0013-7227/06/$15.00/0
Printed in U.S.A.

Endocrinology 147(11):5340–5351

doi: 10.1210/en.2006-0536

Preadipocytes Mediate Lipopolysaccharide-Induced Inflammation and Insulin Resistance in Primary Cultures of Newly Differentiated Human Adipocytes

Soonkyu Chung, Kathleen LaPoint, Kristina Martinez, Arion Kennedy, Maria Boysen Sandberg, and Michael K. McIntosh

Department of Nutrition (S.C., K.L., K.M., A.K., M.K.M.), University of North Carolina at Greensboro, Greensboro, North Carolina 27402-6170; and Department of Biochemistry and Molecular Biology (M.B.S.), University of Southern Denmark, Odense DK-5230, Denmark

Recent data suggest that proinflammatory cytokines secreted from adipose tissue contribute to the morbidity associated with obesity. However, characterization of the cell types involved in inflammation and how these cells promote insulin resistance in human adipocytes are unclear. We simulated acute inflammation using the endotoxin lipopolysaccharide (LPS) to define the roles of nonadipocytes in primary cultures of human adipocytes. LPS induction of the mRNA levels of proinflammatory cytokines (*e.g.* IL-6, TNF-α, and IL-1β) and chemokines (*e.g.* IL-8, monocyte chemoattractant protein-1) occurred primarily in the nonadipocyte fraction of newly differentiated human adipocytes. Nonadipocytes were characterized as preadipocytes based on their abundant mRNA levels of preadipocyte markers preadipocyte factor-1 and adipocyte enhancer protein-1 and only trace levels of markers for macrophages and myocytes. The essential role of preadipocytes in inflammation was confirmed by modulating the degree of differentiation in the cultures from approximately 0–90%. LPS-induced proinflammatory cytokine/chemokine expression and nuclear factor-κB and MAPK signaling decreased as differentiation increased. LPS-induced cytokine/chemokine expression in preadipocytes was associated with: 1) decreased adipogenic gene expression, 2) decreased ligand-induced activation of a peroxisome proliferator activated receptor (PPAR)-γ reporter construct and increased phosphorylation of PPARγ, and 3) decreased insulin-stimulated glucose uptake. Collectively, these data demonstrate that LPS induces nuclear factor-κB- and MAPK-dependent proinflammatory cytokine/chemokine expression primarily in preadipocytes, which triggers the suppression of PPARγ activity and insulin responsiveness in human adipocytes. (*Endocrinology* 147: 5340–5351, 2006)

OBESITY AND ITS associated metabolic pathologies are the most common metabolic diseases in the United States, affecting over 50% of the adult population. One emerging feature of obesity is the linkage between obesity and chronic inflammation characterized by increased cytokine and chemokine production and acute-phase inflammatory signaling in adipose tissue (1, 2). Thus, white adipose tissue (WAT) is no longer considered an inert depot of stored energy but also an active endocrine organ secreting a diverse array of proinflammatory adipokines such as leptin, IL-1β, IL-6, IL-8, TNF-α, monocyte chemoattractant protein (MCP)-1, and macrophage migration inhibitory factor, all of which have been linked to insulin resistance. However, the exact role of cells comprising adipose tissue in mediating inflammation and causing insulin resistance is still unclear.

Adult human WAT has been reported to be composed of approximately 50–70% adipocytes, approximately 20–40% stromal vascular (SV) cells (*i.e.* preadipocytes, fibroblasts, nondifferentiated mesenchymal cells), and approximately 1–30% infiltrated macrophages (3). However, less is known about the localization and secretory pattern of cytokines in WAT. It has been suggested that nonadipocytes (*e.g.* SV cells and/or cells from the supporting matrix) in human WAT are the major producers of IL-6 and TNF-α rather than adipocytes (4, 5). Similarly, preadipocytes have been reported to act as macrophage-like cells and secrete an array of cytokines (6). Conversely, it has been proposed that macrophages residing in adipose tissue are responsible for most of the secreted cytokines (7). Weisberg *et al.* (8) reported that adipose tissue recruits circulating monocytes/macrophages from bone. Intriguingly, Charrière *et al.* (9) reported plasticity of preadipocytes showing evidence that 3T3-L1 cells have the ability to acquire phagocytic phenotypes and properties in the presence of macrophages.

Given these emerging data linking cross talk between nonadipocytes and adipocytes with the development of obesity and insulin resistance, the use of primary cultures of

First Published Online July 27, 2006

Abbreviations: AD0, Preadipocyte; AD50, approximately 50% differentiation; AD90, approximately 90% differentiation; ADF, adipocyte fraction; AEBP-1, adipocyte enhancer protein-1; AM1, adipocyte media; aP2, adipocyte-specific fatty acid-binding protein; APM-1, adiponectin; BMI, body mass index; COX, cyclooxygenase; DM1, differentiation media; DMAP, 6-dimethylaminopurine; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBSS, Hanks' balanced salt solution; IκB, inhibitory-κB protein; IKK, IκBα kinase; JNK, c-Jun NH_2-terminal kinase; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MEK, MAPK kinase; NFκB, nuclear factor-κB; PI3K, phosphatidylinositol-3-kinase; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; Pref-1, preadipocyte factor-1; qPCR, quantitative PCR; SV, stromal vascular; SVF, stromal vascular fraction; TLR, Toll-like receptor; WAT, white adipose tissue.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

newly differentiated human adipocytes as a cell model to investigate this linkage is timely. These heterogeneous cultures contain various percentages of nonadipocytes and adipocytes, depending on the isolation, growth, and differentiation protocols used. However, data on the types of cells in these cultures, and their role in triggering inflammation and insulin resistance are lacking.

Based on our previous findings demonstrating that cultures of newly differentiated human adipocytes robustly secrete cytokines/chemokines that impair insulin sensitivity (10, 11) and reports showing that preadipocytes are targets of inflammatory stimuli (6, 12), we focused on delineating the role of nonadipocytes from WAT in inflammation and insulin resistance. To simulate acute inflammation, we treated the cultures with the bacterial endotoxin lipopolysaccharide (LPS). LPS has been reported to induce nuclear factor-κB (NFκB) signaling through Toll-like-receptors (TRLs) in macrophages (13) and (pre)adipocytes (14, 15) and has been linked to insulin resistance. However, the mechanism by which LPS induces inflammation and insulin resistance in human WAT is less clear.

To this end, we tested the hypothesis that LPS induces proinflammatory cytokine/chemokine expression predominantly in nonadipocytes in our cultures, which subsequently triggers insulin resistance in adipocytes. We found that cytokine/chemokine expression was predominantly in the nonadipocyte fraction, which were primarily preadipocytes based on marker analyses. We also demonstrated that LPS-stimulated endotoxemia activated proinflammatory cytokine/chemokine production via NFκB and MAPK signaling, predominantly in preadipocytes, and decreased peroxisome proliferator activated receptor (PPAR)-γ activity and insulin responsiveness in adipocytes. These data demonstrate that human preadipocytes play a pivotal role in the development of insulin resistance in human adipocytes via increasing proinflammatory cytokine/chemokine expression involving NFκB and MAPK signaling.

