Interleukin-1β mediates macrophage-induced impairment of insulin signaling in human primary adipocytes

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Gao D, Madi M, Ding C, Fok M, Steele T, Ford C, Hunter L, Bing C. Interleukin-1β mediates macrophage-induced impairment of insulin signaling in human primary adipocytes. Am J Physiol Endocrinol Metab 307; E289–E304, 2014. First published June 10, 2014; doi:10.1152/ajpendo.00430.2013.—Adipose tissue expansion during obesity is associated with increased macrophage infiltration. Macrophage-derived factors significantly alter adipocyte function, inducing inflammatory responses and decreasing insulin sensitivity. Identification of the major factors that mediate detrimental effects of macrophages on adipocytes may offer potential therapeutic targets. IL-1β, a proinflammatory cytokine, is suggested to be involved in the development of insulin resistance. This study investigated the role of IL-1β in macrophage-adipocyte cross-talk, which affects insulin signaling in human adipocytes. Using macrophage-conditioned (MC) medium and human primary adipocytes, we examined the effect of IL-1β antagonism on the insulin signaling pathway. Gene expression profile and protein abundance of insulin signaling molecules were determined, as was the production of proinflammatory cytokine/chemokines. We also examined whether IL-1β mediates MC medium-induced alteration in adipocyte lipid storage. MC medium and IL-1β significantly reduced gene expression and protein abundance of insulin signaling molecules, including insulin receptor substrate-1, phosphoinositide 3-kinase p85α, and glucose transporter 4 and phosphorylation of Akt. In contrast, the expression and release of the proinflammatory markers, including IL-6, IL-8, monocyte chemotactic protein-1, and chemokine (C-C motif) ligand 5 by adipocytes were markedly increased. These changes were significantly reduced by blocking IL-1β activity, its receptor binding, or its production by macrophages. MC medium-inhibited expression of the adipogenic factors and -stimulated lipolysis was also blunted with IL-1β neutralization. We conclude that IL-1β mediates, at least in part, the effect of macrophages on insulin signaling and proinflammatory response in human adipocytes. Blocking IL-1β could be beneficial for preventing obesity-associated insulin resistance and inflammation in human adipose tissue.

human adipocytes; macrophages; IL-1β; insulin signaling; cytokines

Obesity is a major risk factor for the development of insulin resistance and the progression to type 2 diabetes (23). Growing evidence suggests that a state of low-grade chronic inflammation links excess fat to metabolic disorders (31, 39). During adipose tissue expansion, there is an increase in infiltration of macrophages together with other immune cells, and these cells may constitute the major sources of adipose-derived proinflammatory cytokines/chemokines (4, 8, 29, 38). It is estimated that over 20–30 million macrophages accumulate per kilogram of excess fat in human subjects (31). The interaction between macrophages and adipocytes has been demonstrated to modify adipocyte function, such as inhibiting preadipocyte differentiation (5, 13), promoting inflammatory responses (27), and reducing insulin sensitivity (30).

Identification of the key factors that mediate the effect of macrophages on adipocytes is challenging, but it may provide potential therapeutic targets for obesity-related insulin resistance. Interleukin-1β (IL-1β), a master proinflammatory cytokine produced mainly by monocytes and macrophages, is activated through caspase-1 via the NLRP3 inflammasome complex (1). Recent studies suggest IL-1β as a putative candidate in the development of insulin resistance and type 2 diabetes (49, 57). An elevation in circulating levels of IL-1β together with IL-6 has been shown to increase the risk of type 2 diabetes (44). IL-1β inhibition reduces hyperglycemia and tissue inflammation in obese mice and diabetic rats (7, 33, 40, 42). Furthermore, IL-1β may constitute a cell-cell mediator in metainflammation, as IL-1β produced by TNFα-stimulated mouse adipocytes has been shown to induce insulin resistance in liver cells (36).

Adipose tissue, in addition to skeletal muscle and liver, is a key organ that displays insulin resistance in obesity (15). The decreased responsiveness to insulin in adipocytes may promote fatty acid release into the circulation, leading to hepatic and muscle insulin resistance (43). IRS-1, one of the major substrates of the insulin receptor, is essential to activate PI3K in response to insulin, which leads to the phosphorylation of protein kinase B (also known as Akt) and subsequent glucose uptake (50). In adipose tissue, gene expression of IL-1β is upregulated in obese mice and humans (18, 19, 28). IL-1β is also released by human adipose tissue explants but is due primarily to the nonfat cells (9, 25), and the levels released are enhanced in obesity (36). Previous studies, mostly using murine 3T3-L1 adipocytes, have shown that IL-1β at a very high dose (20 ng/ml) decreased protein expression of IRS-1 and GLUT4 transcripts, and prolonged treatment blunted insulin-induced phosphorylation of IRS-1 and Akt (18, 28, 58). However, little is known about the impact of macrophage-adipocyte cross-talk on insulin signaling in human adipose tissue. More importantly, whether IL-1β is responsible for macrophage-induced insulin resistance in human and rodent adipocytes has not been reported.

This study was therefore to determine the role of IL-1β in macrophage-adipocyte cross-talk, which affects insulin signaling in human adipocytes. By using in vitro human cell models, we have provided novel evidence that IL-1β is a critical factor that mediates the detrimental effect of macrophages on insulin signal transduction in adipocytes; this was demonstrated by a series of experiments by inhibiting IL-1β activity, receptor
binding, and production. We also found that IL-1β blockade can substantially reduce macrophage-stimulated release of the proinflammatory cytokines and lipid mobilization in human adipocytes, which could provide a mechanistic link between IL-1β and insulin resistance at local and also systemic levels.

