Insulin Receptor Substrate-2-dependent Interleukin-4 Signaling in Macrophages Is Impaired in Two Models of Type 2 Diabetes Mellitus*

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We have shown previously that hyperinsulinemia inhibits interferon-α-dependent activation of phosphatidylinositol 3-kinase (PI3-kinase) through mammalian target of rapamycin (mTOR)-induced serine phosphorylation of insulin receptor substrate (IRS)-1. Here we report that chronic insulin and high glucose synergistically inhibit interleukin (IL)-4-dependent activation of PI3-kinase in macrophages via the mTOR pathway. Resident peritoneal macrophages (PerMφs) from diabetic (db/db) mice showed a 44% reduction in IRS-2-associated PI3-kinase activity stimulated by IL-4 compared with PerMφs from heterozygote (db/+ ) control mice. IRS-2 from db/db mouse PerMφs also showed a 78% increase in Ser/Thr-Pro motif phosphorylation without a difference in IRS-2 mass. To investigate the mechanism of this PI3-kinase inhibition, 12-O-tetradecanoylphorbol-13-acetate-matured U937 cells were treated chronically with insulin (1 nM, 18 h) and high glucose (4.5 g/liter, 48 h). In these cells, IL-4-stimulated IRS-2-associated PI3-kinase activity was reduced by 37.5%. Importantly, chronic insulin or high glucose alone did not impact IL-4-activated IRS-2-associated PI3-kinase activity in the absence of basal serine phosphorylation of IRS-2. Chronic insulin + high glucose did reduce IL-4-dependent IRS-2 tyrosine phosphorylation and p85 association by 54 and 37%, respectively, but did not effect IL-4-activated Jak/Stat signaling. When IRS-2 Ser/Thr-Pro motif phosphorylation was examined, chronic insulin + high glucose resulted in a 92% increase in IRS-2 Ser/Thr-Pro motif phosphorylation without a change in IRS-2 mass. Pretreatment of matured U937 cells with rapamycin blocked chronic insulin + high glucose-dependent IRS-2 Ser/Thr-Pro motif phosphorylation and restored IL-4-dependent IRS-2-associated PI3-kinase activity. Taken together these results indicate that IRS-2-dependent IL-4 signaling in macrophages is impaired in models of type 2 diabetes mellitus through a mechanism that relies on insulin/glucose-dependent Ser/Thr-Pro motif serine phosphorylation mediated by the mTOR pathway.

The first member of the insulin receptor substrate (IRS) family, IRS-1, was initially discovered in Fao hepatoma cells as a tyrosine-phosphorylated substrate of the insulin receptor (1). In addition to insulin signaling, IRS proteins are integrally linked to intracellular signaling pathways initiated by IGF-I and the cytokines IL-2, 3, 4, 7, 9, 10, 13, 15 and IFN-α and IFN-γ (2–10). Importantly, serine phosphorylation of IRS-1 blocks insulin, IGF-I, and cytokine signaling through IRS-1 (11–15) and appears critical to the initiation of proteasome-dependent IRS-1 degradation (16, 17). We have shown that chronic insulin in the presence of high glucose leads to serine phosphorylation of IRS-1 through an mTOR-dependent mechanism and that this renders IRS-1 a poorer substrate for JAK1 (18). In addition, we have shown that serine phosphorylation targets IRS-1 for proteasome-dependent degradation in L6 muscle cells (19). However, these same mechanisms have not been investigated in relation to IRS-2-dependent cytokine signaling.

IRS-2 is expressed in a wide variety of tissues and appears, in IRS-1 knock-out mice, to duplicate many of the functions of IRS-1 (20–22). Although IRS-1 is most important to insulin action in skeletal muscle, IRS-2 appears to be required for insulin/IGF-I and, importantly, cytokine signaling in liver, muscle, fat, pancreatic β cells, B cells, T cells, and macrophages (9, 23–25). IRS-2 knock-out studies in mice have shown that IRS-2 is important for neuroendocrine function, and a lack of IRS-2 leads to enhanced neointima formation (26, 27). Furthermore, IRS-2 is necessary for IL-4-induced proliferation and differentiation of T cells (28). Sequence alignment shows a high degree of homology between IRS-1 and IRS-2, including the presence of two Src homology 2 domain binding sites for the p85 subunit of PI3-kinase (20). As with IRS-1, serine phosphorylation appears to regulate IRS-2 tyrosine phosphorylation negatively. Removal of basal serine phosphorylation of IRS-2 increases IGF-I-stimulated tyrosine phosphorylation of IRS-2, and hyperphosphorylation of IRS-2 on serine residues impairs insulin receptor-dependent IRS-2 tyrosine phosphorylation (29). In addition, Ser/Thr phosphorylation of IRS-2 induced by either TNF-α or prolonged insulin exposure results in a reduced ability of IRS-2 to interact with the juxtamembrane region of the insulin receptor (15).