Materials and Methods

Materials

All cell cultureware and scintillation cocktail (ScintiSafe) were purchased from Fisher Scientific (Norcross, GA). Fetal bovine serum (FBS) was purchased from Cambrex/BioWhittaker (Walkersville, MD). Monoclonal antibody for CD68 was purchased from Research Diagnostics Inc. (Boston, MA), and CD11b/MAC-1 was purchased from BD PharMingen (San Jose, CA). Rodamine red and fluorescein isothiocyanate-conjugated IgGs were purchased from Jackson ImmunoResearch (West Grove, GA). Gene-specific primers for real-time PCR were purchased from IDT (Coralville, IA). 6-Dimethylaminopurine (DMAP) and MG132 were purchased from Calbiochem (San Diego, CA). LY294002, U0126, and primary antibodies for p-inhibitory-κB (IκBα) kinase (IKK)-α/β (Ser 180/ser181, rabbit), p-stress-activated protein kinase/Jun-N-terminal kinase (JNK) (Thr183/Tyr185, mouse), p-AKT (protein kinase B) (Ser473, rabbit), p-ERK-1/2 (Thr 202/Tyr204 rabbit) were purchased from Cell Signaling Technology (Beverly, MA). Preadipocyte factor-1 (Pref-1/Dlk1) monoclonal antibody was obtained from R&D Systems (Minneapolis, MN). All other chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO), unless otherwise stated.

Cell cultures

Abdominal adipose tissue was obtained from females with a body mass index (BMI) of 30 or less during elective surgery with approval from the Institutional Review Board at University of North Carolina-Greensboro. SV cells were isolated and cultured as previously described with minor modification (10). To induce approximately 50% differentiation (*e.g.* 50% of cells containing visible lipid droplets), confluent cultures of SV cells were supplemented with differentiation media-1 [DM1; 97% DMEM/Ham F-12, 3% FBS, 1 μM rosiglitazone, 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, 33 μM biotin, 17 μM panthothenate, 100 nM insulin] for the first 3 d (referred to as DM1 in Fig. 1 or AD50 in the other figures). To obtain maximum differentiation, cultures of SV cells were exposed to DM1 for 6 d, which resulted in approximately 90% differentiation (AD90). To generate cultures that did not differentiate into adipocytes (referred to as −DM1 in Fig. 1 or AD0 in other figures), cultures of SV cells were supplemented with adipocyte media (AM1; 97% DMEM/Ham F-12, 3% FBS, 1 μM dexamethasone, 33 μM biotin, 17 μM panthothenate, 100 nM insulin) beginning on d 1 of differentiation until the assays were performed.

For the coculture experiment (see Fig. 4C), SV cells were initially seeded as a monoculture in individual cell culture inserts (Falcon 0.4 μm pores catalog no. 3090; Fisher Scientific) and suspended in six-well Multiwell plates containing AM1 for 12 d (AD0). On d 14, inserts containing the AD0 cultures were transferred to six-well Multiwell plates and cocultured above AD50 cultures for 8 h, thereby allowing the AD0 and AD50 cultures to communicate with one another by sharing the same media during treatment with LPS. For the positive controls of macrophages, the U937 human monocyte line (CRL1593; American Type Culture Collection, Manassas, VA) was used. U937 cells were supplemented with RPMI 1640 media containing 10% FBS and induced to differentiation by adding 10 nM phorbol 12-myristate 13-acetate for 72 h.

Fractionation of SV cells and adipocytes using density gradient

For fractionation of lipid-laden adipocytes from the nondifferentiated SV cells, cultures grown in 100-mm plates (~3 million cells) were washed with Hanks' balanced salt solution (HBSS)/0.5 mM EDTA and trypsinized with trypsin-like enzyme at 37 C. Cells were layered onto the 6% iodixanol (Optiprep; Axis-Shield, Oslo, Norway; ~1.03 g/ml) in 0.5% BSA/HBSS in a 15-ml centrifuge tube and centrifuged at 650 × *g* for 20 min at 4 C. SV cells were collected from the pellet. The floating adipocytes were harvested from the top and delivered to microfuge tubes. To remove SV cell contamination and dead cell debris, adipocytes were resuspended with ice-cold HBSS and centrifuged at 5000 × *g* for 5 min. Adipocytes were collected from the top of the microfuge tube in which fat cells formed a fat film. TriReagent (Molecular Research Center Inc., Cincinnati, OH) was added to each fraction for RNA extraction.

Immunostaining

Cells were cultured on coverslips for immunofluorescence microscopy and stained as described previously (10). For double staining of Pref-1 and adipose tissue fatty acid binding protein (aP2), coverslips were first incubated with mouse-anti Pref-1 (1:10) overnight and stained with fluorescein isothiocyanate-conjugated secondary antibody (1:500). Then coverslips were blocked again and incubated with rabbit-anti aP2 for 2 h and stained with Rodamine red-conjugated secondary antibodies (1:500). For MAC-1 and CD68 immunostaining, 1:10 diluted antibodies were incubated overnight at 4 C. Fluorescent images were captured with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI) mounted on a BX60 fluorescence microscope (Olympus, Tokyo, Japan).

Immunoblotting and 4 M urea-SDS-PAGE

Immunoblotting was conducted as we previously described (10) using NuPage precasted gels (Invitrogen, Carlsbad, CA). To resolve PPARγ phosphoproteins, total cell extracts (75 μg protein) were subjected to 10% SDS-PAGE [acrylamide to bisacrylamide 100:1 (wt/wt)] containing 4 M urea and electrophoresis at 80 V for 20 h. Separated proteins were subsequently transferred to polyvinyl difluoride membranes and immunoblotted with a monoclonal PPARγ antibody (Santa Cruz Inc., Santa Cruz, CA). The abundance of PPARγ was quantified from exposed x-ray film using the Kodak image station 440 (Eastman Kodak Co., Rochester, NY).

