INSULIN ANTAGONIZES IL-6 SIGNALING AND IS ANTI-INFLAMMATORY IN 3T3-L1 ADIPOCYTES

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Running Title: Anti-inflammatory effect of insulin

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The adipose tissue secretes different adipokines, including interleukin-6 (IL-6), which have been implicated in the insulin resistance and inflammatory state characterizing obesity. We examined the putative cross-talk between insulin and IL-6 in adipose cells and found that insulin exerts an inhibitory effect on the IL-6 signaling pathway by altering the post-translational modifications of the signal transducer and activator of transcription 3 (STAT3).

Insulin reduces the tyrosine phosphorylation and increases the serine phosphorylation of STAT3, thereby reducing its nuclear localization and transcriptional activity. Signaling through the MEK/MAPK pathway plays an important role as treatment with the MEK inhibitor PD98059 reduces the effects of insulin on IL-6 signaling. We also show that the protein tyrosine phosphatase SHP2 is activated upon insulin signaling and is required for the dephosphorylation of STAT3 and that insulin exerts a synergistic effect with IL-6 on SOCS3 expression. As a consequence, the IL-6-induced expression of the inflammatory markers serum amyloid A 3 (SAA3) and haptoglobin are significantly decreased in cells incubated with both IL-6 and insulin. Thus, insulin exerts an important anti-inflammatory effect in adipose cells by impairing the IL-6 signal at several levels.

The adipose tissue has long been regarded as important only for the storage and release of lipids. It is now known to be the largest endocrine organ in the body, secreting cytokines, hormones, and growth factors (commonly referred to as adipokines) that are important paracrine/endocrine regulators (1). Adipose tissue is also thought to play an important role in the low-grade chronic inflammation associated with obesity and insulin resistance (1). Obesity and enlarged fat cells alter the pattern of secreted adipokines, favoring release of the inflammatory cytokine, interleukin-6 (IL-6), and reducing the anti-inflammatory and insulin-sensitizing adipokine, adiponectin (1).

IL-6 is an important activator of inflammation in both liver and adipose tissue. Interestingly, the IL-6 concentration in the interstitial fluid of the adipose tissue is ~50-fold higher than in circulation, which strongly supports its role as an important regulator in this tissue (2). In addition, the adipose tissue accounts for around 30% of the circulating IL-6 concentration (3).

Several studies have shown that IL-6 impairs insulin signaling. For example, IL-6 has been shown to reduce expression of adiponectin in human adipose tissue and the insulin receptor substrate-1 (IRS-1) and glucose transporter 4 (GLUT4) in 3T3-L1 cells (2, 4). IL-6 is also a well-known inducer of suppressor of cytokine signaling (SOCS) proteins (5), and induction of SOCS1 and SOCS3 has been shown to inhibit insulin signaling by binding to the insulin receptor and IRS-1 (6-8).

Intracellular IL-6 signaling is initiated by the interaction of IL-6 with a plasma membrane receptor complex containing the signal transducer, glycoprotein 130 (gp130). This results in the activation of Janus kinases (JAKs), allowing tyrosine phosphorylation of gp130. In turn, this recruits signal transducers and activators of transcription (STAT) family members, such as STAT3, but also other Src homology 2 domain (SH2)-containing proteins, including the protein tyrosine phosphatase SHP2. STAT3 is phosphorylated by JAK at Tyr705, which is considered crucial
for the homo- or heterodimerization of STAT3 to either STAT3 or STAT1, enabling nuclear translocation (9). Phosphorylation of Ser727 in STAT3 has been reported to be important for the transcriptional activation, but the evidence is contradictory. Some reports have shown that serine phosphorylation increases the transcriptional activation of STAT3 (10-14), whereas others have shown that it reduces or has no effect on transcriptional activity (15-18). There are also reports indicating that the serine and tyrosine phosphorylations may negatively regulate each other (16). The effect of Ser727 phosphorylation of STAT3 may vary according to cell type, the kinase pathway activated, and the cytokine or growth factor used.