MATERIALS AND METHODS

Culture of adipocytes. Human white preadipocytes derived from subcutaneous adipose tissue of a female Caucasian subject (BMI 21 kg/m²; age 44 yr) were obtained from PromoCell (Heidelberg, Germany). Cells were cultured in preadipocyte growth medium supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B (Lonza, Tewkesbury, UK) at 37°C in a humidified atmosphere of 5% CO₂/95% air. Preadipocytes were seeded at 40,000/cm² and grown in 6- or 24-well plates until confluence. At confluence, cells were induced to differentiate (day 0) by incubation for 3 days in Dulbecco’s modified Eagle’s medium (DMEM)-Ham’s F-12 (1:1) medium containing 320 F-12 (1:1) medium containing 320 PSI0.001 RPMI medium, and 0.25 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. Following induction, cells were cultured in maintenance medium containing 3% fetal calf serum (FCS, Sigma), 100 µM insulin, 32 µM rosiglitazone (GlaxoSmithKline, Uxbridge, UK), and 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B. After induction, cells were cultured in maintenance medium containing 3% fetal calf serum (FCS, Sigma), 100 µM insulin, 32 µM rosiglitazone, and 1 µM dexamethasone until full differentiation into adipocytes.

Generation of THP-1 macrophage-conditioned medium. The human THP-1 monocytic cell line was purchased from Health Protection Agency Culture Collections (Porton Down, Salisbury UK). THP-1 monocytes (1 × 10⁶ cells/ml) were cultured in Roswell Park Memorial Institute (RPMI-1640) medium with 10% FCS and 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B (all from Sigma) at 37°C. To prepare macrophage-conditioned (MC) medium, THP-1 monocytes were first differentiated by the addition of 100 ng/ml phorbol 12-myristate 13-acetate (Sigma) for 48 h. After removing the medium, THP-1 macrophages were washed twice with RPMI medium (without FCS, without PMA) and then cultured with FCS-free RPMI-1640 (without PMA) for 24 h; the MC medium was harvested, filtered through a 0.22 µm filter, and stored at −80°C for later use. The protein level of IL-1β in THP-1 MC medium was 1,936 ± 190 pg/ml, determined using an ELISA kit (R&D Systems, Abingdon, UK).

Culture of blood monocyte-derived macrophages and generation of MC medium. Monocytes were obtained from the peripheral blood of six healthy male and female donors (BMI 20–28 kg/m²). Blood sampling was approved by local and national ethics committees (UK National Research Ethics Service reference 11/NW/0313). Twenty-five milliliters of blood taken from the antecubital vein was layered on 15 ml of Ficoll-Paque Premium (GE Healthcare, Amersham, Buckinghamshire, UK) and centrifuged at 350 g for 30 min. The PBMCs (peripheral blood mononuclear cells) were isolated from the buffy layer and washed once with RPMI-1640 (without FBS) or RPMI-1640 (without FCS, without PMA) and then cultured with FCS-free RPMI-1640 (without PMA) for 24 h; the MC medium was harvested, filtered through a 0.22 µm filter, and stored at −80°C for later use. The total cellular protein concentration in PBMC-derived MC medium was 387–603 pg/ml, determined as described above.

Cell treatment. To assess the effect of macrophage-derived factors on insulin signaling, differentiated adipocytes were incubated with RPMI-1640 (25%) as control or THP-1 MC medium (25%) for 24 h. To assess the effect of IL-1β on insulin signaling, differentiated adipocytes were treated with RPMI-1640 or IL-1β (2 ng/ml) for 24 h. To investigate whether IL-1β mediates the effects of MC medium, the following experiments were carried out. First, MC medium was preincubated with a human IL-1β neutralizing antibody (2 µg/ml; R&D Systems, Abingdon, UK) for 1 h at 37°C to inactivate IL-1β activity; differentiated adipocytes were then incubated with either RPMI-1640 (control), MC medium, or MC medium neutralized by IL-1β antibody or mouse IgG (Sigma) for 24 h. Second, to inhibit IL-1 production by macrophages, THP-1 cells were incubated with RPMI-1640 (serum free) as controls or 50 µM caspase-1 inhibitor (Ac-YVAD-CMK; Calbiochem, Watford, UK) in RPMI-1640 (serum free) for 48 h, with fresh medium replenished at 24 h; the medium was collected from macrophages without treatment (MC medium) or treated with caspase-1 inhibitor (MC medium plus caspase-1 inhibitor). Differentiated adipocytes were then incubated with RPMI-1640 (control), MC medium, or MC medium plus caspase-1 inhibitor for 24 h. Finally, to block IL-1β receptor in adipocytes, differentiated adipocytes were pretreated with a recombinant human IL-1β receptor antagonist (IL-1RA, Sigma) at 1 µg/ml for 2 h and then incubated with MC medium in the presence or absence of IL-1RA for 24 h. To further examine whether IL-1β mediates the effect of primary macrophages on adipocyte insulin signaling and inflammatory response, MC medium generated from human PBMC-derived macrophages was used. Differentiated human adipocytes were incubated with either RPMI-1640 (control), MC medium, MC medium neutralized by an IL-1β antibody (R&D), MC medium neutralized by an IL-1β antibody and a TNFα antibody (R&D), mouse IgG (Sigma), or MC medium with recombinant IL-1RA (Sigma) for 24 h. At the end of each experiment, cells and the culture media were collected and stored at −80°C until analysis.

