity-related insulin resistance, type 2 diabetes, and downstream complications (1–4). Recent work has revealed a role for adipose tissue macrophages in adiposity (5, 6). In early obesity, resident macrophages shift from a non-inflammatory, regulatory M2 phenotype toward the classical, pro-inflammatory M1 (CCR2+) phenotype (5). A high-fat diet increases circulating and adipose MCP1 (7) and promotes monocyte recruitment/retention in adipose (6, 8). Paracrine adipose tissue macrophage-adipocyte cross-talk induces adipocyte inflammation, modulates adipocytokines (9), and drives local and systemic insulin resistance and type 2 diabetes (10).

The triggers for adipose macrophage switching are poorly understood. Emerging reports demonstrate loss of regulatory T-cells (Treg) (11–13) and infiltration of inflammatory T-cells, particularly interferon (IFN) γ-secreting T helper type 1 (TH1) cells (11) and effector CD8+ T-cells (13, 14), with increasing adipose expression of T-cell chemokines (15). Furthermore, infiltration of T-cells into adipose tissue during obesity has been shown to precede macrophage recruitment (16). T-cells cytokines, in particular pro-inflammatory IFNγ (17), promote the macrophage M1 phenotype (18). Rocha et al. (19) recently identified a role for IFNγ in diet-induced adipose inflammation, obesity, and glucose intolerance in vivo. However, the role and mechanism of IFNγ modulation of adipocyte metabolic functions are poorly understood.

The IFNγ receptor consists of two subunits (α and β) that dimerize upon ligand occupation leading to transactivation of Janus family kinases 1 and 2 (JAK1 and 2), tyrosine phosphorylation of signal transducers and activator of transcription (STAT) molecules with translocation to the nucleus where they regulate gene transcription (20). Activation of JAK-STAT pathways potently induces suppressor of cytokine signaling molecules 1 and 3 (SOCS1 and 3) (21), which have been implicated in tumor necrosis factor α-induced insulin resistance in adipocyte and hepatic insulin resistance in vitro and in vivo (22–24). Thus, IFNγ and its JAK-STAT signaling are plausible candidates for...
inducing adipocyte inflammation and insulin resistance in diet-induced obesity.

In the current study we demonstrate that IFNγ induces insulin resistance in mature human adipocytes. This effect was time-dependent and remarkably coincided with suppression of insulin signaling molecules, markers of adipocyte differentiation and reduced triglyceride storage. Furthermore, IFNγ completely prevented pre-adipocyte differentiation to mature adipocytes. Inhibition of the JAK/STAT pathway with a non-selective JAK inhibitor abolished all adverse effects of IFNγ in mature adipocytes. In contrast, specific inhibition of JAK2 failed to alleviate IFNγ effects suggesting an important role for JAK1-STAT1 signaling. These studies establish the JAK-STAT pathway as a novel integrative mechanism, and therefore a potential therapeutic target, for modulation of T-cell-mediated adipose inflammation and insulin resistance in human obesity and type 2 diabetes.

**EXPERIMENTAL PROCEDURES**

**Glucose Uptake Assays**

2-[1,2,3-H]Deoxy-d-glucose was purchased from PerkinElmer Life Sciences. Simpson-Golabi-Behmel syndrome (SGBS) human cells were a gift from Dr. Martin Wabitsch, University of Ulm, Germany. Primary human pre-adipocytes were harvested from fresh subcutaneous adipose collected during elective bariatric surgeries at the hospital of the University of Pennsylvania. JAK inhibitor I (active against all JAK1, -2, -3, and Tyk2), AG490 (JAK2 inhibitor), JAK3 inhibitor L, SB203580 (p38 MAPK inhibitor), recombinant human leptin, and bovine serum albumin (Fraction V, low heavy metals) were purchased from Calbiochem (EMD, Germany). Recombinant human IFNγ was purchased from R&D Biosystems (Minneapolis, MN) and recombinant human interleukin-6 (IL-6) was purchased from Peprotech (Rocky Hill, NJ). The PPARγ agonist, GW347845, was purchased from GlaxoSmithKline (King of Prussia, PA). The ApoStrand™ ELISA was purchased from Enzo Life Sciences International, Inc. (Plymouth Meeting, PA). All other reagents, unless otherwise stated, were obtained from Sigma.