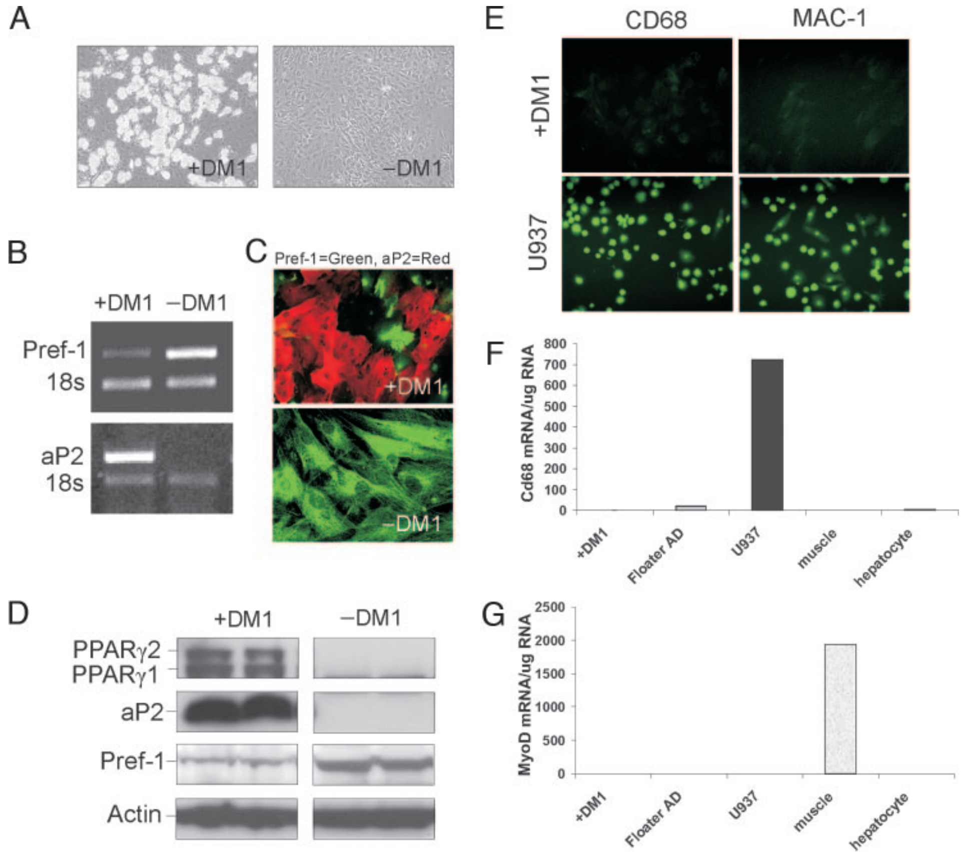

FIG. 1. Primary cultures of newly differentiated human adipocytes are composed of adipocytes and preadipocytes. SV cells were isolated from human sc adipose tissue. Confluent SV cells were either induced to differentiation (+DM1) or kept in adipocyte media (−DM1) for 12 d. A, Morphological changes obtained using phase contrast microscopy (×10). B, Gene expression of Pref-1 and aP2 using RT-PCR. C, Immunolocalization of Pref-1 (*green*) and aP2 (*red*). D, Protein expression of PPARγ, aP2, Pref-1, and actin using Western blotting. E, Differentiated cultures of human adipocytes (+DM1) were immunostained with the macrophage-specific markers CD68 or MAC-1. Differentiated U937 cells (human macrophage cell line) were used as positive controls. F, Gene expression profiles of CD68 from RNA from differentiated cultures (+DM1), compared with RNA from freshly isolated, floating human adipocytes (Floater AD), differentiated human U937 cells (U937), human muscle, and human primary hepatocytes by real-time qPCR, G, Gene expression profiles of the muscle marker MyoD in differentiated cultures (+DM1) were compared with RNA from the cultures or tissues described in F. Data are representative of at least two independent experiments in all panels. Data in F and G were obtained using RNA from a single subject.

[2-^{3}H deoxyglucose] uptake

Basal and insulin-stimulated glucose uptakes were measured as we described previously (11).

RNA isolation and PCR

Total RNA was isolated from the cultures using TriReagent according to manufacturer's protocol for RT-PCR. Total RNA (0.5 μg) from each RNA sample was used with the One-Step RT-PCR kit (QIAGEN, Valencia, CA). Primer sets for aP2 were previously described (42). Primer sequences for Pref-1 (accession no. NM_003836) were forward (5′-TACGAGTGTCTGTGCAAGC), reverse (5′-ACACAAGAGATAGCGAACACC) and running conditions were 37 cycles of 95 C for 30 sec, 56 C for 30 sec, and 72 C for 30 sec.

For real time quantitative PCR (qPCR), 1 μg total RNA was converted into first-strand cDNA using Omniscript RT kit (QIAGEN). qPCR was performed in a Smartcycler (Cepheid, Sunnyvale, CA) using the QuantiTect SYBR Green PCR kit (QIAGEN) for 40 cycles. To account for possible variation related to cDNA input amounts or the presence of PCR inhibitors, the endogenous reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was simultaneously quantified in a separate tube for each sample. Initial real-time amplifications were examined by agarose gel electrophoresis to confirm the sizes of the products. After PCR amplification, a melting curve was generated for every PCR product to check the specificity of the PCR. Primer sequences and running conditions are summarized in Table 1.

Transient transfection and PPAR activity

For measuring PPARγ activity, primary human adipocytes were transiently transfected with the PPAR-responsive luciferase reporter construct pTK-PPRE3x-luc (16) using the Amaxa Nucleofactor (Amaxa, Cologne, Germany) according to the manufacturer's protocol. On d 6 of differentiation, 1×10^6 cells from a 60-mm plate were trypsinized and resuspended in 100 μl of nucleofector solution (Amaxa) and mixed with 2 μg of pTK-PPRE3x-luc and 25 ng pRL-CMV for each sample. Electroporation was performed using the V-33 nucleofector program (Amaxa). Cells were replated in 96-well plates after 10 min recovery in calcium-free RPMI 1640 media. Two hours later, cultures were supplemented with charcoal-stripped AM1. LPS stimulation was performed 20 h after transfection for 3 h. Firefly luciferase activity was measured using the Dual-Glo luciferase kit (Promega, Madison, WI) and normalized to *Renilla* luciferase activity from the cotransfected control pRL-CMV vector. All luciferase data are presented as a ratio of firefly luciferase to *Renilla* luciferase activity.

Statistical analysis

Unless otherwise indicated, data are expressed as the mean ± SEM (n = 3–8) using a pool of cells obtained from three to five different human subjects. Data were analyzed using one-way ANOVA, followed by Student's t tests for each pair for multiple comparisons. Differences were considered significant if $P < 0.05$. All analyses were performed using JMP IN 4.04 software (SAS Institute, Cary, NC).

Results

Primary cultures of newly differentiated adipocytes contain preadipocytes and adipocytes

Our normal differentiation protocol using DM1 for the first 3 d of differentiation resulted in a cell population on d 12 containing approximately 50% adipocytes and approximately 50% nonadipocytes (cells without visible lipid droplets). Based on our findings that the nonadipocyte fraction of our cultures robustly express and/or secrete cytokines (*e.g.* IL-6, TNF-α) and chemokines (*e.g.* IL-8) in response to *trans*-10, *cis*-12 CLA treatment (10, 11), we wanted to know whether preadipocytes were present in this nonadipocyte or SV fraction. To answer this question, we first cultured the

TABLE 1. List of human gene-specific primers for qPCR

Target mRNA	Sense/Antisense primers	GeneBank accession no.	Annealing temperature (C)	Size (bp)
AEBP-1	F-atg ggt gat gta cac caa cgg cta R-agt ggg tag atg cgg atg aaa cga	NM_001129	61	123
aP2	F- cct ggt aca tgt gca gaa at R, aga gtt caa tgc gaa ctt ca	NM_001442	56	169
CD68	F, gct ggc tgt gct ttt ctc g R, gtc acc gtg aag gat ggc a	NM_001251	60	111
MAC-1	F, act tgc agt gag aac acg tat g R, aga gcc atc aat caa gaa ggc	NM_000632	58	141
MyoD	F, cgg cgg aac tgc tac gaa g R, gcg act cag aag gca cgt c	NM_002478	60	172
IL-6	F, aaa tgc cag cct gct gac gaa R, aac aac aat ctg agg tgc cca tgc tac	NM_000600	63	150
IL-8	F, gaa tgg gtt tgc tag aat gtg ata R, cag act agg gtt gcc aga ttt aac	NM_000584	60	129
IL-1β	F, cgc caa tga ctc aga gga aga R, agg gcg tca ttc agg atg aa	NM_000576	60	144
TNFα	F, tct tct cga acc ccg agt ga R, cct ctg atg gca cca cca g	NM_000592	60	151
COX-2	F, agt ccc tga gca tct acg gtt t R, ccc att cag gat gct cct gtt	NM_00963	58	123
APM-1	F, gca gag atg gca ccc ctg R, ggt ttc acc gat gtc tcc ct	NM_004797	60	80
GAPDH	F, gag aag gct ggg gct cat R, tgc tga tga tct tga ggc tg	NM_002046	59	130
MCP-1	F, tgt ccc aaa gaa gct gtg atc t R, gga atc ctg aac cca ctt ctg	NM_002982	58	84
TLR2	F, ggc cag cca att acc tgt gtg R, agg cgg aca tcc tga acc t	NM_003264	52	68
TLR4	F, aag ccg aaa ggt gat tgt tg R, ctg agc agg gtc ttc tcc ac	NM_138554	58	153