Western blotting. Total cellular protein was prepared with lysis buffer (50 mM Tris·HCl pH 6.7, 10% glycerol, 4% SDS, 2% 2-mercaptoethanol) with freshly added protease inhibitor cocktail and phosphatase inhibitor cocktail (both from Sigma). Protein concentrations were determined with the BCA method. Protein samples (20 µg/lane) were resolved by 10% Tricine-SDS polyacrylamide slab gels (Mini Proton Tetra, Bio-Rad, Hemel Hempstead, UK) and transferred onto a nitrocellulose membrane (Hybond C Extra; Amersham Bioscience, Little Chalfont, UK) by wet transfer (Trans Blot, Bio-Rad) at 100 V for 1 h. The transfer of proteins onto the membrane was assessed by Ponceau S staining (Sigma). For immunodetection, the membrane was blocked for 1 h at room temperature with Tris-buffered saline (TBS) containing 0.1% Tween 20 and 5% BSA (Sigma) and incubated overnight at 4°C with the antibody for GLUT4 (1:2,500 dilution (Sigma), IRS-1 (1:1,000 dilution), PI3K p85 (1:1,000 dilution), and p-Akt (1:1,000 dilution) (New England BioLabs, Hitchin, UK) in 5% BSA and TBS and 0.1% Tween 20 followed by an anti-rabbit secondary antibody (New England BioLabs, Hitchin, UK) at 1:2,000 dilution. Signals were detected by chemiluminescence (West Pico kit; Pierce, Loughborough, UK) and scanned using a Molecular Imager ChemiDoc XR5 + System (Bio-Rad). The size of the protein bands detected was estimated with PageRuler protein markers (Fermentas, York, UK). The membrane was further probed with GAPDH (Abcam, Cambridge, UK) or total Akt (New England BioLabs) as a loading control.

Real-time PCR array. Total RNA was extracted from cells using TRIzol (Invitrogen, Paisley, UK) and the RNA concentration determined from absorbance at 260 nm. First-strand cDNA was reverse transcribed from 0.5 µg of total RNA using an iScript first-strand synthesis kit (Bio-Rad) in a final volume of 10 µl. The expression profile of 84 genes involved in insulin signaling, insulin sensitivity,
and glucose metabolism was examined with a Human Diabetes RT² Profiler PCR Array (SABiosciences, QIAGEN, West Sussex, UK) according to the manufacturer’s protocol. Briefly, a total volume of 25 μl of reaction mixture containing cDNA (0.25 μg) and RT² SYBR Green Mastermix were added to 96-well PCR plates precoated with the primers for target genes. Real-time PCR applications were performed using a Stratagene Mx3005P instrument, and PCR cycling conditions were as follows: 95°C for 10 min followed by 40 cycles (95°C for 15 s, 60°C for 1 min). The results were expressed as fold changes of CT value relative to controls, using the data analysis software from the manufacturer.

Glucose consumption. Glucose consumption was performed as previously described (61). Briefly, adipocytes were cultured in 96-well plates and incubated with RPMI-1640 (control), MC medium, or MC medium neutralized by IL-1β antibody (2 μg/ml) or mouse IgG (2 μg/ml, as negative control) for 24 h. The glucose concentration in the culture medium was analyzed using the glucose oxidase method. Glucose consumption was calculated by subtracting the glucose value from the control (blank well).

Measurement of cytokine/chemokine release. The secretion levels of IL-1β by THP-1 macrophages or PBMC-derived macrophages and of IL-6, monocyte chemoattractant protein-1 (MCP-1), IL-8, and RANTES [CCL5 chemokine (C-C motif) ligand-5] by adipocytes were determined as the protein concentrations in cell culture medium, using ELISA kits (R&D Systems, Abingdon, UK).

Measurement of glycerol release. Lipolysis was determined as the levels of glycerol in adipocyte culture medium using a colorimetric method. Cell culture medium (25 μl) or serial dilutions of glycerol standard solution (Sigma) were incubated with a free glycerol reagent (200 μl, Sigma) at room temperature for 10 min. The absorbance of the samples and standard were then measured using a spectrophotometer (Bio-Rad) at a wavelength of 540 nm. The concentration of glycerol was calculated by a glycerol standard curve.

Statistical analysis. Data are expressed as means ± SE. Differences between two groups were analyzed by Student’s unpaired t-test. One-way ANOVA coupled with Bonferroni’s t-test was employed for comparison of multigroups. Differences were considered statistically significant when \( P < 0.05 \).

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**Fig. 1.** Macrophage-conditioned (MC) medium reduces protein expression of insulin signaling molecules in human adipocytes. Adipocytes (at day 12 postdifferentiation) were treated with RPMI 1640 medium (control) or THP-1 MC medium (25%) for 24 h. Cell lysates were analyzed by Western blotting and densitometry, using antibodies to IRS-1 and PI3K p85α (A, B, C) and GLUT4 (D, E). For measuring basal and insulin-stimulated Akt phosphorylation, adipocytes were incubated with RPMI 1640 or MC medium for 24 h before being stimulated with insulin (167 nmol/l) for 5 min; Akt phosphorylation at Ser473 (pAkt) was analyzed by Western blotting and densitometry. Total Akt and GAPDH were used as loading controls (F, G). Representative blots are shown; data are means ± SE (n = 3 per group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. controls. Results were confirmed by 3 independent experiments.
RESULTS

Macrophage-derived factors inhibit the insulin signaling pathway in human adipocytes. The initial experiments were to establish an in vitro model to study the effects of macrophage-derived factors on the insulin signaling pathway in human adipocytes. As shown in Fig. 1, A–E, treatment with MC medium for 24 h led to a significant reduction in protein abundance of IRS-1 (by 47%, \( P < 0.001 \)), PI3K p85α (by 33%, \( P < 0.05 \)), and GLUT4 (by 30%, \( P < 0.01 \)) in adipocytes. We then examined the level of serine phosphorylation of Akt, a key insulin signaling molecule downstream of IRS-1. A time course experiment was performed to verify the time point of maximum response to insulin, which showed that 5-min stimulation with insulin was optimal (data not shown).