**Adipocyte Culture**—SGBS human adipocytes were cultured as previously described (25). Primary human pre-adipocytes were extracted from freshly isolated adipose tissue. Adipose was minced and digested with collagenase (1 mg/ml) (Roche Applied Science). Cells were centrifuged and the Stromal vascular pellet resuspended in Dulbecco’s modified Eagle’s medium/F-12 media containing 20% fetal bovine serum. Human pre-adipocytes were differentiated identically to SGBS cells. Briefly, confluent cells were incubated in differentiation media (Dulbecco’s modified Eagle’s medium/F-12, panthothenate (4 mg/liter), biotin (8 mg/liter), insulin (20 nm), hydrocortisone (1 μM), dexamethasone (250 nm), isobutylmethylxanthine (500 μM), PPARγ agonist (GW347845) (2 μM), triiodothyronine (0.2 nm), human transferrin (10 mg/liter), and penicillin/streptomycin) for 7 days. Differentiation media was replaced with 3FC media (differentiation media excluding PPARγ agonist and dexamethasone) for a further 7 days. 3T3L1 fibroblasts were differentiated to adipocytes as previously described (26).

**Immunoblot Analysis**—Protein concentration was quantified by the Pierce bicinchoninic acid (BCA) protein assay kit (Thermo, Fisher Scientific). Equal concentrations of protein lysate (10 μg) were reduced, separated by SDS-PAGE electrophoresis, transferred to nitrocellulose membranes, and blocked in 5% BSA in TBS-Tween prior to overnight incubation (at 4 °C) in primary antibody. Blots were probed with antibodies to phosphorylated Akt, whole cell Akt, phosphorylated STAT1, phosphorylated STAT3, phosphorylated JAK1, phosphorylated JAK2 (Cell Signaling Technology), whole cell IRS1 (Upstate, Millipore, MA), or β-actin primary antibodies (Abcam). Blots were washed, incubated in secondary antibody, and visualized by chemiluminescence.

**RNA Isolation and Quantitative Real-time PCR Analysis**—Mature adipocytes (day 14) ± the indicated treatments were harvested in TRIzol (Invitrogen) and RNA was isolated according to the manufacturer’s instructions and quantified spectrophotometrically. Treatments, and mock control, were added consecutively from −48 h, and at the experimental end point all cells were lysed simultaneously. RNA (500 ng) was reverse transcribed using an Applied Biosystems High Capacity cDNA archive kit. Expression levels of various genes were assessed by quantitative real-time PCR using an Applied Biosystems 7300 sequence detector. Primers, probes, and TaqMan Universal Mastermix were purchased from Applied Biosystems (ABI). To control the between sample variability, mRNA levels were normalized to β-actin for each sample by subtracting the Ct for β-actin from the Ct for the gene of interest, producing a ΔCt value. The ΔCt for each treatment sample was compared with the mean ΔCt for control samples using the relative quantitation 2−(ΔΔCt) method to determine fold-change.
Oil Red Staining and Triglyceride Measurement—Adipocytes were treated with IFNγ (20 ng/ml) or PPARγ agonist (GW347845) (10 μM) prior to fixing in formalin. Cells were washed, incubated with oil red stain for 1 h, washed again, and 4',6-diamidino-2-phenylindole nuclear stain was added. Cells were visualized at 100 magnification using a Nikon diaphot 300 fluorescent microscope. After visualization, cells were left to dry, lipid stain was extracted by addition of isopropyl alcohol, and quantitated by reading absorbance at 490 nm. In selective experiments, identically treated cells were lysed in RIPA buffer prior to triglyceride quantitation using the Affinity Triglyceride Assay Kit (Thermo Electron).