In this study, we have further investigated the cross-talk between insulin and IL-6 and asked if insulin alters the IL-6 signaling pathway in 3T3-L1 cells. It has previously been implicated that insulin has an anti-inflammatory effect in vivo and in PBMC cells through an effect on ROS generation and NFκB activation (19). Our results show that preincubation of 3T3-L1 cells with insulin significantly reduces the transcriptional activity of STAT3, thus reducing the transcription of the IL-6-induced inflammatory proteins SAA3 and haptoglobin. This is accomplished through a reduced tyrosine phosphorylation of STAT3 and, consequently, nuclear translocation and transcriptional activity in response to IL-6. We also provide evidence that STAT3 tyrosine and serine phosphorylations are regulated by insulin through the protein tyrosine phosphatase SHP2 and the MEK/MAPK signaling pathway, respectively. We conclude that insulin exerts anti-inflammatory effects in 3T3-L1 adipocytes by modulating the intracellular IL-6 signaling pathway.

**Experimental Procedures**

**Cell Cultures** – 3T3-L1 cells and mouse embryonic fibroblasts (MEFs) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (PEST) and 2 mM L-glutamine. Differentiation was induced in post-confluent 3T3-L1 cells by adding a differentiation cocktail (500 μM 3-isobutyl-1-methylxantine, 5 μg/ml insulin and 10 μM dexamethasone) for two days. The cells were then allowed to grow for another two days in supplemented DMEM with 5 μg/ml insulin followed by four days in normal supplemented DMEM (i.e. no insulin or differentiation cocktail). On day eight, medium was changed to DMEM with 1.5% FBS for 12 h. The cells were then washed in phosphate-buffered saline (PBS) and grown for another 12 h in DMEM with 0.5% bovine serum albumin (BSA) before starting the experiment. The MEFs were starved for 16 h in DMEM with 0.5% BSA before the insulin/IL-6 additions. The cells were then incubated with 20 ng/ml IL-6 with or without 100 nM insulin for different times. Insulin was usually added 10 min before IL-6. PD98059 (Calbiochem, San Diego, CA) (40 μM) was added 30 min before IL-6.

**Cell Lysates, Immunoprecipitation and Western Blot** – Cells were washed in PBS and lysis buffer (25 mM Tris-HCl, pH 7.4, 0.5 mM EGTA, 25 mM NaCl, 1% Nonidet P-40, 10 mM NaF, 0.01 mg/ml leupeptin, 1 mM orthovanadate, 100 nM okadaic acid, 1 mM benzamidine, 2 mM AEBSF) was then added to the cells and the lysate was centrifuged at 12,000 rpm for 10 min. Preparation of nuclear lysate followed the recommendation of the manufacturer of the STAT3 Transcription Factor Assay (Chemicon International, Temecula, CA). Purity of the nuclear fraction was verified by immunoblotting for proteins abundant in the cytoplasm. Protein concentration was measured by the BCA protein assay kit (Pierce, Rockford, IL). Immunoprecipitations were made by adding 30 μl of Protein G-coupled Dynabeads (Dynal Biotech, Oslo, Norway), that had been preincubated for 1 h with 1 μg of αgp130 antibody (Santa Cruz Biotechnology, Santa Cruz, CA), to 100 μg of cell lysates and incubated over night at 4°C. The lysate (or immunoprecipitated proteins) was boiled in SDS loading buffer and applied on 7.5% SDS-PAGE (Cambrex, Walkersville, MD). Following gel transference, the nitrocellulose membranes were blocked with 5% fat-free milk powder in PBS-Tween20 for 1 h. Membranes were probed with primary antibodies over night at 4°C followed by incubation for 1 h with secondary HRP-conjugated antibodies (Amersham Biosciences, Buckinghamshire, U.K.), then incubated over night at 4°C followed by incubation for 1 h with secondary HRP-conjugated antibodies (Amersham Biosciences, Buckinghamshire, U.K.), diluted 1:1000 in blocking solution. Proteins were visualized by ECL substrate (Amersham Biosciences,
The primary antibodies (pY705-STAT3, pY542-SHP2, pY694-STAT5 and pT202/Y204-p44/42 MAPK) were all from Cell Signaling Technologies (Beverly, MA) and diluted 1:1000 according to the manufacturer’s protocol. STAT3 (BD Biosciences, Palo Alto, CA) mAb was diluted 1:2000 and pS727-STAT3 (Santa Cruz Biotechnology, Santa Cruz, CA) was diluted 1:500.

Transcriptional Activity of STAT3 – Nuclear lysates from differentiated 3T3-L1 cells treated with IL-6 and insulin for 15, 30 and 60 min were analyzed using a STAT3 Transcription Factor Assay kit (Chemicon International, Temecula, CA) according to the recommendations of the manufacturer.