R, Reverse; F, forward.

cells in the absence or presence of DM1 for the first 3 d of differentiation, followed by 9 d of exposure to AM1 used to maintain the adipocyte phenotype. As shown in Fig. 1A, approximately 50% of the cells were differentiated into lipid-containing adipocytes by d 12 when exposed to DM1. In contrast, cells not supplemented with DM1 had few lipid-containing cells. Expression analyses and/or immunolocalization of gene markers associated with preadipocytes (Pref-1, a trans-membrane protein containing an epidermal growth factor-like domain exclusively expressed in preadipocytes) and adipocytes (*e.g.* aP2, PPARγ) on d 12 revealed that cultures receiving DM1 had lower mRNA (Fig. 1B) and protein (Fig. 1, C and D) levels for Pref-1 and higher mRNA (Fig. 1B) and protein (Fig. 1, C and D) levels of aP2 and PPARγ, compared with cultures not receiving DM1. Taken together, these data demonstrate that cultures exposed to DM1 for 3 d and then AM1 for 9 more days contain both adipocytes and preadipocytes. In contrast, cultures receiving only AM1 for 12 d contained primarily preadipocytes.

Primary cultures of newly differentiated adipocytes do not express markers of macrophages or myocytes

Next, we wanted to determine which cell types (other than preadipocytes) with the potential to produce cytokines/chemokines were present in our cultures. To answer this question, we measured the expression and/or localization of markers of human macrophages (*e.g.* CD68, MAC-1) and myocytes (*e.g.* MyoD), cells known to secrete cytokines/chemokines, in our differentiated cultures (+DM1). We measured CD68/MAC-1 and MyoD in differentiated human macrophages (U937 cells) and RNA obtained from muscle as positive controls for macrophages and myocytes, respectively. We measured CD68 and MyoD in RNA from muscle and hepatocytes (generously provided by Zen Bio Inc., Research Triangle Park, NC) as negative controls for macrophages and myocytes, respectively. Very little mRNA or protein for CD68 (Fig. 1, E and F) or MAC-1 (Fig. 1E) were detected in our newly differentiated cultures of human adipocytes. mRNA levels of the myocyte marker MyoD were not detectable in our differentiated cultures (Fig. 1G). Interestingly, RNA obtained from freshly isolated floating adipocytes (generously provided by Zen Bio Inc.) expressed significant amounts of mRNA for CD68, suggesting the presence of monocytes or lipid-laden macrophages in this fraction (Fig. 1F). Collectively, these data suggest that our cultures of newly differentiated adipocytes contain negligible amounts of macrophages or myocytes.

Preadipocytes play an essential role in LPS-induced cytokine gene expression and insulin resistance

To determine the capacity of preadipocytes and adipocytes in our differentiated cultures to express cytokine/chemokine genes (and secrete cytokines/chemokines) reported to cause insulin resistance, we first developed a procedure to separate SV cells (preadipocytes) from adipocytes obtained from our differentiated cultures (Fig. 2A). Next, we treated the cultures with LPS; separated the SV cells from the adipocytes; and measured the mRNA levels for several cyto-