Cell Viability Assays—Mature SGBS adipocytes were seeded in 24-well plates (for trypan blue exclusion assay) or 96-well plates (for ApoStrand ELISA) and were differentiated as described above. Mature adipocytes (day 14) were treated with IFNγ (20 ng/ml) for up to 48 h. Positive control cells were incubated with PBS for 24 h to induce cell death. To test for cell viability, cells were washed with PBS, trypsinized, pelleted, and resuspended in trypan blue (0.4% for 5 min). Live and dead cells were counted using a hemocytometer. To test for apoptosis, adipocytes were treated as above and apoptosis was evaluated using the ApoStrand ELISA kit, a single-stranded DNA ELISA kit specific for apoptotic cells. Positive control wells were coated with single-stranded DNA (30 ng/well).

Statistical Analysis—Data are reported as mean ± S.E. For experiments with multiple treatments, analysis of variance was used to test for differences in means. When analysis of variance was significant, post hoc Bonferroni t tests were applied for group comparisons. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA).

RESULTS

IFNγ-induced Insulin Resistance in Human Adipocytes in a Time-dependent Manner—IFNγ (20 ng/ml) induced a progressive, time-dependent attenuation of insulin-stimulated glucose uptake in mature human SGBS adipocytes, with complete inhibition after 48 h treatment (Fig. 1A). On average, insulin-stimulated glucose uptake was 2.12 ± 0.12-fold higher than basal (non-insulin-stimulated cells) (n = 3, p < 0.001 versus insulin alone). In selective experiments, identically treated cells were lysed in RIPA buffer prior to triglyceride quantitation using the Affinity Triglyceride Assay Kit (Thermo Electron).

FIGURE 1. A, mature SGBS adipocytes were treated with IFNγ (20 ng/ml), or control (SFM + 0.2% BSA), for the indicated time points prior to stimulation with insulin (100 nM) for 15 min. [3H]Glucose uptake was monitored over a further 15-min period. Fold-increase in insulin-stimulated [3H]glucose uptake over basal (non-insulin treated cells) is presented (n = 4, ***, p < 0.001 versus insulin alone). B, mature SGBS adipocytes were treated with increasing concentrations of IFNγ (in SFM + 0.2% BSA) for 48 h and insulin-stimulated [3H]glucose uptake subsequently monitored. Fold-increase in insulin-stimulated glucose uptake over basal is presented (*, p < 0.05; **, p < 0.01; ***, p < 0.001 versus insulin alone, n = 3). C, SGBS adipocytes were treated with IFNγ for the indicated time points and the effects on mRNA expression of insulin signaling genes were measured by real-time PCR analysis (n = 3, **, p < 0.01; ***, p < 0.001 versus control; white circles, insulin receptor; black squares, insulin receptor substrate 1; black triangles, Glut4). Error bars represent S.E.

D, adipocyte protein lysates from time course glucose uptake assay were probed for levels of phosphorylated AKT and whole cell AKT by immunoblot analysis.

E, the effect of IFNγ on protein levels of IRS1 and β-actin was assessed by immunoblot analysis.

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p < 0.001). Also, compared with control, IFNγ reduced, in a similar time-dependent manner, insulin-stimulated phosphorylation of Akt, a key indicator of insulin sensitivity (Fig. 1D). Increasing concentrations of IFNγ (0.1 to 30 ng/ml) progressively reduced insulin-stimulated glucose transport with effects at concentrations as low as 0.3 ng/ml (Fig. 1B). Furthermore, IFNγ had no significant effect on adipocyte apoptosis or cellular viability (supplemental Fig. S1, A and B) suggesting specific IFNγ modulation of adipocyte functions rather than cytotoxic effects.