RT-PCR and Quantitation Analysis – RNA was isolated with the RNasey Mini Kit (Qiagen, Valencia, CA) and the following reverse transcriptase (RT) PCR for cDNA preparation was made using High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Relative quantification was made using ABI Prism 7700 Sequencing Detection System (Applied Biosystems, Foster City, CA). Primers and probes were designed using Primer Express software and sequences are available upon request.

Statistical Analysis – All statistical analyses were performed in Microsoft Excel and statistical significances were evaluated with the paired Student’s t test when appropriate.

RESULTS

Effect of Insulin on Tyr705 Phosphorylation of STAT3 – To study the effects of insulin on IL-6-induced activation of STAT3, Western blot analysis was used to determine the phosphorylation of Tyr705 of STAT3 in differentiated 3T3-L1 adipocytes following incubation with IL-6 and/or insulin. The IL-6 induced tyrosine phosphorylation of STAT3 occurred rapidly (data not shown) and insulin did not exert an immediate effect on the phosphorylation of Tyr705 of STAT3. However, after 30 min a consistent reduction was seen in cells treated with both IL-6 and insulin compared with cells treated with IL-6 alone (Fig. 1A, upper panel). The STAT3 protein content was not altered by any of the additions (Fig. 1A, lower panel).

Effect of Insulin on Immunoprecipitation of gp130 and STAT3 – As IL-6-induced tyrosine phosphorylation occurs when STAT3 is bound to the gp130 receptor, we investigated the effect of insulin on the interaction between STAT3 and gp130 using co-immunoprecipitation experiments. Lysates from differentiated 3T3-L1 cells treated for 120 min with insulin and/or IL-6 were immunoprecipitated with an anti-gp130 antibody followed by immunoblotting for STAT3. The amount of STAT3 associated with gp130 was reduced in samples treated with both IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 1B, upper panel). The blots were probed with αgp130 ab to verify that equal amounts of gp130 had been immunoprecipitated (Fig. 1B, lower panel). These results show that the reduction in IL-6-induced tyrosine phosphorylation of STAT3 in response to insulin is associated with a decreased binding of STAT3 to gp130.

Effect of Insulin on Nuclear localization of STAT3 – We investigated the effect of insulin on IL-6-induced dimerization and nuclear translocation of STAT3 as these events are dependent on tyrosine phosphorylation (20, 21). Nuclear lysates were prepared and analyzed for STAT3 after 15-60 min incubations with IL-6 and/or insulin. The level of STAT3 in the nucleus was reduced after 15 min in samples treated with both IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 2A). This reduction was seen throughout the study period.

Effect of Insulin on the transcriptional activation of STAT3 – Using a STAT3 transcription factor assay, nuclear lysates from differentiated 3T3-L1 cells that had been incubated with IL-6 and/or insulin were analyzed. A significant reduction of the transcriptional activation was detected after 15 min in samples treated with IL-6 and insulin compared with samples treated with IL-6 alone (Fig. 2B). These results are in agreement with the results of nuclear translocation (Fig. 2A) and the insulin-induced reduction was also observed throughout the studied period.

IL-6-induced gene expression – The inflammatory proteins serum amyloid A (SAA) and haptoglobin are strongly upregulated by cytokines such as IL-6 (22, 23). We analyzed the gene expression of SAA3 and haptoglobin in differentiated 3T3-L1 cells that had been incubated with insulin and/or IL-6 for 24 h. Insulin significantly reduced the IL-6-
induced expression of both SAA3 and haptoglobin mRNA (Fig 3A and 3B).

IL-6 also upregulates expression of the suppressor of cytokine signaling SOCS3 (5). Exposure of differentiated 3T3-L1 cells to IL-6 for 60 min significantly increased the gene expression of SOCS3 ~25-fold (Fig. 3C). In contrast to the gene expression of SAA3 and haptoglobin, addition of insulin increased the IL-6-induced expression of SOCS3 (Fig. 3C). In an attempt to explain this finding, we examined the effect of insulin on STAT5 as previous studies have shown that STAT5B is activated by insulin and enhances SOCS3 expression (7). Immunoblots showed an increased tyrosine phosphorylation of STAT5 in samples treated with IL-6 or insulin alone for 60 min, but the phosphorylation was greater in the presence of both insulin and IL-6 (Fig. 3D). This indicates that IL-6 and insulin can synergistically activate STAT5, supporting the suggestion that SOCS3 expression can be induced by both STAT3 and STAT5 activation.