IL-1β inhibits expression of insulin signaling molecules and lipogenic genes in human adipocytes. We next determined the effects of IL-1β, a major product of macrophages, on the expression of insulin signaling molecules in human adipocytes. First, we assessed the effect of 24-h treatment of IL-1β/H9251 on gene expression of insulin signaling molecules in human adipocytes. Treatment with IL-1β/H9251 led to a significant reduction in insulin-stimulated phosphorylation of Akt Ser473 (by 10.22±0.33-fold, \( P < 0.001 \)), intercellular adhesion molecule-1 (ICAM1; 8-fold, \( P < 0.01 \)), and NF-κB1 (2-fold, \( P < 0.01 \)) (Fig. 2B) compared with controls. Consistent with the gene expression data, IL-1β significantly reduced protein abundance of IRS-1 (by 63%), PI3K p85α (by 33%), and GLUT4 (by 45%) (all \( P < 0.001 \)) (Fig. 3, A–D). Furthermore, IL-1β significantly decreased insulin-stimulated phosphorylation of Akt, PI3K p85α, and IRS-1.

Table 1. Genes whose expressions were up- or downregulated in human adipocytes by IL-1β

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Results are means of fold change in mRNA levels (n = 3 each group). *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) vs. controls.
Blocking IL-1β activity reduces the effects of MC medium on expression profile of genes involved in insulin signaling, insulin sensitivity and glucose metabolism in human adipocytes. To examine whether IL-1β mediates the effects of MC medium, we used a neutralizing antibody to block IL-1β activity and determined the expression levels of 84 genes in human adipocytes by a PCR array. MC medium led to a downregulation of 27 genes (Table 2); most of those are related to glucose metabolism, insulin signaling, insulin sensitivity, lipid metabolism and inflammation (Fig. 4A), including GLUT4 (14-fold, \(P < 0.001\); Fig. 3, E and F).

However, blocking IL-1β activity significantly reduced the effects of MC medium as the expression of 22 of 27 genes being downregulated by MC medium was partially restored (Fig. 4A), including GLUT4 (76%, \(P < 0.001\)), IRS-1 (54%, \(P < 0.05\)), IRS-2 (53%, \(P < 0.01\)), PI3K (53%, \(P < 0.01\)), SREBF1 (82%, \(P < 0.01\)), PPARα (40%, \(P < 0.01\)), and PGC-1β (77%, \(P < 0.01\)). In contrast, 15 genes were upregulated by MC medium (Table 2) with a marked increase in proinflammatory factors, including IL-6 (822-fold, \(P < 0.001\)), CCL5 (144-fold, \(P < 0.001\)), ICAM1 (22-fold, \(P < 0.001\)), VEGFA (4-fold, \(P < 0.001\)), and NF-KB1 (23–41%) (Fig. 4).

Blocking IL-1β activity reverses the effects of MC medium on protein expression of insulin signaling molecules and cytokine release in human adipocytes. Since blocking IL-1β activity reduced effects of MC medium on expression of genes involved in insulin signaling pathway, we subsequently assessed whether it is effective at the protein levels. MC medium significantly reduced protein abundance of IRS-1 (46%, \(P < 0.01\)), PI3K p85α (31%, \(P < 0.01\)), and GLUT4 (>2-fold, \(P < 0.05\)) in adipocytes (Fig. 5, A–F); however, blocking IL-1β activity with a neutralizing antibody abolished the effect elicited by 10.2 ng/ml or vehicle (control) for 24 h. Expression levels of genes involved in glucose metabolism, insulin signaling, insulin sensitivity, lipid metabolism and inflammation (B) were measured using a PCR array. Data are expressed as fold changes (\(n = 3\) per group). \(*P < 0.05, **P < 0.01, ***P < 0.001\) vs. controls.

**Fig. 2. Effects of IL-1β on expression profile of genes involved in glucose metabolism, insulin signaling, lipid metabolism and inflammation in human adipocytes.** Differentiated adipocytes were cultured in the presence of IL-1β (2 ng/ml) or vehicle (control) for 24 h. Expression levels of genes involved in glucose metabolism, insulin signaling, insulin sensitivity, lipid metabolism and inflammation (A) and inflammation (B) were measured using a PCR array. Data are expressed as fold changes (\(n = 3\) per group). \(*P < 0.05, **P < 0.01, ***P < 0.001\) vs. controls.
MC medium also led to a reduction in insulin-stimulated phosphorylation of Akt at Ser473 compared with controls (H11022 3-fold, P/0.001), but this effect was largely reversed by IL-1 neutralization (P/0.05; Fig. 5G). Treatment with IgG (as a negative control) did not affect the effects of MC medium (Fig. 5, A–H). In addition, we evaluated whether blocking IL-1 activity inhibits the effects of MC medium on the release of proinflammatory factors known to impair insulin signaling by adipocytes. As shown in Fig. 6, A–D, basal secretion of cytokines was barely detectable (IL-6, IL-8, and RANTES) or low (MCP-1, with a mean value of 140 pg/ml) by adipocytes; MC medium potently stimulated the release of IL-6 (19,623 pg/ml), MCP-1 (42,776 pg/ml), IL-8 (88,806 pg/ml), and RANTES (5,618 pg/ml), but this induction was blunted by IL-1 neutralization (all P/0.001).