**IFNγ Suppressed Insulin Signaling Genes**—The effects of IFNγ, compared with mock control, on expression of genes involved in insulin signaling were assessed in mature SGBS adipocytes. Treatments were added sequentially and all cells were lysed simultaneously to eliminate the time-dependent variation in gene expression. A significant reduction in GLUT4, insulin receptor (IR), and insulin receptor substrate-1 (IRS1) mRNA levels were observed as early as 4 h and sustained at 48 h post-IFNγ (Fig. 1C). IFNγ also reduced whole cell IRS1 protein levels (Fig. 1E). Similar effects of IFNγ on expression of insulin signaling genes were observed in human primary and mouse 3T3L1 adipocytes (supplemental Fig. S2, A and C).

**IFNγ Reduced Triglyceride Storage in Mature Adipocytes Coincident with Suppression of Adipogenic Gene Expression**—Because IFNγ modulated multiple components of the adipocyte insulin signaling pathway, we hypothesized that it may more broadly impact basic adipocyte functions including differentiation and lipid storage. In mature SGBS adipocytes, treatment with IFNγ reduced, in a time-dependent manner, triglyceride content (Fig. 2A) and lipid droplet number at oil red staining (Fig. 5E), but had no effect on the number of cells by nuclear staining. The effect of IFNγ on lipid content was paralleled by suppression of mRNA levels for important adipogenic genes (e.g. PPARγ and adiponectin) as well as genes involved in lipid synthesis and storage (e.g. lipoprotein lipase, fatty acid synthase, and perilipin) (Fig. 2B). The time course of these IFNγ effects coincided with maximal attenuation of insulin signaling and down-regulation of insulin signaling genes. Control mock treatment for 48 h had no effect on triglyceride storage or gene expression.

**IFNγ Blocks Pre-adipocyte Maturation to Fully Differentiated Adipocytes**—The effect of IFNγ on SGBS pre-adipocyte differentiation was assessed by adding IFNγ (20 ng/ml) to the differentiation media for the full 2-week treatment period. At day 14, compared with control, IFNγ markedly suppressed triglyceride accumulation (Fig. 3B) and abolished insulin-stimulated glucose uptake (Fig. 3C), both established markers of adipocyte differentiation and maturity.

**IFNγ Activated JAK/STAT Signaling and Induced Suppressor of Cytokine Signaling Molecules in Adipocytes**—The IFNγ receptor couples to the JAK/STAT pathway but specific IFNγ signaling in adipocytes is poorly defined. Therefore, phosphorylation of JAK1, JAK2, STAT1, and STAT3 was examined. Basal levels of phosphorylated JAK1 were detectable, consistent with previous non-adipocyte literature (29), whereas IFNγ induced a further sustained increase in JAK1 phosphorylation. In contrast, JAK2 phosphorylation was absent at baseline but was transiently induced by IFNγ (Fig. 4A). Similarly, IFNγ induced robust and sustained phosphorylation of STAT1, whereas STAT3 phosphorylation was modest and transient.

Previous work implicates STAT1 in SOCS1, and STAT3 in SOCS3, expression; lack of SOCS1 induction was observed in STAT1−/− cells (30). In mature SGBS adipocytes, IFNγ induced robust and sustained expression of SOCS1, tracking the sustained activation of STAT1 (Fig. 4, A and B), whereas SOCS3 induction was modest and transient consistent with the pattern of STAT3 phosphorylation. A similar trend for SOCS expression was observed in human primary and 3T3L1 adipocytes (supplemental Fig. S2, B and D). These data suggest that activation of JAK-STAT’s, particularly STAT1, may mediate IFNγ effects on human adipocytes.