The IL-4 receptor is expressed ubiquitously on monocytes and macrophages (30, 31), and, as with other class I cytokine

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¶¶ The abbreviations used are: IRS, insulin receptor substrate; Ch. Ins, chronic insulin; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; JAK, janus kinase; mTOR, mammalian target of rapamycin; PerMφs, peritoneal macrophages; PI3-kinase, phosphatidylinositol 3-kinase; STAT, signal transducers and activators of transcription; TNF, tumor necrosis factor; TPA, 12-O-tetradecanoylphorbol-13-acetate.
receptors (hematopoietin receptor family), the IL-4 receptor lacks intrinsic kinase activity and requires receptor-associated kinases for initiation of intracellular signaling (32, 33). Binding of IL-4 to its receptor leads to JAK1 and JAK3 activation. One or both of these tyrosine kinases phosphorylate the IL-4a chain on residues Tyr<sup>137</sup>, Tyr<sup>157</sup>, Tyr<sup>160</sup>, Tyr<sup>161</sup>, and Tyr<sup>193</sup>. Tyr<sup>193</sup> is within the insulin/IL-4 receptor motif and is responsible for IRS-2 recruitment (34). IL-4 is a potent anti-inflammatory cytokine and leads to an "alternative activation phenotype" in macrophages. This IL-4-dependent macrophage activation results in an absence of nitric oxide production, generation of IL-10 and IL-1 receptor antagonist, and suppressive activity directed toward T cells (35).

Recently, subacute chronic inflammation (36, 37) has been identified as a significant contributor to the complications associated with type 2 diabetes mellitus (38). Cardiovascular problems such as accelerated and exacerbated atherosclerosis appear especially responsive to the proinflammatory environment of diabetes (37, 39). The diabetic state is known to alter macrophage function resulting in increased lipoprotein lipid production and T NF-a release (40). In addition, hyperglycemia, through the generation of advanced glycation end products, increases macrophage secretion of IL-1 and TNF-a while enhancing intercellular adhesion molecule 1 expression and decreasing phagocytic activity (41). Because the IRS-2/PI3-kinase pathway is common to both IL-4 and insulin signaling in macrophages, we wanted to demonstrate that IL-4-dependent IRS-2/PI3-kinase signal transduction was inhibited in diabetic macrophages and that the likely mechanism was a result of mTOR-dependent serine phosphorylation of IRS-2 on Ser/Thr-Pro motifs.

**EXPERIMENTAL PROCEDURES**

**Materials**—The U937 promonocytic cell line was purchased from American Type Culture Collection (Rockville, MD). All cell culture reagents and chemicals were purchased from Sigma except as noted below. Fetal calf serum (0.05 mg/ml, 0.48 enzyme unit/ml of endotoxin) was purchased from Atlanta Biologicals (Norcross, GA). [γ-<sup>32</sup>P]ATP was purchased from PerkinElmer Life Sciences. Protein G-Sepharose and the ECL Western blotting analysis system were purchased from Amer sham Biosciences. Silica Gel 60 thin layer chromatography plates were purchased from EM Science (Gibbstown, NJ). Bio-Rad protein reagent was purchased from Bio-Rad. Anti-IRS-1, anti-IRS-2, anti-p85, anti-JAK1, anti-JAK3, anti-anti-phosphorylation protein monoclonal #2, antiphosphotyrosine, and antiphospho-STAT6 antibodies and TF-1 cell lysate were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). IL-4 and rapamycin were purchased from Calbiochem. N-CB antibody was purchased from Bio-Rad. Anti-IRS-1, anti-IRS-2, anti-p85, anti-p85, and antiphosphotyrosine antibodies were purchased from EM Science (Gibbstown, NJ). Bio-Rad protein reagent and the ECL Western blotting analysis system were purchased from Amer sham Biosciences.