Blocking IL-1 receptor binding in human adipocytes restores MC medium-suppressed protein expression of IRS-1, PI3K p85α, and GLUT4 and reverses the effects of MC medium on cytokine release by human adipocytes. To provide further evidence whether IL-1β has a key role in macrophage-induced impairment of insulin signaling, recombinant IL-1RA was used to block IL-1 receptor binding in adipocytes. Adipocytes exposed to MC medium displayed a marked reduction in protein expression of IRS-1 (P < 0.001; Fig. 7, A and B), PI3K p85α (P < 0.05; Fig. 7, C and D), and GLUT4 (P < 0.001; Fig. 7, E and F) compared with controls. However,
Table 2. Genes whose expressions were up- or downregulated in human adipocytes by MC- medium or MC with IL-1β neutralizing antibody (MC+IL-1β Ab)

<table>
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<th>Gene Symbol</th>
<th>Description</th>
<th>GenBank ID</th>
<th>MC</th>
<th>MC+IL-1β Ab</th>
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| GPD1 | Glycerol-3-phosphate dehydrogenase | NM_005276 | −23.59*** | −5.02***$
| ACLY | ATP citrate lyase | NM_001096 | −22.47** | −8.21***$
| MEI | Malic enzyme 1 | NM_002395 | −14.32** | −4.30***$
| GLUT4 | Solute carrier family 2 (facilitated glucose transporter), member 4 | NM_001042 | −13.77*** | −3.25***$
| SREBF1 | Sterol regulatory element binding transcription factor 1 | NM_004176 | −10.73*** | −1.94***$
| NOS3 | Nitric oxide synthase 3 | NM_00603 | −9.19*** | −3.37***$
| PGC-1β | Peroxisome proliferator-activated receptor-γ coactivator 1β | NM_133263 | −7.00*** | −1.59***$
| CEBPα | CCAAT/enhancer binding protein (C/EBP)α | NM_003644 | −6.02*** | −1.57***$
| PYGL | Phosphorylase, glycogen; liver | NM_002863 | −5.28*** | −2.75***$
| HMOX1 | Heme oxygenase (decycling) 1 | NM_003749 | −4.50*** | −3.08***$
| IRS-2 | Insulin receptor substrate 2 | NM_003374 | −4.47*** | −1.66***$
| IGFBP5 | Insulin-like growth factor-binding protein-5 | NM_000598 | −4.17*** | −1.11***$
| AGT | Angiotensinogen | NM_000162 | −4.07** | −1.63***$
| GSK-3β | Glycogen synthase kinase 3 | NM_002093 | −3.52*** | −2.33***$
| PIK3R1 | Phosphoinositide 3-kinase, regulatory subunit 1 (p85α) | NM_181504 | −3.47** | −1.63***$
| ENPP1 | Ectonucleotide pyrophosphatase-1 | NM_002608 | −3.05** | −1.80***$
| PIK3C2B | Phosphoinositide 3-kinase, class 2b polypeptide | NM_002646 | −2.89*** | −1.57***$
| PIK3Rα | Peroxisome proliferator-activated receptor-α | NM_005036 | −2.85*** | −1.71***$
| AGT | Angiotensinogen | NM_000029 | −2.73** | −1.18***$
| IL-10 | Interleukin 10 | NM_000572 | −2.39** | −1.05***$
| PPARα | Peroxisome proliferator-activated receptor-α | NM_001618 | −2.33** | −1.14***$
| TRIB3 | Tribbles homolog 3 | NM_021158 | −2.27*** | −1.93***$
| IRS-1 | Insulin receptor substrate 1 | NM_005554 | −2.22*** | −1.03***$
| IL-1β | Interleukin-1β | NM_006178 | −2.08*** | −1.64***$
| MAPK14 | Mitogen-activated protein kinase-14 | NM_004578 | −2.00*** | −1.48***$
| SACOPB | Synaptosomal-associated protein, 23 kDa | NM_003825 | −1.97*** | −1.26***$
| SNAP23 | Synaptosomal-associated protein, 23 kDa | NM_003825 | −1.40** | −1.10***$

Results are means of fold change in mRNA levels ($n = 3$ each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. controls; §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$ vs. MC medium-treated group.

**Blocking IL-1 signal transduction by IL-1RA abolished the inhibitory effect of MC medium on IRS-1 ($P < 0.01$), PI3K p85α ($P < 0.05$), and GLUT4 ($P < 0.01$) in adipocytes (Fig. 7, A–F). Moreover, IL-1RA was able to significantly reverse the stimulatory effect of MC medium on protein release of IL-6 (97%), MCP-1 (93%), and IL-8 (90%) by human adipocytes (all $P < 0.001$; Fig. 7, G–I).

**Inhibiting IL-1β production by macrophages reverses the effects of MC medium on insulin signaling pathway in human adipocytes.** To further examine the importance of IL-1β in mediating the effects of MC medium on the insulin signaling pathway, IL-1β release by THP-1 macrophages was blocked with an inhibitor of caspase-1 (IL-1β converting enzyme). Caspase-1 inhibitor significantly reduced IL-1β production by THP-1 macrophages by 84% (317 ± 56 vs. 1,936 ± 190 pg/mL, $P < 0.001$). Adipocytes were then incubated with RPMI medium (control) or the medium from THP-1 macrophages treated with or without the caspase-1 inhibitor. As illustrated in Fig. 8, A–D, MC medium led to a significant decrease in protein expression of IRS-1, PI3K p85α, and GLUT4 in adipocytes (all $P < 0.001$); however, this inhibition was partially (for IRS-1 and GLUT4) or totally (GLUT4) reversed in adipocytes exposed to MC medium (Fig. 8, E–G).

**Human primary macrophage-derived factors inhibit insulin signaling and stimulate IL-6 release by human adipocytes, and the effect of blocking IL-1β.** To further examine whether IL-1β mediates the effect of primary macrophages on adipocyte
insulin signaling and inflammatory response, MC medium generated from human PBMC-derived macrophages was used. MC medium significantly reduced protein expression of IRS-1 (Fig. 9, A and B) and GLUT4 (Fig. 9, C and D) in adipocytes (all \( P < 0.01 \)); this inhibition was partially (for IRS-1) or totally (for GLUT4) reversed by IL-1\( \beta \) neutralization (both \( P < 0.05 \)) and totally reversed by both IL-1\( \beta \) and TNF\( \alpha \) neutralization (both \( P < 0.05 \)) or an IL-1\( \beta \) RA (\( P < 0.01 \)). MC medium also decreased insulin-stimulated phosphorylation of Akt (Ser473) compared with controls (2-fold, \( P < 0.05 \); Fig. 9, E–F). Furthermore, as shown in Fig. 9G, the MC medium-elicted substantial release of IL-6 by adipocytes was largely reversed by IL-1\( \beta \) neutralization (90%) or IL-1\( \beta \) and TNF\( \alpha \) neutralization (92%) or an IL-1\( \beta \) RA (95%) (all \( P < 0.001 \)).