**JAK Inhibition Blocks IFNγ-induced Adipocyte Dysfunction**—Cells lacking either JAK1 or JAK2 fail to transduce IFNγ signals highlighting the importance of these isoforms in IFNγ signaling (31). However, little is known of the role for specific JAK isoform signaling in adipocyte biology. Therefore, we assessed the role of various kinases in mediating IFNγ effects in human adipocytes using pharmacological inhibitors. SGBS adipocytes were pre-treated with a JAK inhibitor I (10 μM) (activity against all four JAK kinases: JAK1, JAK2, JAK3, and Tyk2), JAK2 inhibitor (100 μM), JAK3 inhibitor (50 μM), or a p38 MAPK inhibitor (5 μM) for 2 h

**FIGURE 2. A,** mature SGBS adipocytes were treated with IFNγ (20 ng/ml), or control (SFM + 0.2% BSA), for the indicated time points prior to lysing in RIPA buffer. Triglyceride levels were measured using the Affinity Triglyceride Assay Kit (n = 3, ***p < 0.001 versus control). B, the effect of IFNγ (20 ng/ml) on mRNA levels of adipocyte differentiation genes over a 48-h time period was assessed by real-time PCR analysis (n = 3, *, p < 0.05; ***, p < 0.001; and ***, p < 0.001 versus control; black circles, fatty acid synthase (FAS); white circles, lipoprotein lipase (LPL); black triangles, perilipin; white triangles, adiponectin, black squares, PPARγ). Error bars represent S.E.
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FIGURE 3. A, confluent SGBS pre-adipocytes were incubated in differentiation media ± IFNγ (20 ng/ml) for 14 days; cells were fixed and lipid accumulation monitored by oil red staining. Images presented are at ×100 magnification. B, lipid stain from cells was extracted using isopropyl alcohol and oil red accumulation quantified using spectrophotometry (***, p < 0.001 versus control, n = 6). C, at day 14 post-initiation of differentiation, insulin-stimulated [3H]glucose uptake into the cells was assessed. Cells were stimulated with insulin (100 nM) for 15 min and [3H]glucose uptake monitored over a further 15-min period. Cells were lysed and [3H]glucose uptake measured by liquid scintillation counting; fold-increase in glucose uptake over non-insulin treated cells is presented (***, p < 0.001 versus non-insulin stimulated control, n = 4). Error bars represent S.E.

prior to co-treatment with IFNγ (20 ng/ml) for 4 or 48 h. Specific JAK1 inhibitors are not commercially available. Remarkably, pre-treatment with JAK inhibitor I, but not other inhibitors (including JAK2 inhibitor), resulted in complete attenuation of IFNγ effects on insulin-stimulated glucose transport (Fig. 5A), AKT phosphorylation (Fig. 5B), down-regulation of IRS1 (Fig. 5C), and induction of SOCS1 (Fig. 5D) and SOCS3 (which blocked IFNγ-induced SOCS3 from the 23.09 ± 1.03-fold increase over control to 0.82 ± 0.01-fold, p < 0.001). Pre-treatment with JAK inhibitor I also blocked the IFNγ-induced loss of triglyceride stores (Fig. 5E) as well as down-regulation of PPARγ, adiponectin, lipoprotein lipase, fatty acid synthase, and perilipin mRNA (Fig. 5F).

The lack of effect of JAK2 inhibition was surprising given placement of JAK2 downstream of IFNγ in this (Fig. 4A) and other (31) studies. To ensure pharmacological activity of the JAK2 inhibitor, we pre-treated SGBS adipocytes with the JAK2 inhibitor (100 μM for 2 h), prior to stimulation with IL-6 (30 ng/ml for 1 h). IL-6 specifically activates JAK1 and induces STAT3 phosphorylation (32). Pre-treatment with the JAK2 inhibitor attenuated IL-6-induced STAT3 phosphorylation validating its bioactivity (supplemental Fig. 3E). Overall, the potent effects of the JAK inhibitor I coupled with the absence of effect of JAK2 inhibition, and several other kinase inhibitors, on IFNγ effects suggest a central role for JAK1-STAT1 coupling in IFNγ-mediated adipocyte dysfunction.