Involvement of SHP2 – We examined the effect of insulin on the protein tyrosine phosphatase SHP2, as this enzyme is known to exert a regulating effect on the tyrosine phosphorylation of STAT3 (9). Immunoblotting of lysates from differentiated 3T3-L1 cells that had been incubated with IL-6 and/or insulin showed that insulin slowly increased the tyrosine phosphorylation of SHP2 which, in turn, is associated with enzyme activation (Fig. 4A). To further investigate the importance of SHP2 in STAT3 phosphorylation, we studied MEFs from mice expressing a mutant variant of SHP2, where the SH2-domains that are necessary for SHP2 activity had been deleted (24). The kinase-dead MEFs showed a high degree of tyrosine phosphorylation of STAT3 in the basal state and this was not altered by IL-6 and/or insulin (Fig. 4B). These findings support the importance of SHP2 for regulating the tyrosine phosphorylation of STAT3.

Effect of Insulin on Ser727 Phosphorylation of STAT3 – Ser727 phosphorylation of STAT3 has been shown to either activate or inhibit STAT3 depending on cell type (10-18, 25). Western blot analysis was used to determine the phosphorylation of Ser727 of STAT3 in differentiated 3T3-L1 adipocytes following incubation with insulin and/or IL-6. Insulin alone caused a rapid (≤15 min) phosphorylation of Ser727 (data not shown) which remained throughout the 120 min experiment, whereas the IL-6-induced phosphorylation of Ser727 declined after 60 min (Fig. 5). Insulin also increased the IL-6-induced phosphorylation of Ser727 compared with the effect of IL-6 alone (Fig. 5).

Interaction of MAPK signaling pathway on IL-6 signaling – It has previously been reported that the MEK inhibitor PD98059 inhibits serine phosphorylation of STAT3 in a Raf-inducible fibroblast cell line (16). To investigate if the MAPK signaling pathway affects STAT3 signaling in 3T3-L1 cells, these cells were pre-treated with PD98059 30 min before addition of insulin and/or IL-6 for 120 min. Insulin caused an increase in the levels of phosphorylated ERK1 and ERK2, and this was reduced by the addition of PD98059 (Fig. 6A). The results showed that the insulin-induced serine phosphorylation of STAT3 was not only antagonized by the presence of PD98059 but, interestingly, the ability of insulin to antagonize the IL-6-stimulated tyrosine phosphorylation of STAT3 was also prevented in cells pre-treated with PD98059 (Fig. 6A).

To further analyze the cross-talk between the MEK/MAPK pathway and the effect of insulin on IL-6 signaling, the nuclear localization and transcriptional activation of STAT3 were studied in 3T3-L1 cells pre-treated with PD98059 before addition of insulin and/or IL-6 for 15 min. The ability of insulin to reduce the IL-6-stimulated nuclear localization of STAT3 was almost completely inhibited in the presence of PD98059 (Fig. 6B). Similarly, insulin had no significant inhibitory effect on the IL-6-induced transcriptional activation of STAT3 in cells pre-treated with PD98059 (Fig. 6C).

**DISCUSSION**

In this report, we show that preincubation of 3T3-L1 cells with insulin: 1) reduced the IL-6-induced Tyr705 phosphorylation of STAT3; 2) reduced the IL-6-induced co-precipitation of gp130 and STAT3; 3) reduced the IL-6-induced nuclear localization and transcriptional activity of STAT3; 4) reduced the IL-6-induced gene expression of SAA3 and haptoglobin and; 5) increased the IL-6-induced Ser727 phosphorylation of STAT3. In addition, we show the inhibitory effect of insulin was
regulated by SHP2 and the MEK/MAPK signaling pathway.

IL-6 induced tyrosine phosphorylation of STAT3 is required for the dimerization of STATs through their SH2-domains (21), and formation of STAT dimers is required for nuclear translocation (9, 20). This is in agreement with our study showing that the insulin-induced reduction of IL-6-stimulated STAT3 tyrosine phosphorylation was accompanied by a reduced nuclear localization of STAT3 as well as a reduced activation of the proinflammatory genes SAA3 and haptoglobin. The reduction in tyrosine phosphorylation of STAT3 seems, in part, to be mediated through SHP2 as this protein tyrosine phosphatase is activated by insulin with a similar time-course as the reduced STAT3 tyrosine phosphorylation. SHP2 does not only interact with STAT3 (26, 27), but it also binds to Y759 of gp130 that is reported to be important for STAT3 recruitment (28-30). Additionally, SHP2 has also been described to bind to both STAT1 and STAT3 (27, 31). Our results support this as we found a reduced co-precipitation of gp130 and STAT3 in the presence of IL-6 and insulin. The same samples also showed an increased co-precipitation between SHP2 and STAT3 (data not shown). The importance of SHP2 was further supported by the finding that MEFs lacking a functional SHP2 activity showed an increased Tyr705 phosphorylation of STAT3 in both the basal and IL-6-stimulated states. In addition, overexpression of wild-type SHP2 in MEFs completely inhibited IL-6-induced expression of the inflammatory marker genes, SAA3 and haptoglobin (data not shown).