**DISCUSSION**

During the course of obesity, there is a rise in accumulation of macrophages and other immune cells in adipose tissue (56, 59). The enhanced macrophage-adipocyte cross-talk in obesity affects adipose tissue biology, but the molecular mechanisms and the key mediators, particularly in human adipose tissue, remain largely unknown. In the present study, we used in vitro models of human macrophages (derived from a monocytic cell line and also PBMCs) and primary fat cells to examine the insulin signaling and inflammatory response, MC medium generated from human PBMC-derived macrophages was used. MC medium significantly reduced protein expression of IRS-1 (Fig. 9, A and B) and GLUT4 (Fig. 9, C and D) in adipocytes (all \( P < 0.01 \)); this inhibition was partially (for IRS-1) or totally (for GLUT4) reversed by IL-1\( \beta \) neutralization (both \( P < 0.05 \)) and totally reversed by both IL-1\( \beta \) and TNF\( \alpha \) neutralization (both \( P < 0.05 \)) or an IL-1\( \beta \) RA (\( P < 0.01 \)). MC medium also decreased insulin-stimulated phosphorylation of Akt (Ser473) compared with controls (2-fold, \( P < 0.05 \); Fig. 9, E–F). Furthermore, as shown in Fig. 9G, the MC medium-elicted substantial release of IL-6 by adipocytes was largely reversed by IL-1\( \beta \) neutralization (90%) or IL-1\( \beta \) and TNF\( \alpha \) neutralization (92%) or an IL-1\( \beta \) RA (95%) (all \( P < 0.001 \)).

**IL-1\( \beta \) mediates macrophage-induced alteration of glucose and lipid metabolism in human adipocytes.** MC medium also led to a reduction in glucose consumption (28%, \( P < 0.01 \)) in adipocytes, but this effect was abolished by IL-1\( \beta \) neutralization (\( P < 0.01 \); Fig. 10A). As macrophage-derived factors enhance adipocyte lipolysis, which may induce insulin resistance in multiple organs (47), we also investigated whether IL-1\( \beta \) mediates macrophage-induced lipolysis in human adipocytes. As shown in Fig. 10, B and C, both IL-1\( \beta \) and MC medium significantly increased glycerol release from adipocytes (1.5 and >2-fold, both \( P < 0.001 \)). However, MC medium-stimulated lipolysis was completely abolished by blocking IL-1\( \beta \) activity with a neutralizing antibody (\( P < 0.001 \); Fig. 10C). Again, treatment with IgG (negative control) had no effect (Fig. 10C).

**Fig. 4.** Inhibition of IL-1\( \beta \) activity reduces the effect of macrophages on expression profile of genes related to glucose metabolism, insulin signaling, and inflammation in human adipocytes. To block the activity of IL-1\( \beta \), MC medium was preincubated with an IL-1\( \beta \) neutralizing antibody (2 \( \mu \)g/ml) for 1 h at 37°C. Differentiated adipocytes were then treated with RPMI 1640 (control), MC medium, or MC medium neutralized by IL-1\( \beta \) antibody for 24 h. Expression levels of genes involved in glucose metabolism, insulin signaling, lipid metabolism, insulin sensitivity (A), and inflammation (B) were determined using a PCR array. Data are expressed as fold changes (\( n = 3 \) per group). \( * P < 0.05 \), \( ** P < 0.01 \), \( *** P < 0.001 \) vs. controls; \( # P < 0.05 \), \( ## P < 0.01 \), \( ### P < 0.001 \) vs. MC medium treatment.
influence of macrophage-derived factors on the insulin signaling pathway and the role of IL-1β in human adipocytes. We observed that MC medium significantly reduced protein abundance of insulin signaling molecules, including IRS-1, PI3K p85α, GLUT4, and insulin-stimulated phosphorylation of Akt. These results are in agreement with a previous study in murine 3T3-L1 fat cells (30), suggesting that macrophage-derived factors can impair insulin signaling in human adipocytes.

Identification of the major factors that mediate the detrimental effect of macrophages on adipocytes is crucial for developing effective therapeutic targets. IL-1β has been implicated as a key regulator in the translation of obesity-associated inflammation into insulin resistance in rodent models (7, 18, 33, 40). The present study has demonstrated that in human adipocytes IL-1β powerfully repressed insulin signal transduction. This was initially revealed by the PCR array analysis, which showed that IL-1β downregulated expression of genes involved in insulin signaling, insulin sensitivity, glucose metabolism, and lipid metabolism, including IRS-1, IRS-2, PPARα, PPARγ, PGC-1β, GLUT4, GPD1, GSK-3β, ACLY, and CEBPα. Furthermore, IL-1β (2 ng/ml) showed a similar potency as the MC medium in reducing protein expression of IRS-1, PI3K p85α, GLUT4, and insulin-stimulated phosphorylation of Akt. These data led us to further investigate whether IL-1β is responsible for the inhibitory effect of MC medium on the insulin signaling pathway in human adipocytes.