Leptin, a JAK2/STAT3 Activator, Induces Differential Effects in Adipocytes Compared with IFNγ—Our findings suggest a minor role for JAK2, and its downstream target STAT3, in IFNγ-mediated insulin resistance. We sought independent support for this finding in mature adipocytes with leptin (100 ng/ml), which signals primarily via JAK2-STAT3 (33, 34). In contrast to IFNγ, we found that leptin induced SOCS3 to a greater extent than SOCS1 (supplemental Fig. S3A), consistent with the expected greater activation of STAT3 than STAT1 by leptin (33, 35). Furthermore, relative to IFNγ, leptin induced less marked down-regulation of IRS1 and GLUT4 (supplemental Fig. S3B), more modest suppression of PPARγ and mRNA markers of adipocyte differentiation (supplemental Fig. S3C), whereas having no effect on adipocyte lipid content (supplemental Fig. S3D). These studies suggest that JAK1-STAT1 activation by IFNγ induces a more profound and prolonged effect on adipocyte functions than that observed with JAK2-STAT3 activation and support the concept that JAK2-STAT3 is unlikely to
mediate the marked IFNγ modulation of human adipocyte functions.

**PPARγ Agonist Partially Alleviates IFNγ-induced Insulin Resistance**—PPARγ is required for adipocyte differentiation and insulin sensitivity (27, 28). To explore the role of PPARγ in IFNγ effects, we co-treated mature adipocytes ± IFNγ (20 ng/ml) ± PPARγ agonist (GW34784; 10 μM) for 48 h. The PPARγ agonist only partially ameliorated the effects of IFNγ on insulin-stimulated glucose transport (24% increase in glucose transport with IFNγ/PPARγ compared with IFNγ alone, Fig. 6A). There remained a significant decrease in glucose transport in IFNγ/PPARγ-treated cells compared with control cells suggesting non-PPARγ-dependent effects of IFNγ. Furthermore, the PPARγ agonist failed to block IFNγ reduction of lipid stores in mature adipocytes (Fig. 6B).
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Recent studies suggest that loss of Treg cells (11–13) coincident with accumulation of inflammatory T-cells in adipose (11, 13, 14) are early events in obesity that drive adipose inflammation and induce adipocyte insulin resistance even prior to macrophage infiltration (16). We demonstrate that IFNγ, a prototypical inflammatory T-cell cytokine, induced insulin resistance, down-regulated markers of differentiation, and reduced lipid storage in human adipocytes via the JAK-STAT pathway, most likely mediated through sustained JAK1-STAT1 activation. Our studies establish IFNγ, and the JAK-STAT pathway, as novel mediators of, and potentially therapeutic target, for inflammatory T-cell-induced human adipose inflammation and insulin resistance.

Adipose tissue inflammation has emerged as a primary mediator of diet and obesity-driven insulin resistance (4, 36). Elevated systemic levels of adipose-derived cytokines IL-6 and tumor necrosis factor-α (37, 38) are found in obesity, impair glucose transport in vitro (39, 40), and attenuate insulin sensitivity in vivo (41, 42). Hyperthrophied adipocytes, observed in obesity, secrete abundant monocyte chemoattractant protein-1, which can promote macrophage recruitment into adipose (43). Furthermore, resident non-inflammatory M2 macrophages switch to a pro-inflammatory M1 phenotype augmenting adipose tissue inflammation (5, 8). The trigger that causes adipose macrophage phenotype switching is poorly understood. IFNγ, a primary T-cell-derived cytokine, stimulates macrophages toward an M1 phenotype (18). Thus, it is conceivable that inflammatory T-cell infiltration of adipose, with secretion of IFNγ, plays a role in regulation and switching to pro-inflammatory macrophages.