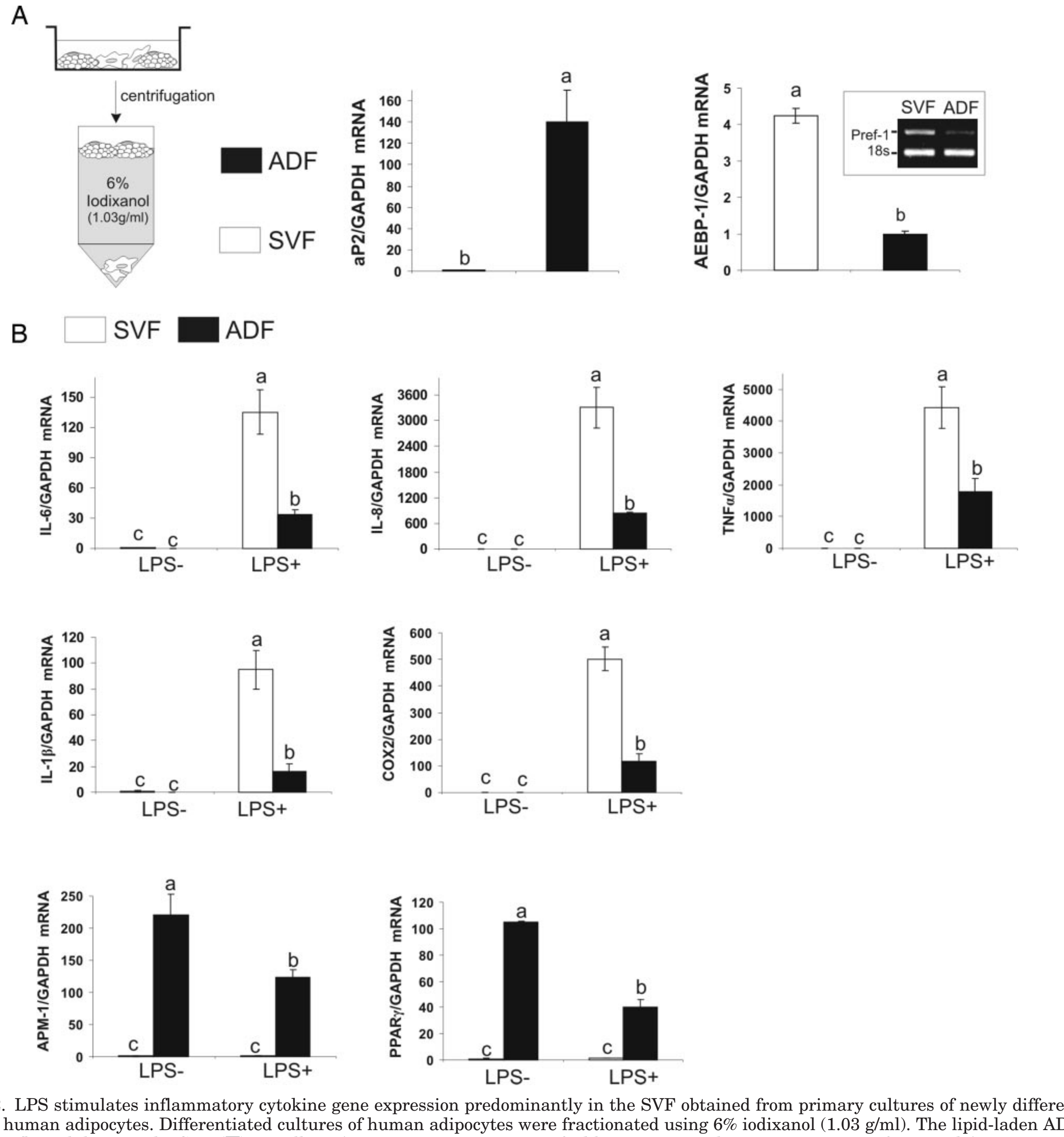

FIG. 2. LPS stimulates inflammatory cytokine gene expression predominantly in the SVF obtained from primary cultures of newly differentiated human adipocytes. Differentiated cultures of human adipocytes were fractionated using 6% iodixanol (1.03 g/ml). The lipid-laden ADF (■) was floated, leaving the SVF (□) as pellets. A, Fractionations were verified by measuring the gene expression of aP2 and AEBP-1 using real-time qPCR and Pref-1 using RT-PCR. B, Cultures of differentiated human adipocytes (d 14) were incubated in the presence or absence of LPS (10 ng/ml) for 3 h before fractionation. Relative mRNA expression of IL-6, IL-8, TNF-α, IL-1β, COX-2, APM-1, and PPARγ were investigated using qPCR. Means (± SEM, n = 4) not sharing a *common superscript* differ significantly ($P < 0.05$).

kines, preadipocyte markers, and adipogenic genes in these two fractions (Fig. 2B). As shown in Fig. 2A, our fractionation procedure using 6% Iodixanol yielded an SV fraction (SVF) in the pellet containing cells with little mRNA for the adipocyte marker aP2 and significantly more mRNA for the preadipocyte markers adipocyte-enhancer binding protein (AEBP-1) and Pref-1, compared with the buoyant adipocyte fraction (ADF), which had more aP2 and less AEBP-1 and Pref-1. LPS robustly induced the expression of IL-6, IL-8, TNF-α, IL-1β, and cyclooxygenase (COX)-2, genes positively associated with inflammation and NFκB activation, in the SVF, compared with the ADF (Fig. 2B). Conversely, the expression levels of adiponectin (APM-1) and PPARγ, almost exclusively expressed in the ADF, were attenuated by LPS treatment. These data demonstrate the capacity of preadipocytes to generate inflammatory signals and their association with the suppression of markers of insulin sensitivity in human adipocytes.

To further investigate the role of preadipocytes in inflammation, we established three human (pre)adipocyte models by manipulating the duration and exposure to DM1. Using this protocol, cultures on d 14 had 0, 50, or 90% adipocytes (Fig. 3A). LPS-stimulated TNF-α, IL-6, and IL-8 expression decreased as the degree of differentiation increased to 90% (Fig. 3B). A similar trend was observed for TNF-α and IL-6 mRNA levels under basal conditions.

The capacity of preadipocytes to recruit monocytes was examined in the same cultures by measuring the mRNA levels of MCP-1, a chemokine associated with monocyte recruitment and inflammation. LPS robustly induced MCP-1 gene expression in cultures containing exclusively preadipocytes (AD0) but decreased as the degree of differentiation increased (Fig. 3C). These data provide further support for the hypothesis that preadipocytes are important inducers of inflammation in primary cultures of newly differentiated human adipocytes. In addition, these data also suggest that preadipocytes have the capacity to initiate macrophage infiltration into adipose tissue given their ability to express MCP-1.

Given the role of TLRs in mediating inflammation induced by LPS, the relative mRNA levels of TLR4 and TLR2 were determined in our cultures (Fig. 3D). In the absence of LPS, AD0 (preadipocyte) cultures expressed approximately 3.0 and approximately 1.9 times more TLR4 and TLR2 mRNA, respectively, compared with the AD90 (adipocyte) cultures. Whereas LPS stimulation had only a marginal impact on TRL4 expression, TLR2 mRNA levels were robustly increased by LPS. The expression of both TLR4 and TLR2 decreased as the degree of differentiation increased, consistent with the proinflammatory capacity of preadipocytes demonstrated in Fig. 3, B and C.

To determine the impact of LPS-induced cytokine production in preadipocytes on insulin responsiveness in adipocytes, we measured [2-^{3}H] deoxyglucose uptake in our three models (Fig. 4). As expected, AD0 cultures, which are primarily preadipocytes, showed a blunted response to insulin-stimulated glucose uptake (Fig. 4, A and B). However, even this small increase in insulin-stimulated glucose uptake