A major finding of our study is that blocking the actions of IL-1β can reverse the effects of MC medium on insulin signaling in human adipocytes. When IL-1β activity was inhibited with a neutralizing antibody, the expression of 22 of 27 genes being downregulated by MC medium was partially restored, including GLUT4, GPD1, IRS-1, IRS-2, PI3Kα,
sensitive (53). To further examine the importance of IL-1β pose tissue, and caspase-1 knockout mice are more insulin also been reported to regulate IL-1β/H9251 phages isolated from human adipose tissue (46). Caspase-1 has reduction in protein expression of IRS-1, PI3K p85α, and kine release in human adipocytes. We demonstrated that the caspase-1 inhibitor on insulin signaling and cytokine/chemo-

Fig. 6. Inhibition of IL-1β activity reverses macrophage-induced cytokine release by hu-

man adipocytes. Differentiated adipocytes were incubated with RPMI 1640 (control), MC medium, MC medium neutralized by IL-1β antibody (2 μg/ml), mouse IgG (2 μg/ml, as negative control), or MC medium alone (without cells) for 24 h. The release of IL-6 (A), MCP-1 (B), IL-8 (C) and RANTES (D) by adipocytes was measured as protein concentrations in cell culture medium by ELISAs. Data are means ± SD (n = 6 per group). ***P < 0.001 vs. indicated groups. Results were confirmed by 3 independent experiments.

ACLY, CEBPα, SREBF1, PPARα, and PGC-1β (by 40–82%; Fig. 4A). In addition, IL-1β depletion totally abolished the inhibitory effect of MC medium on protein abundance of insulin signaling molecules (IRS-1, PI3K p85α, and GLUT4) and insulin-stimulated phosphorylation of Akt, suggesting that IL-1β blockade can restore insulin signal transduction in human adipocytes. This is consistent with the result of glucose consumption, which was reduced by MC medium but restored by IL-1β neutralization (Fig. 10A). IL-1β could therefore be a major contributor to macrophage-induced inhibition on the insulin signaling pathway. To substantiate the role of IL-1β in mediating the effect of macrophages, we used an IL-1RA to prevent IL-1β binding to its receptor in adipocytes, and it restored protein expression of IRS-1, PI3K p85α, and GLUT4 suppressed by MC medium. Collectively, our results suggest that IL-1β is required for the inhibitory effect of macrophages on insulin signaling in human adipocytes.

Biologically active IL-1β is produced through cleavage of pro-IL-1β by IL-1β converting enzyme (caspase-1), activated via the NLRP3 inflammasome complex (1). Recent studies have shown that IL-1β production is caspase-1 dependent in macrophages isolated from human adipose tissue (46). Caspase-1 has also been reported to regulate IL-1β production in mouse adipose tissue, and caspase-1 knockout mice are more insulin sensitive (53). To further examine the importance of IL-1β in MC medium, we assessed the effects of IL-1β inhibition with a caspase-1 inhibitor on insulin signaling and cytokine/chemo-
kine release in human adipocytes. We demonstrated that the reduction in protein expression of IRS-1, PI3K p85α, and GLUT4 by MC medium was partially or totally reversed when IL-1β production by macrophages was inhibited. In contrast, macrophage-stimulated release of IL-6, IL-8, and RANTES by adipocytes was significantly reduced by blocking IL-1β production. These results further support a key role for IL-1β in mediating macrophage-induced inhibition of insulin signaling and upregulation of the release of proinflammatory cytokines in human adipocytes. It is debatable whether blocking caspase-1 may also affect IL-18 release, as both IL-1β and IL-18 production within the inflammasome involve the activation of caspase-1. However, the effect of IL-18 on the insulin signaling pathway remains to be identified. Although plasma IL-18 was reported to be higher in obese subjects and correlated with insulin resistance (HOMA) (2), IL-18 release by adipose tissue explants was reduced in obese women (10). IL-18 in vitro stimulated phosphorylation of Akt and glucose uptake by 3T3-L1 adipocytes (60). Further studies are needed to clarify whether IL-18 modulates insulin sensitivity in human adipose tissue.

The mechanisms by which IL-1β modulates the macrophage-adipocyte cross-talk on insulin sensitivity in adipose tissue remain to be elucidated. Evidence suggests that local inflammation exemplified by macrophage accumulation in adipose tissue could be crucial, leading to the impairment of insulin sensitivity (31). In the present study, we demonstrated that MC medium potently upregulated the expression of the proinflammatory factors, including IL-6, CCL5, ICAM1, VEGFA, and NF-KB1 with IL-6 (822-fold) and CCL5 (144-fold) being the most strongly induced, which indicates a
substantial increase in inflammatory response in adipocytes. Strikingly, this upregulation was largely reversed by IL-1β neutralization, consistent with a marked reduction in protein release of IL-6, CCL5, MCP-1, and IL-8 by adipocytes (Fig. 6). In addition, we observed that preventing IL-1β signaling in adipocytes or blocking IL-1β production by macrophages can reduce macrophage-induced release of these cytokine/chemokines. Similar to the effect of THP-1 macrophages, conditioned medium from PBMC-derived macrophages markedly induced IL-6 release by adipocytes, but this can be largely reversed by IL-1β neutralization or blocking IL-1 receptors (Fig. 9G). Our results implicate IL-1β as a key mediator for the proinflammatory activity of adipose tissue macrophages. IL-6 is suggested to play a role in insulin resistance, as its circulating levels are positively related to adiposity (44, 54) and IL-6 is overexpressed in fat cells from insulin-resistant subjects (41). In vitro, IL-6 inhibits gene transcription of IRS-1, GLUT4, and PPARγ and reduces insulin-stimulated glucose uptake in 3T3-L1 adipocytes (41). In addition to IL-6, recent studies suggest that CCL5 is another key player in obesity-related adipose tissue inflammation (32); gene expression of CCL5 in adipose tissue is increased in obese subjects, and CCL5 increases monocyte migration and macrophage survival in human adipose tissue (22). Furthermore, obese mice with deletion of CCL5 receptor are protected from insulin resistance, and this is related to reduced adipose tissue macrophage content and an M2 type-dominant polarization (24). Like CCL5, MCP-1 (or CCL2) also belongs to CC chemokines with a chemotactic activity for monocytes (21). Overexpression of MCP-1 enhances macrophage accumulation and insulin resis-
tance (20), whereas MCP-1 or its receptor (CCR2) knockout in mice reduces macrophage infiltration in adipose tissue (21, 51). IL-8, known as neutrophil chemotactic factor, also induces chemotaxis in other cell types including macrophages (55), and gene expression of IL-8 is upregulated in mammary adipose tissue of obese women, in parallel with increased macrophage infiltration (48). In diet-induced obese mice, IL-8 receptor (CXCR2) knockout prevents macrophage recruitment in adipose tissue and insulin resistance (35). Collectively, our data suggest that the deleterious effects of macrophage-derived IL-1β on insulin signaling could be mediated through an upregulated inflammatory response in adipocytes, especially the production of proinflammatory cytokines/chemokines. In addition to the endocrine and autocrine effects of these adipocyte-derived cytokines, adipocyte-derived factors may affect macrophage function in a paracrine manner. We have reported recently that conditioned medium from human preadipocytes and adipocytes can increase THP-1 monocyte migration, and this is probably due to chemoattractants, such as MCP-1, secreted by preadipocytes and adipocytes (6, 12). A very recent work has shown that lipid-induced fetuin-A from adipocytes is a chemokine for macrophage migration and polarizes M2 macrophages to M1 type (3). Moreover, chemoattractants are also produced by macrophages and would increase further macrophage infiltration into adipose tissue. It is probable that the cross-talk between macrophages and adipocytes may induce a vicious cycle of monocyte recruitment and also M1 macrophage polarization, thereby increasing the inflammatory potential and reducing insulin sensitivity of adipose tissue in obesity.