Recent work strongly supports this model and goes further by directly implicating inflammatory T-cells and IFNγ in early adipose inflammation and adipocyte dysfunction (11–13, 16, 19). Rocha et al. (19) found an increased number of CD4+ and CD8+ cells in adipose tissue of diet-induced obese mice. Furthermore, obese IFNγ-deficient mice exhibited less accumulation of inflammatory cells in adipose tissue and improved glucose tolerance than their control counterparts. The presence of T-cells, observed at week 5, correlated with early onset insulin resistance, whereas the presence of macrophages was not evident until week 10 (16). Three recent independent reports (11–13) all demonstrate an increased ratio of CD8+/CD4+ T cells in adipose tissue with obesity and suggest that resident adipose CD4+ T cells (in particular TH2 and Treg cells) protect against obesity and insulin resistance under resting conditions. Nishimura et al. (13) also demonstrated that depletion of CD8+ T-cells lowered macrophage infiltration and adipose tissue inflammation while enhancing insulin sensitivity. Winer and colleagues (11) reported visceral adipose accumulation of pathogenic IFNγ producing CD8+ T-cells and that immunotherapy with CD4+ T-cells in Rag1-deficient mice with diet-induced obesity reversed weight gain and insulin resistance, predominantly through TH2 cells. Feuerer et al. (12) found that a unique population of CD4+ Treg cells, which restrain adipose inflammation, are present in normal adipose but markedly depleted in adipose of obese insulin-resistant rodents. Ablation of Treg cells in lean mice reduced adipose insulin sensitivity (12).

Despite these emerging data of T-cell involvement in adipose inflammation, little is known of the mechanisms of IFNγ modulation of adipocyte functions. In the present study, we examined the effects of IFNγ on insulin sensitivity, lipid storage, and differentiation in human adipocytes. We found that IFNγ induced a time-dependent reduction of insulin-stimulated glucose uptake and AKT phosphorylation, both indicative of insulin resistance. Dose-response studies demonstrated that IFNγ-induced insulin resistance at concentrations as low as 0.3 ng/ml suggesting modulation of adipocyte functions at local concentrations observed in physiological and pathophysiological settings in vivo. Notably, reduced glucose uptake was not evident until at least 8 h post-treatment suggesting that direct kinase modulation of insulin signaling proteins, such as serine phosphorylation of IRS1, is unlikely to be the primary mechanism of the IFNγ effect. Rather, IFNγ markedly reduced expression of insulin signaling proteins including IR, IRS1, and GLUT4; indeed, reduced IRS1 protein levels were observed after 24 h coincident with reduced glucose uptake.

In mature adipocytes, longer term IFNγ treatment markedly reduced triglyceride storage, coincident with striking and sustained suppression of mRNAs for adipogenic genes including PPARγ, fatty acid synthase, lipoprotein lipase, adiponectin, and perilipin. Furthermore, during the 2-week treatment with differentiation medium, addition of IFNγ to pre-adipocytes completely blocked lipid accumulation and insulin-stimulated glucose uptake, both hallmarks of adipocyte differentiation. Because PPARγ is required for adipocyte differentiation and insulin sensitivity (27, 28), we hypothesized that modulation of PPARγ might play a key role in IFNγ effects on adipocyte functions. Indeed, the addition of PPARγ agonist during IFNγ treatment partially restored insulin-stimulated glucose uptake. However, PPARγ activation failed to block the effects of IFNγ.
on triglyceride storage in mature adipocytes. Parenthetically, the presence of PPARγ agonist in differentiation medium did not prevent IFNγ inhibition of pre-adipocyte differentiation (Fig. 3). These data provide preliminary support for both PPARγ-dependent and -independent IFNγ effects in adipocytes. Besides PPARγ, there are several potential mediators of IFNγ effects in adipocytes, including interferon regulatory factors (44, 45), transcription factors with emerging roles in adipocyte biology (46).