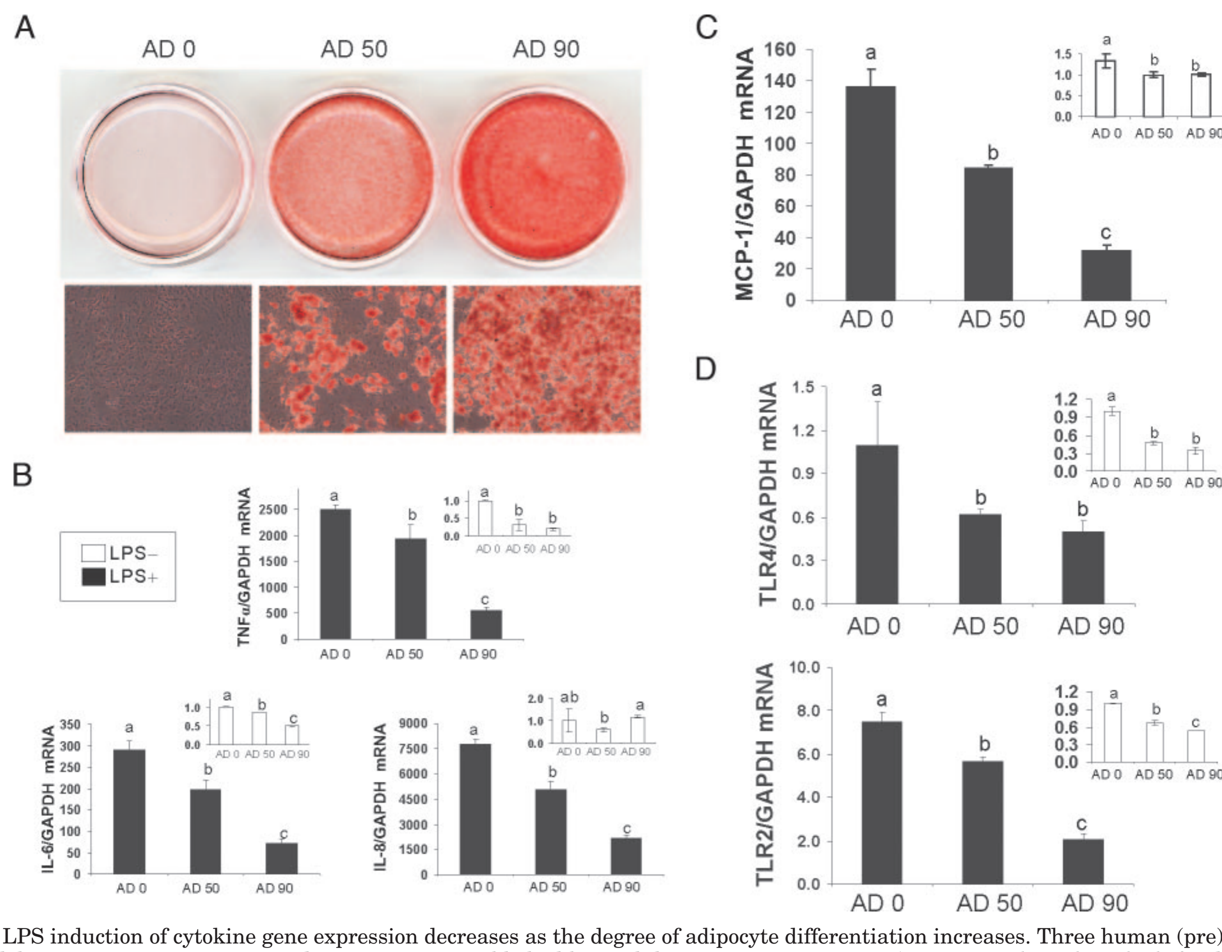

FIG. 3. LPS induction of cytokine gene expression decreases as the degree of adipocyte differentiation increases. Three human (pre)adipocyte cell models containing 0, 50, or 90% adipocytes were established by modulating exposure to DM1 (see *Materials and Methods* for details). A, On d 14, each culture was stained with oil-red-O to show neutral lipid accumulation and are representative of two independent experiments. B–D, On d 14, cultures of human (pre)adipocytes were incubated in the absence (□) or presence (■) with LPS for 3 h and total RNA was harvested for the mRNA analyses of TNF-α, IL-6, and IL-8 (B), MCP-1 (C), or TLR4 and TLR2 (D) using qPCR in each cell model. Data in B–D are normalized to the basal (−LPS) gene expression level of AD0. Means (± SEM, n = 4) not sharing a *common superscript* differ significantly ($P <$ 0.05).

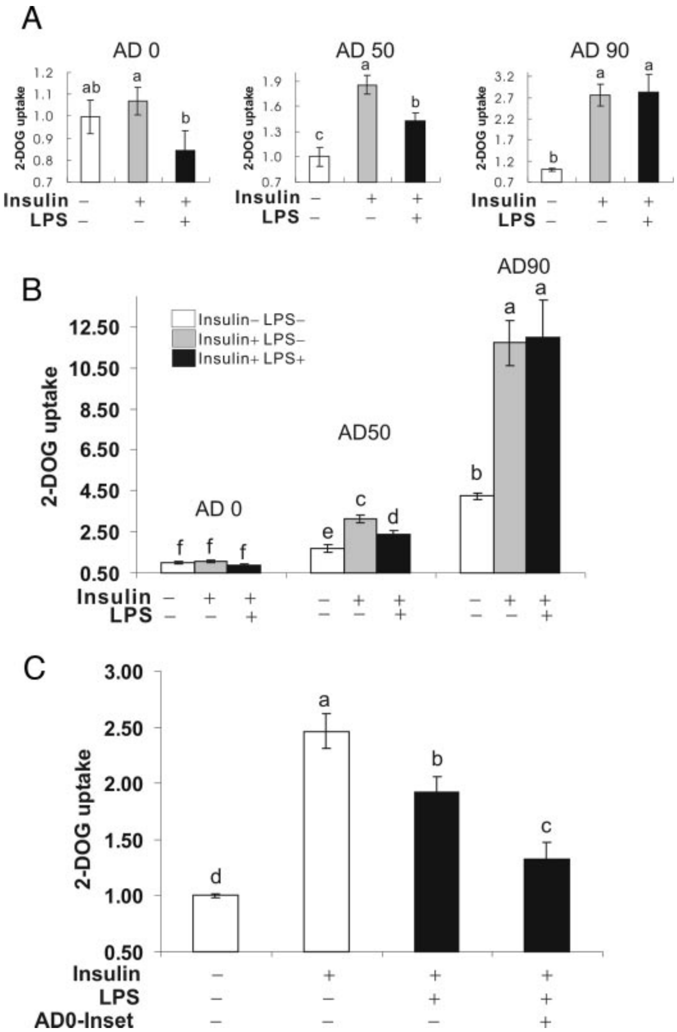

FIG. 4. LPS suppression of insulin-stimulated glucose uptake and adipogenic gene expression decreases as the degree of adipocyte differentiation increases. Three human (pre)adipocyte cell models containing 0, 50, or 90% adipocytes were established by modulating exposure to DM1 (see *Materials and Methods* for details). A, Cultures were incubated on d 12 with low glucose media (1000 mg/ml) for an additional 48 h and stimulated with LPS for 8 h. Basal and insulin (100 nM)-stimulated glucose uptake of 4 nmol [2-^{3}H]deoxyglucose (2-DOG) were measured for 90 min. Data are normalized to the basal glucose uptake level (−Insulin, −LPS) in each cell model. B, Data from A were normalized to the AD0 basal glucose uptake level to demonstrate the differences in absolute amounts of glucose uptake in each model. C, Cultures were grown and treated as in A with the following exceptions. Preadipocytes (AD0) were grown independently on inserts (AD0-inserts) for 12 d and then suspended above the AD50 cultures during the 8 h of LPS treatment. Means ($\pm$ SEM, n = 4) not sharing a common superscript differ significantly ($P < 0.05$).

was attenuated by LPS. In AD50 cultures, insulin's stimulation of glucose uptake was suppressed approximately 30% by LPS (Fig. 4A), which was consistent with its attenuation of adiponectin gene expression shown in Fig. 2B. Intriguingly, in our AD90 model in which insulin-stimulated glucose uptake was 3-fold higher than in the AD50 model (Fig. 4B), LPS had no adverse effect on glucose uptake (Fig. 4A). Consistent with these data, coculturing preadipocytes (AD0) using inserts (AD0-insert) with the AD50 cultures suppressed LPS-mediated glucose uptake by another 30%, compared with LPS-treated cultures without inserts (Fig. 4C). Collectively, these data demonstrate that preadipocytes are required for LPS suppression of insulin-stimulated glucose uptake and suggest that proinflammatory cytokines originating in preadipocytes mediate insulin resistance in adipocytes.

LPS decreases the activity and increases the phosphorylation of PPARγ

LPS suppression of adipogenic gene expression (Fig. 2B) and insulin-stimulated glucose uptake (Fig. 4) suggested that LPS may decrease the activity of PPARγ, which is essential for insulin-stimulated glucose uptake and triglyceride (TG) synthesis in adipocytes. To answer this question, basal and ligand-induced activation of PPARγ activity were measured in AD50 cultures transfected transiently with a luciferase reporter construct containing a multimerized peroxisome proliferator-responsive element (PPRE). We consistently obtained approximately 65% transfection efficiency revealed by parallel transfections with a green fluorescent protein reporter construct (data not shown). Both adipocytes and preadipocytes were equally transfectable using this protocol, based on aP2 immunostaining and 4′,6′-diamino-2-phenylindole nuclear staining. Although basal levels of PPARγ activity were not affected by LPS, it decreased rosiglitazone (BRL49653)-stimulated PPARγ activity in a dose-dependent manner (Fig. 5A).

To determine the extent to which LPS decreased PPARγ activity by increasing PPARγ phosphorylation, AD50 cultures were treated with and without LPS for 3 h, and the isolated cell proteins were separated by SDS-PAGE-urea gel electrophoresis to detect band shifts in PPARγ. As seen in Fig. 5B, LPS caused a band shift of PPARγ 1 and 2, which was attenuated by treatment with alkaline phosphatase. These data indicate that LPS may decrease the activity of PPARγ by increasing its degree of phosphorylation, and suggest a mechanism by which LPS impairs insulin responsiveness. However, the upstream signaling mechanism linking LPS-induced cytokine production in preadipocytes to decreased PPARγ activity and insulin sensitivity in adipocytes remains unknown.