Whether adipocyte-derived IL-1β modulates adipocyte insulin sensitivity in an autocrine manner is not known. One study has demonstrated that conditioned medium from 3T3-L1 adipocytes stimulated with TNFα induced insulin resistance in
hepatocytes, and this could be prevented by blocking TNFα-induced IL-1β production by 3T3-L1 cells (36). It is therefore suggested that mouse adipocyte-derived IL-1β may mediate the perturbed adipose-liver cross-talk. However, in our study, basal IL-1β secretion by human primary adipocytes was very low (0–14 pg/ml; data not shown) compared with IL-1β released by THP-1 macrophages (>1,900 pg/ml), and furthermore, treatment of adipocytes with a caspase-1 inhibitor did not alter the protein expression of IRS-1 and GLUT4 by human adipocytes (data not shown). This is consistent with the previous report that the release of interleukins including IL-1β by human adipose tissue is enhanced in obesity and is primarily due to the nonfat cells (8, 9). In our study, although the autocrine effect of human adipocyte-derived IL-1β cannot be excluded it is unlikely to be crucial.

Reduced lipid storage capacity in adipocytes is a critical event in promoting ectopic fat deposition in muscle and liver, which may lead to metabolic derangement such as insulin resistance in obesity (14, 26, 47). Previous studies have shown that macrophage-conditioned medium inhibited adipogenesis in 3T3-L1 and human preadipocytes (11, 17). Moreover, IL-1β KO mice being high-fat fed (HFF) had lower mRNA levels of proinflammatory factors, increased expression of the adipogenic genes (Pparα, Cebpα, Cebpβ, Fabp4) in adipose tissue and less hepatic steatosis compared with wild-type HFF mice, which suggests that IL-1β could impair fat storage and promote ectopic fat accumulation by limiting adipose tissue expandability (37). It has been shown recently that the markers of macrophage infiltration positively correlate with the rate of lipolysis in human adi-
IL-1β mediates macrophage-induced alteration of glucose and lipid metabolism in human adipocytes. Adipocytes were treated with RPMI 1640 (control), THP-1 MC medium, MC medium neutralized by IL-1β antibody (2 ng/ml), MC medium with IgG (2 μg/ml), RPMI 1640 only (control), or RPMI 1640 with IL-1β (2 ng/ml) for 24 h. Glucose consumption was measured as the glucose concentration in culture medium by glucose oxidase method (A). Lipolysis was determined as glycerol release into culture medium (B, C). Data are means ± SE (n = 6 per group). **P < 0.01, ***P < 0.001 vs. indicated groups. Results were confirmed by 3 independent experiments. D: schematic diagram of IL-1β in mediating the effect of human macrophages on insulin signaling in human adipocytes.
IL-1β and IL-6 was suggested to be predictive of type 2 diabetes (44). A combined effect of the two cytokines on insulin sensitivity in adipose tissue could be possible, but further work is required to determine whether such an interaction occurs.

In conclusion, our study demonstrates that IL-1β is a key factor in mediating macrophage-induced insulin resistance in human adipocytes (Fig. 10D). Blocking IL-1β activity, its receptor binding, and production can partially or totally restore insulin signaling and responsiveness in adipocytes. IL-1β antagonism also protects against macrophage-stimulated production of the proinflammatory cytokines/chemokines, including IL-6, CCL5, MCP-1, and IL-8. Finally, macrophage-induced lipolysis and inhibition in the expression of the adipogenic factors are reversed by IL-1β depletion. These results suggest that targeting IL-1β may have therapeutic benefits in the prevention of obesity-associated insulin resistance in human adipose tissue.

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AUTHOR CONTRIBUTIONS

C.B. and D.G. designed study, analyzed and interpreted data, and wrote manuscript; D.G., M.M., C.D., M.F., T.S., and L.H. executed experiments and contributed to data analysis; M.F. and T.S. contributed to drafting manuscript; D.G., M.M., C.D., M.F., T.S., C.F., and L.H. approved final version of the manuscript.

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