**Cell Culture/Insulin and Glucose Treatment**—U937 cells were grown in low glucose growth medium. Cells were passaged 1:1 with fresh medium every 3 days. All cell counts were performed on a Coulter ZM (Miami, FL). For high glucose treatments, cells were resuspended at 5 × 10<sup>5</sup> cells/ml in low glucose growth medium with the addition of 5 g/liter glucose and grown for 24 h. For chronic insulin treatment, cells were resuspended at 5 × 10<sup>5</sup> cells/ml in low glucose growth medium with or without the addition of 3.5 g/liter glucose and grown for 48 h.

**Statistical Analysis**—Data are presented as the mean ± S.E. The significance of differences was determined by one-way analysis of variance. Statistical significance was denoted at *p* < 0.05.

**RESULTS**

IL-4-activated IRS-2-associated PI3-Kinase Activity Is Impaired in PerM<sub>0</sub> cells from db/db Mice—We have shown previously that chronic insulin exposure-dependent serine phosphorylation of IRS-1 can block cytokine signaling in myeloma cells in vitro (13). To investigate whether macrophages from type 2 diabetic animals had impaired IRS-2-dependent cytokine signaling, we examined PerM<sub>0</sub> cells from db/db mice (Table I). Fig. 1A

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**References**

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are representative of three independent experiments. PI3-kinase activity was assayed in IRS-2 immunoprecipitates. Results isolated from db mice were then treated (anti-IRS-2 antibody) in the presence of 100 nM insulin for 10 min. PI3-kinase activity was measured in IRS-1 and IRS-2 immunoprecipitates. Results are representative of three independent experiments. C, matured U937 cells were grown in high glucose medium (4.5 g/liter) for 48 h. During the last 18 h, cells were treated (+) or not (−) with 1 nM insulin (Ch.Ins). Cells were then stimulated with 5.5 ng/ml IL-4 for the indicated times. PI3-kinase activity was measured in IRS-2 immunoprecipitates. Results are representative of three independent experiments. D, matured U937 cells were grown in low glucose medium (1 g/liter) (Con.Ins) or high glucose medium (4.5 g/liter) (Glc) for 48 h. During the last 18 h, cells were treated with Ins or without (Con, Glc) 1 nM insulin. Cells were then stimulated with 5.5 ng/ml IL-4 for 15 min. Phosphotyrosine (pY), PI3-kinase p85α (p85), and IRS-2 were detected by Western analysis in IRS-2 immunoprecipitates using an anti-pY, anti-p85α, and anti-IRS-2 antibody, respectively. Results are representative of three independent experiments.

shows that PerMΦs from 8-week-old db/db mice treated with IL-4 (5.5 ng/ml, 15 min) had a 44% (db/+), P < 0.05) reduction in IRS-2-associated PI3-kinase activity compared with similarly treated PerMΦs from db/+ control mice. Activity of PI3-kinase associated with IRS-2 basally was not different in db/db and db/+ PerMΦs. Fig. 1, A and C, demonstrates that, when insulin (100 nM, 10 min) or IGF-I (10 ng/ml, 10 min) was used in place of IL-4, PerMΦ PI3-kinase activity associated with IRS-2 was also reduced by 34% (db/+), P < 0.05) and 39% (db/+), P < 0.01) in db/db mice, respectively. To determine the serine phosphorylation state of IRS-2 in db/db mouse PerMΦs, Western analysis was performed. Fig. 1D demonstrates that IRS-2 Ser/Thr-Pro motif phosphorylation was increased 78% in db/db mouse PerMΦs compared with PerMΦs from db/+ mice (db/+), P < 0.001). Importantly, IRS-2 mass of both db/db and db/+ mice was similar (Fig. 1E; db/+), P < 0.05). Combined, these results indicate that PerMΦs from type 2 diabetic mice have a reduced ability to form active IRS-2/PI3-kinase complexes coupled with augmented basal Ser/Thr-Pro motif IRS-2 phosphorylation.

Fig. 2. Chronic insulin and high glucose synergize to block IL-4-activated IRS-2-associated PI3-kinase activity.
cells. Fig. 2C demonstrates that chronic insulin + high glucose treatment led to a 37.5% (Ch.Ins, 100 ± 3.3%; +Ch.Ins, 62.5 ± 3.3%; p < 0.01) reduction in peak IL-4-activated (5.5 ng/ml, 15 min) IRS-2-associated PI3-kinase activity. Importantly, IL-4-activated IRS-2-associated PI3-kinase activity was not affected by chronic insulin or high glucose alone (Fig. 2D).