Ser727 phosphorylation of STAT3 has previously been described to either activate or inhibit the transcriptional properties of STAT3 in different cells (10-18, 25). The controversial effect of Ser727 phosphorylation of STAT3 may depend on cell type, the kinase pathway activated and/or the cytokine/growth factor used. Our study suggests that serine phosphorylation is inhibitory for the transcriptional activity of STAT3 in 3T3-L1 cells. We found that Ser727 phosphorylation of STAT3 was rapidly increased by insulin, which is in agreement with a previous study showing that insulin-induced serine phosphorylation of STAT3 occurs within 5 minutes (32). IL-6-induced Ser727 phosphorylation of STAT3 declined more rapidly than that seen with insulin, which indicates that Ser727 phosphorylation by IL-6 and insulin are mediated through different serine/threonine kinases. As several serine/threonine kinases have been described to phosphorylate Ser727, including ERK, JNK, p38 MAPK, PKCδ and mTOR kinase (10-12, 14-16, 18, 25, 33), we used specific inhibitors to some of the known pathways involved in order to define the mechanism involved. Specifically we inhibited the p38, JNK, MEK, PI3K and mTOR pathways. However, the MEK inhibitor (PD98059), which blocks signaling upstream of ERK1/2, was found to reduce all the studied effects of insulin on STAT3 signaling (i.e., alterations of Ser and Tyr phosphorylations, nuclear translocation and transcriptional activity). Also other studies have found that inhibition of the MEK signaling pathway alters STAT3 phosphorylation and that this can alter the transcriptional activity (16, 34).

Since we were unable to find any insulin-associated changes in the JAK2/STAT3 interactions (data not shown), we focused on the role of SHP2 for the reduction of STAT3 tyrosine phosphorylation in response to insulin. However, it is unlikely that the rather late effect of insulin on SHP2 activation can solely account for the rapid inhibitory action on STAT3 transcriptional activation as seen in figure 2B. To explain this difference, we speculate that the rapid induction of Ser727 phosphorylation of STAT3 by insulin plays a role in the early reduction of nuclear translocation and transcriptional activation of STAT3 in response to IL-6. Such an effect could be mediated by the impaired binding of STAT3 to gp130 seen in the presence of insulin. The later activation of SHP2 by insulin and the associated gradual reduction in tyrosine phosphorylation of STAT3 would then serve as a mechanism to turn off the IL-6 signal. Interestingly, preliminary experiments indicate that SHP2 can directly interact with STAT3 in an insulin-dependent manner (data not shown) which supports that STAT3 is a substrate for the phosphatase SHP2. This is in accordance with data previously presented by Wang et al. (27).

STAT3 regulates expression of SOCS1-7. This family of proteins contains a central SH2 domain that interacts with Tyr759 of gp130 to inhibit STAT3 activation, thus acting as negative feedback regulators of STAT
signaling (5, 9, 29). SOCS3 is rapidly induced by IL-6 stimulation and expression of this enzyme peaks within 1 h (5). As we found that insulin reduced the transcriptional activity of STAT3, one would expect a reduced expression of SOCS3. We found, however, that the IL-6-induced expression of SOCS3 was increased by insulin. To address the possible mechanisms for this effect of insulin, we investigated other transcription factors (STATs) that target induction of SOCS3 including STAT5B (7). In contrast to Emanuelli et al. (7), we did not find a clear increase of SOCS3 expression in samples incubated with insulin alone, but we showed that IL-6 and insulin synergistically activated SOCS3, probably through an increased activation of STAT5. These findings provide a mechanism whereby insulin can increase SOCS3 expression while, concomitantly, reducing STAT3 tyrosine phosphorylation and transcriptional activity. The synergistic effect of IL-6 and insulin on SOCS3 expression is an additional mechanism whereby insulin can turn off the IL-6 signal and, in addition, the insulin signal as SOCS3 is also a feed-back inhibitor to insulin (7, 35).