LPS-induced cytokine/chemokine gene expression depends on NFκB signaling in preadipocytes

Although well characterized in macrophages, the regulation of NFκB activation and signaling in adipose tissue is less clear. Berg *et al.* (15) reported altered NFκB sensitivity to LPS-induced signaling in adipocytes during differentiation using the murine 3T3-L1 cell line. However, NFκB sensitivity to LPS in primary cultures of human adipocytes has not yet been established. To determine the extent to which preadipocytes and adipocytes contribute to NFκB and MAPK activation and subsequent cytokine/chemokine expression in the mixed cultures (AD50), we measured protein phosphorylation kinetics associated with NFκB and MAPK (*e.g.* JNK, ERK1/2) signaling in AD0, AD50, and AD90 cultures treated with LPS. Consistent with the inflammatory cytokine expression profile in Fig. 3B, the degree to which LPS induced

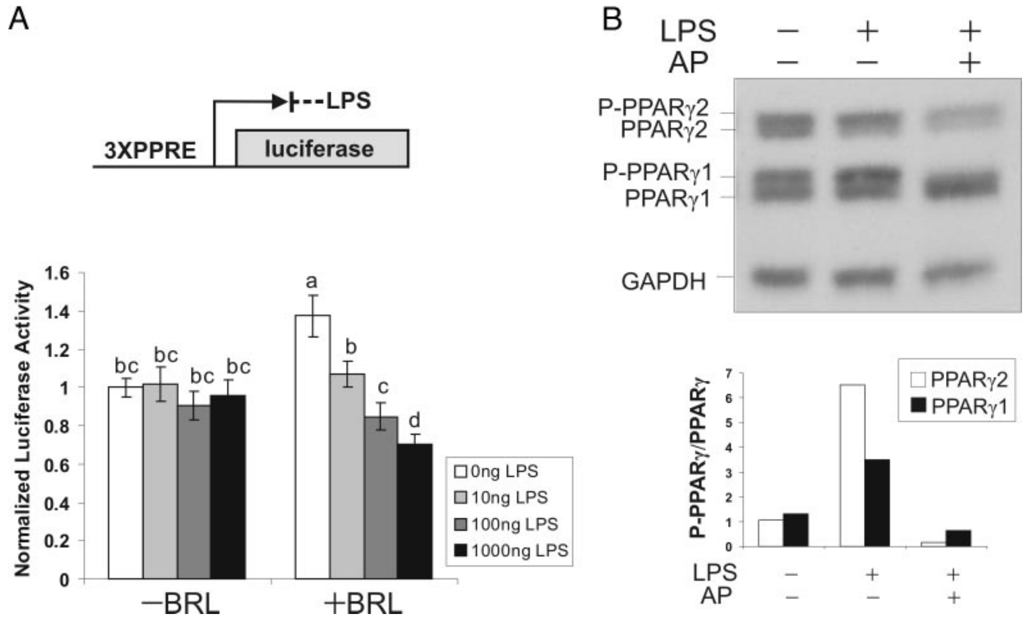

FIG. 5. LPS suppresses PPARγ activity and induces its phosphorylation. Cultures of human SV cells were induced to differentiate for 3 d and then kept in AM1 for 3 more days. On d 6 of differentiation, cultures were transfected using Amaxa's Nucleofactor. A, Cultures were transiently transfected with PPRE reporter construct and stimulated with 0, 10, 100, and 1000 ng/ml LPS in the absence or presence of rosiglitazone (BRL49653; 0.1 μM) for 3 h before the luciferase assay. Means ($\pm$ SEM, n = 8) not sharing a *common superscript* differ significantly ($P < 0.05$). B, Cultures of human adipocytes (d 12–14) were stimulated with LPS (10 ng/ml) for 3 h. To remove phosphorylated protein, 75 μg total protein were incubated with alkaline phosphatase (AP; 20 U per 50 μl) for 30 min at 37 C and 15 min at 55 C and then subjected to 4 M urea-SDS-PAGE for the separation of phosphorylated (P) PPARγ bands. Blots were quantified by densitometry, and the ratio of P-PPARγ/PPARγ was expressed as a bar graph under each lane. B is a representative blot from two independent experiments.

the phosphorylation of IKK, JNK, and ERK, and the degradation of IκB decreased as the degree of differentiation increased from approximately 0 to 90% (Fig. 6). NFκB and MAPK activation reached its maximum after 1 h of LPS treatment in all three models, albeit at much lower levels in the AD50 and AD90 cultures, compared with the AD0 cultures. These data provide additional evidence that the presence of preadipocytes in cultures of human adipocytes modulates the susceptibility of LPS-induced NFκB and MAPK activation that trigger cytokine/chemokine production.

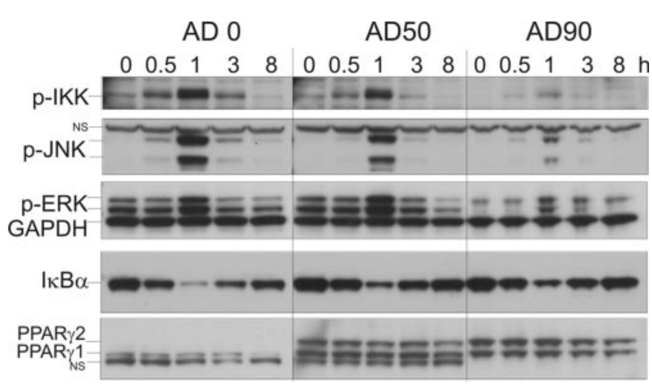

FIG. 6. LPS-induced NFκB activation decreases as the degree of differentiation increases. Three human (pre)adipocyte cell models containing 0, 50, or 90% adipocytes were established by modulating exposure to DM1 (see *Materials and Methods* for details). Each culture was stimulated with LPS (10 ng/ml) for 0, 0.5, 1, 3, or 8 h and 15 μg of total proteins used for immunoblotting. Transferred polyvinyl difluoride membranes were probed with antibodies targeting to phosphorylated (P)-IKK, P-JNK, P-ERK, IκBα, PPARγ, and GAPDH (loading control). These data are representative of two independent experiments.

Next, we determined the extent to NFκB and MAPK activation contributed to the LPS-inducted cytokine/chemokine expression using selective chemical inhibitors of NFκB and MAPK. LPS is known to act as an agonist for TLR4/2 in (pre)adipocytes (14), which triggers NFκB activation through MAPK and phosphatidyl inositol 3-kinase (PI3K)/AKT pathway (17, 18). As seen in Fig. 7A, the proteasome inhibitor MG132 abolished LPS-induced TNF-α gene expression, suggesting that proteasomal degradation of IκBα is crucial for NFκB activation and subsequent induction of TNF-α gene expression in human adipocytes. MG132 treatment also decreased the expression of IL-6 and IL-8, genes also regulated by NFκB, but not to the extent of TNF-α (Fig. 7, B and C). The MAPK kinase (MEK)/ERK inhibitor U0126, and the JNK inhibitor DMAP also attenuated LPS-induced cytokine production. The PI3K inhibitor LY-294002 blocked LPS-induced TNF-α gene expression by approximately 50% but had only minimal effects on IL-6 or IL-8. Collectively, these results suggest that NFκB and MAPK activation and signaling play an essential role in LPS-mediated proinflammatory cytokine/chemokine expression in preadipocytes and insulin resistance in human adipocytes.