Fig. 2E shows that chronic insulin + high glucose reduced by 89% (Ch.Ins, 100 ± 12.2%; +Ch.Ins, 14.2 ± 12.2%; p < 0.05) IL-4-dependent (5.5 ng/ml, 15 min) IRS-2 tyrosine phosphorylation and inhibited by 91% (Ch.Ins, 100 ± 6.3%; +Ch.Ins, 9.2 ± 6.3%; p < 0.01) IL-4-dependent (5.5 ng/ml, 15 min) IRS-2/P13-kinase p85 association. Insulin alone had no impact on the ability of IL-4 to induce IRS-2 tyrosine phosphorylation or p85 association (data not shown). These results indicate that chronic insulin + high glucose synergize to block IL-4 from inducing IRS-2 tyrosine phosphorylation thereby diminishing its subsequent ability to associate with P13-kinase.

JAK/STAT Signaling Activated by IL-4 Is Unaffected by Chronic Insulin + High Glucose—As Fig. 2 shows, IL-4-dependent IRS-2 tyrosine phosphorylation is inhibited in matured U937 cells treated with chronic insulin + high glucose. This finding indicates that the ability of IL-4 to activate JAK may be impaired. Fig. 3A demonstrates that when matured U937 cells were treated with chronic insulin + high glucose, IL-4-dependent (5.5 ng/ml, 15 min) JAK1 autophosphorylation was similar to IL-4-dependent JAK1 activation in cells treated with just high glucose. As in Fig. 2, chronic insulin alone had no effect by itself and did not alter the ability of IL-4 to induce JAK1 autophosphorylation (data not shown). Because IL-4 can also transduce its signal via JAK3 (46), JAK3 was examined. Fig. 3B shows that JAK3 was not detected in matured U937 cells. Finally, to examine whether other JAK1 substrates aside from IRS-2 were impacted by chronic insulin + high glucose IL-4-dependent (5.5 ng/ml, 15 min) IRS-2 tyrosine phosphorylation was examined. Fig. 3C demonstrates that like IL-4-dependent JAK1 autophosphorylation, chronic insulin + high glucose had no effect on IL-4-dependent tyrosine phosphorylation of STAT6. As above, insulin alone did not alter the ability of IL-4 to induce STAT6 phosphorylation (data not shown). Taken together these results indicate that chronic insulin and high glucose target the IRS-2/P13-kinase arm of the IL-4 signaling pathway and not the JAK/STAT arm.

The mTOR Pathway Is Required for the Chronic Insulin + High Glucose Effect on IL-4 Signaling—We have shown previously that mTOR-dependent serine phosphorylation of IRS-1 within Ser/Thr-Pro motifs inhibits IFN-α signaling (18). To determine whether IL-4-dependent IRS-2-associated P13-kinase activation was regulated by mTOR, rapamycin inhibition studies were performed. Fig. 4A shows that chronic insulin (1 nm, 18 h) + high glucose (4.5 g/liter) treatment of matured U937 cells increased Ser/Thr-Pro motif phosphorylation 92% (Ch.Ins, 100 ± 14.2%; +Ch.Ins, 192 ± 14.2%; p < 0.01) over high glucose alone. Importantly, pretreatment of matured U937 cells with 1 nm rapamycin 30 min prior to the addition of insulin completely blocked the increase in IRS-2 Ser/Thr-Pro motif phosphorylation (Rap, +Ch.Ins, 16.2 ± 2.5%; p < 0.001 with +Ch.Ins above). In addition, insulin-independent Ser/Thr-Pro motif phosphorylation was also inhibited by rapamycin (Rap, −Ch.Ins, 18.4 ± 2.5%; p < 0.001 with −Ch.Ins above).
The mass of IRS-2 was unchanged by the addition of rapamycin in the presence or absence of insulin. Similar to Figs. 2 and 3, 18 h of chronic insulin treatment in the presence of low glucose (1 g/liter) did not result in increased phosphorylation of IRS-2 Ser/Thr-Pro motifs (Fig. 4B) (–Ch.Ins, 100 ± 4.65%; +Ch.Ins, 102.8 ± 4.65%; p = not significant). Fig. 4C, like Fig. 1, demonstrates that IL-4-dependent IRS-2-associated PI3-kinase activity was reduced by 41.3% (–Ch.Ins, 100 ± 7.9%; +Ch.Ins, 58.7 ± 7.8%; p < 0.01) in matured U937 cells treated with chronic insulin + high glucose. When cells were pretreated with 1 nm rapamycin for 30 min prior to the chronic insulin addition, IL-4-dependent IRS-2-associated PI3-kinase activity was restored (Rap, –Ch.Ins, 195.3 ± 39.9%; +Ch.Ins, 178.1 ± 39.9%; p = not significant). The mass of p85 was unaffected by rapamycin pretreatment, and, as in Fig. 1, chronic insulin treatment had no effect on IL-4-activated PI3-kinase activity in the presence of low glucose (data not shown). Taken together, these results indicate that the mTOR pathway mediates the suppressive effect of chronic insulin + high glucose on IL-4-dependent IRS-2-associated PI3-kinase activation.