These findings prompted a more detailed study of IFNγ receptor signaling in human adipocytes to elucidate the proximal transduction of these effects. Although IFNγ is known to activate JAK-STAT pathways, its signal transduction in adipocytes is not well characterized. The IFNγ receptor consists of two subunits, IFNR1, which associates with JAK1, and IFNR2, which associates with JAK2 (29). In HeLa cells, JAK1 is constitutively phosphorylated, whereas phosphorylation of JAK2 is induced only after IFNγ treatment (29). Consistent with these studies we detected constitutively phosphorylated JAK1 in human adipocytes, whereas levels of phosphorylated JAK2 were only detected upon IFNγ treatment. Both JAK1 and JAK2 are essential for IFNγ signaling (31, 47). Indeed, activation of JAK1 and -2 by IFNγ results in the coordinated phosphorylation and activation of STAT signaling proteins, in particular STAT1 and to a lesser extent STAT3 (48, 49). These activated transcription factors dimerize and translocate to the nucleus to regulate gene transcription. STAT1 plays an important role in inducing SOCS1 expression, whereas STAT3 mediates SOCS3 expression (30). In our studies, IFNγ induced robust and prolonged phosphorylation of STAT1, which was associated with a marked and sustained increase in SOCS1 mRNA. In contrast, IFNγ induced a transient and lesser phosphorylation of STAT3 coincident with more modest and transient induction in SOCS3. Both SOCS1 and -3 have been shown to bind to tyrosine-phosphorylated insulin receptor blocking IR-IRS1 coupling, thus promoting insulin resistance (22–24, 50). Our data suggest that beyond suppression of insulin signaling proteins and reversal of adipocyte differentiation, that induction of SOCS proteins may contribute to the adverse effects of IFNγ on insulin signaling.

Non-selective JAK inhibition abolished the adverse effects of IFNγ on insulin sensitivity and lipid storage while also blocking all transcriptional effects of IFNγ. In contrast, specific inhibitors of JAK2 or STAT3 (data not shown), as well as inhibitors of JAK3 and p38 MAPK, failed to attenuate the effects of IFNγ. These pharmacological data provide support for the concept that JAK1, but not JAK2, is primarily responsible for IFNγ actions in human adipocytes.

We sought additional evidence in human adipocytes to support an IFNγ-STAT1 signaling effect. Ligands that predominantly activate JAK2, such as growth hormone and leptin, induce more potent phosphorylation of STAT3 than STAT1 (33–35). We found that leptin, a specific JAK2 activator, induced SOCS3 expression to a greater extent than SOCS1 consistent with greater STAT3 activation. Furthermore, leptin induced a distinct response in human adipocytes relative to IFNγ with a modest effect on markers of differentiation and no suppression of lipid storage. Given that STAT1 interacts with the IFNγR1 subunit that is directly bound to JAK1 (51) and that specific activators of JAK2 induce only minimal STAT1 phosphorylation (32–34), these findings provide further evidence that JAK1-STAT1 activation plays the major role in mediating IFNγ effects on human adipocytes. Indeed, recent in vivo studies demonstrating that exanatide, a JAK1-STAT1 inhibitor, prevents high fat diet-induced insulin resistance and glucose intolerance in rats (52) strongly suggest an important role for this pathway in diet-induced insulin resistance.

CONCLUSIONS

We demonstrate that IFNγ attenuates insulin signaling, lipid storage, and differentiation in human adipocytes, an effect likely mediated via sustained STAT1 activation. Pro-inflammatory T-cell derived cytokines, particularly IFNγ, may play an important role in T-cell modulation of diet-induced obesity, insulin resistance, and type 2 diabetes via activation of the adipocyte JAK-STAT pathway.

Acknowledgments—We acknowledge the support of staff and facilities in the Clinical and Translational Science Award (CTSA)-supported Clinical and Translational Research Center and the Diabetes and Endocrinology Research Center (DERC)-supported Human Adipose Resource at the University of Pennsylvania. We thank Dr. Martin Wabitsch (University of Ulm, Germany) for the kind gift of human SGBS cells.

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