The ability of insulin to antagonize IL-6 signaling, and induction of inflammatory proteins like SAA3 and haptoglobin, shows that insulin exerts anti-inflammatory effects in 3T3-L1 adipocytes. This could have important physiological consequences since the adipose tissue plays a major role for the innate inflammatory response (36). Furthermore, the insulin resistance in the adipose tissue can contribute to the increased inflammation seen in obesity and Type 2 diabetes (37, 38).

REFERENCES

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FIGURE LEGENDS

Fig. 1. Effect of insulin on Tyr705 phosphorylation of STAT3. Immunoblots of lysates from differentiated 3T3-L1 cells incubated for 30, 60 and 120 min with addition of insulin, IL-6 and IL-6+insulin. A, immunoblot showing Tyr705 phosphorylated STAT3 and a histogram of the corresponding band intensities from four individual experiments. The lower panel shows total amount of STAT3 protein. B, co-precipitation experiment of gp130 and STAT3. gp130 was immunoprecipitated followed by immunoblot analysis using anti-STAT3 antibody. As a control, the same blot was probed with anti-gp130 ab.
Fig. 2. **Nuclear translocation and transcriptional activity of STAT3.** A, immunoblot showing STAT3 in nuclear lysates from cells incubated with insulin, IL-6 and IL-6+insulin for the indicated times. A histogram of the relative amounts of nuclear STAT3 from three individual experiments is shown (right). B, histogram showing the transcriptional activation of nuclear STAT3 at the indicated times (n=4 experiments).

Fig. 3. **mRNA expression of IL-6-induced genes and the effect of insulin.** Differentiated 3T3-L1 cells were incubated with insulin, IL-6 and IL-6+insulin for 1 or 24 h. The mRNA levels of A, SAA3 (n=10 experiments), B, haptoglobin (n=10 experiments) and C, SOCS3 (n=8 experiments) were measured by real-time PCR. D, immunoblot showing STAT5 tyrosine phosphorylation in lysates from differentiated 3T3-L1 cells incubated with insulin, IL-6 and IL-6+insulin for 1 h. **p<0.01, ***p<0.001.

Fig. 4. **The role of SHP2 on insulin and IL-6 signaling.** A, immunoblot showing tyrosine phosphorylated SHP2 (activated) in differentiated 3T3-L1 cells incubated with insulin for the indicated time points. B, immunoblot analysis of tyrosine phosphorylated STAT3 in wild type MEFs and MEFs expressing a kinase-dead SHP2.

Fig. 5. **Effect of insulin on Ser727 phosphorylation of STAT3.** Immunoblots of lysates from differentiated 3T3-L1 cells incubated for 30, 60 and 120 min with addition of insulin, IL-6 and IL-6+insulin, showing Ser727 phosphorylated STAT3 and a histogram of the corresponding band intensities from five individual experiments.

Fig. 6. **Interaction of MEK/MAPK signaling pathway on IL-6 signaling.** A, immunoblot of differentiated 3T3-L1 cells preincubated or not with PD98059 for 30 min followed by additions of insulin, IL-6 and IL-6+insulin for 120 min. Blots show tyrosine and serine phosphorylated STAT3 and phosphorylated MAPK-ERK1/2. *p<0.05, **p<0.01, ***p<0.001. B, immunoblot showing nuclear STAT3 from differentiated 3T3-L1 cells preincubated or not with PD98059 for 30 min, followed by incubation of IL-6 and/or insulin for 15 min. C, histogram showing transcriptional activation of nuclear STAT3 (n=4 experiments) from cells preincubated or not with PD98059 for 30 min prior to IL-6 and/or insulin that were added for 15 min. *p<0.05, **p<0.01.
FIGURE 1

A

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![Western Blot](pY-STAT3)

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![Western Blot](STAT3)

B

**IP: gp130**

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![Western Blot](STAT3)

![Western Blot](gp130)
FIGURE 3

A  

SAA3

B  

Haptoglobin

C  

SOCS3

D  

pY-STAT5
FIGURE 4

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pY-SHP2

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MEF wt

pY-STAT3

MEF SHP2 kinase-dead
FIGURE 5

[Graph showing relative intensities of pS-STAT3 over time (30, 60, 120 minutes) for Basal, Insulin, IL-6, and IL-6+Insulin conditions, with statistical symbols indicating significance (** and *)]