**DISCUSSION**

Serine phosphorylation has emerged as an important mechanism for counterregulating insulin-dependent IRS-1/PI3-kinase signaling in diabetes (15, 47–50). Serine phosphorylation of IRS-1 occurs constitutively in the cell and is enhanced further by hyperinsulinemia (47, 48), hyperglycemia (51), and proinflammatory cytokines (52, 53). Numerous serine/threonine kinases have been shown to phosphorylate IRS-1 in vitro, including mTOR (18). Serine phosphorylation of IRS-1 can inhibit insulin receptor-dependent tyrosine phosphorylation of IRS-1 (11, 12, 14, 15, 54) and targets IRS-1 to the proteasome for degradation (16, 17, 19). Interestingly, it is not as well recognized that the same mechanisms that result in insulin resistance can, in turn, lead to cytokine resistance through impaired IRS-2 signaling. As Fig. 1 demonstrates, we examined IRS-2/PI3-kinase signaling in PerMΦs from db/db mice, which have both marked hyperglycemia and hyperinsulinemia (Table I). These experiments showed a 44% reduction in IRS-2-associated PI3-kinase activity in response to IL-4 as well as reduced insulin and IGF-1-stimulated IRS-2-associated PI3-kinase activity. Coupled to this reduction in IL-4-dependent IRS-2-associated PI3-kinase activity in db/db mice was a 78% increase in IRS-2 Ser/Thr-Pro motif phosphorylation. Importantly, IRS-2 mass was unaffected in PerMΦs from db/db mice compared with db/+ mice. These data are notable because they are the first to show that cytokine signaling mediated by IRS-2 is reduced in cells from diabetic animals. These results were somewhat unexpected in that reduced IRS-1-dependent insulin signaling is thought to be a result of proteasomal loss of IRS-1 subsequent to its serine phosphorylation (16, 17, 19), but here we show that IRS-2 mass in PerMΦs is unchanged in diabetic db/db mice. These findings indicate that IRS-2 mass loss is not critical to blunted IL-4/IRS-2/PI3-kinase signaling. This is not entirely surprising because diabetes-dependent proteasomal loss of IRS-2 may be tissue-specific, and we have shown in vitro that insulin-dependent inhibition of IFN-α/IRS-1/PI3 kinase signaling does not depend on IRS-1 mass loss (13, 18).

New work by Pirola et al. (55) shows that in L6 muscle cells, prolonged insulin treatment leads to a reduction in IRS-2 protein levels which is dependent on the PI3-kinase/mTOR pathway. In primary skeletal muscle cells from patients with impaired glucose tolerance, however, IRS-2 protein levels were unaffected by high glucose, although insulin-dependent IRS-2 tyrosine phosphorylation and associated PI3-kinase activity were decreased significantly (56). In adipocytes, high glucose treatment when combined with insulin results in a near complete loss of IRS-2 expression (57). Additionally, high glucose in human aortic endothelial cells has been shown to reduce IRS-2 expression (58). To determine the role of insulin and glucose in IL-4/IRS-2/PI3-kinase signaling in PerMΦs, we examined matured U937 cells grown in medium enriched with both insulin and glucose. Fig. 2 shows that blunted IL-4-dependent IRS-2-associated PI3-kinase activation was only present when matured U937 cells were exposed to both chronic insulin and high glucose. These results are consistent with our previous findings in myeloma cells where we demonstrated that 1 nm insulin blunted IFN-α-dependent IRS-1-associated PI3-kinase activation in cells grown in high glucose (4.5 g/liter) medium (13, 19). In our previous work, however, we did not test low glucose conditions to determine its role in blocking cytokine signaling. Our new studies indicate that exposure to elevated glucose concentrations is as critical as insulin is to inducing IRS Ser/Thr-Pro motif phosphorylation and down-regulating IRS-2-dependent PI3-kinase interactions.

As we (18) and others (59, 60) have shown, mTOR phosphorylates proteins within Ser/Thr-Pro motifs. Therefore, to demonstrate that chronic insulin + high glucose-dependent blunting of IL-4/IRS-2/PI3-kinase signaling was the result of mTOR, rapamycin inhibition studies were performed. Fig. 4 shows that rapamycin blocks chronic insulin + high glucose-dependent IRS-2 Ser/Thr-Pro motif phosphorylation and reverses the effect that chronic insulin + high glucose has on IL-4/IRS-2/PI3-kinase signal transduction. It is important to note that rapamycin is considered a highly specific inhibitor, especially at the nanomolar concentrations that were used in this study. Unlike most kinase inhibitors, rapamycin has a unique mechanism that requires the binding of a coreceptor (FKBP12), and it targets a domain unique to mTOR situated outside of the kinase domain (61–65). Grolleau et al. (66) have demonstrated that treatment of Jurkat T cells with 20 nm rapamycin for 3 days results in altered expression of 16 proteins; however, none of these proteins is a serine kinase. In addition, the experiments run by Grolleau et al. (66) demonstrated that a number of kinases, such as members of the mitogen-activated protein kinase cascade, were unaffected by rapamycin treatment. When Davies et al. (67) examined the ability of rapamycin to inhibit various kinases, they found that even at 1 μM, none of the 24 kinases on their panel was affected. In this study, we used a concentration of rapamycin 20–1,000-fold lower than that used in the above two reports. These findings indicate that 1 nm rapamycin is specific for mTOR, and because Ser/Thr-Pro motifs are thought to be consensus sites for mTOR (59, 60), it is likely that the rapamycin effects we demonstrate are mediated directly by mTOR. In addition, chronic insulin + high glucose had no impact on IL-4-dependent JAK1/STAT6 signaling (Fig. 3). Taken together these findings indicate that chronic insulin + high glucose, acting through the mTOR pathway, does not prevent IL-4 from activating JAK1 but does disrupt the ability of IRS-2 from acting as a JAK1 substrate. This work is supported by our previous studies where we demonstrated that serine-phosphorylated IRS-1 was a poorer substrate for JAK1 (13) and that mTOR, but not p70S6K, mediated this effect (18). Also, because PerMΦs from db/db mice have increased IRS-2 Ser/Thr-Pro motif phosphorylation compared with PerMΦs from db/+ mice, and the mass of IRS-2 in PerMΦs from both of these animal types is the same, our data buttress the idea that cytokine-dependent IRS-2/PI3-kinase signaling is perturbed in vivo by a mechanism dependent on IRS-2 serine phosphorylation and not IRS-2 proteasomal mass loss.

Finally, IL-4 plays a key role in inhibiting proinflammatory cytokine production in activated macrophages (68, 69). Prediabetes and type 2 diabetes mellitus are characterized by sub-

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acute chronic inflammation where individuals show elevated serum levels of acute phase proteins such as C-reactive protein and fibrinogen (70) and cytokines such as TNF-α (71) and IL-6 (70, 72). Although TNF-α is produced mainly by adipose tissue in obese prediabetic and diabetic individuals (71, 73), a critical source for the proinflammatory cytokines IL-6 and IL-1β is activated macrophages (74). Furthermore, these macrophage-generated cytokines are potent inducers of liver-produced C-reactive protein and fibrinogen. How chronic inflammation develops in type 2 diabetes mellitus is unclear, but here we suggest that macrophage resistance to anti-inflammatory cytokines may be a potential mechanism. Currently, the role of IL-4/IRS-2/PI3-kinase signaling in macrophages is undefined. Currently, the role of IL-4/IRS-2/PI3-kinase signaling in macrophages is undefined. Currently, the role of IL-4/IRS-2/PI3-kinase signaling in macrophages is undefined.