Discussion

Characterization of nonadipocytes in the cultures

Adult WAT is composed of several cell types. Of the cells residing in WAT, mature adipocytes are by far the largest in size, but their abundance depends on the specific adipose

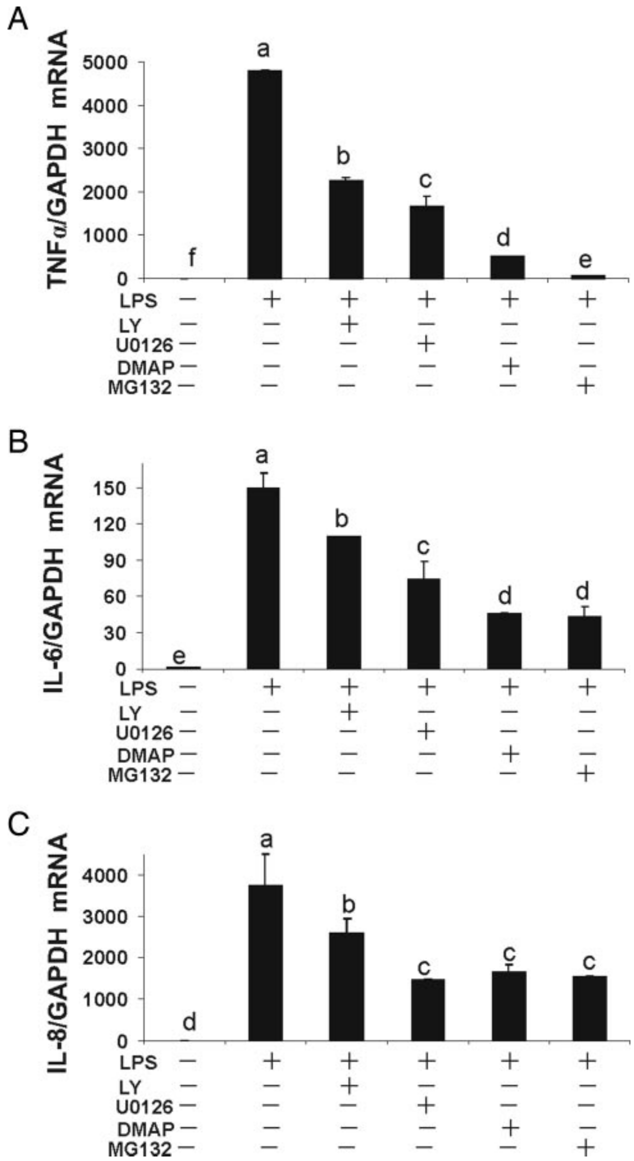

FIG. 7. Inhibitors of NFκB attenuate LPS induction of cytokine gene expression. Cultures of newly differentiated human adipocytes on d 14 were preincubated for 1 h in the absence or presence of the Akt inhibitor of LY-294002 (LY, 10 μM), the MEK-ERK inhibitor U0126 (10 μM), the JNK inhibitor DMAP (250 μM), or the proteasome inhibitor MG132 (10 μM) before LPS treatment (10 ng/ml, 3 h). Total RNA was harvested, and mRNA levels of TNF-α (A), IL-6 (B), and IL-8 (C) were analyzed using qPCR. Means (± SEM, n = 4) not sharing a *common superscript* differ significantly ($P < 0.05$).

depot and species. For example, Hauner (3) reported that human adipocytes represent approximately 50–70% of the cells in human WAT. In contrast, Fain *et al.* (4) reported that approximately 70% of the protein from human WAT digests is associated with tissue matrix, and the remaining 30% was equally divided between SV cells and floating adipocytes. Interestingly, nonadipocytes from human WAT, especially from the tissue matrix, accounted for approximately 90% of the cytokine secretion (4). Furthermore, the more robust cytokine secretion from WAT obtained from morbidly obese subjects (*e.g.* BMI = 45), compared with obese subjects (BMI = 32) was from the nonadipocytes. In contrast, Granneman *et al.* (19) reported that mature adipocytes account for only 16% of murine epididymal WAT cells. Thus, the relative abundance of adipocytes, compared with nonadipocytes, in human adult WAT has yet to be resolved, and the type and function of these nonadipocytes in human WAT are poorly understood.

Based on these gaps, we first characterized the cells in our cultures and then examined the role that the nonadipocytes play in inflammation and their impact on signaling, gene expression, and glucose metabolism in neighboring adipocytes. Initially, we characterized the cultures using DM1 during the first 3 d of differentiation (+DM1), compared with cultures not receiving DM1 (−DM1). Cultures receiving DM1 had about approximately 50% of their cells (Fig. 1A) filled with lipid. In contrast, few of the cells lacking DM1 had visible lipid droplets. As shown in Fig. 1, B–D, cultures receiving DM1 abundantly expressed markers of adipocytes (*e.g.* PPARγ, aP2) and lesser amounts of the preadipocyte marker Pref-1, compared with cultures lacking DM1. Thus, our differentiated cultures contain both adipocytes and preadipocytes.

To determine whether nonadipocytes other than preadipocytes were present in our cultures receiving DM1, we measured the expression levels of markers of macrophages and myocytes using CD68/MAC-1 and MyoD, respectively. As shown in Fig. 1, E–G, neither CD68/MAC-1 nor MyoD mRNA were detectable in our cultures. Thus, we could find no evidence that macrophages or myocytes are present in our cultures on d 12 of differentiation. Clearly this is not the situation *in vivo*, in which immune cells may be recruited to WAT (1, 8) and nonadipocytes in tissue matrix associated with adipose tissue robustly secrete cytokines (4). This discrepancy is most likely due to the loss of macrophages during growth and differentiation of the cultures *in vitro* or because our WAT is from nonobese individuals (*e.g.* BMIs ≤ 30.0). Even though our model fails to mimic cell populations perfectly in human WAT *in vivo* due to the lack of macrophages reported in obese rodents and humans, it provides a model for understanding the role that preadipocytes play in promoting inflammation. Human preadipocytes robustly express MCP-1 when challenged with LPS (Fig. 3C), suggesting that preadipocytes may initiate macrophage recruitment to adipocytes in WAT under inflammatory conditions. Thus, it is plausible that preadipocytes initiate monocyte attraction to adipocytes, resulting in differentiation and anchoring of macrophages to adipocytes due to the high level of expression of macrophage migration inhibitor factor-1 in human adipocytes (20).

To assess the role of nonadipocytes in inflammation and insulin resistance, cultures of newly differentiated human adipocytes (AD50) were initially fractionated after LPS stimulation and the expression of markers of preadipocytes (*e.g.* Pref-1 and AEBP-1) and adipocytes (*e.g.* aP2, APM-1, and PPARγ) and inflammatory cytokines (*e.g.* IL-6, IL-8, TNF-α, and IL-1β) and markers (*e.g.* COX-2) were measured (Fig. 2). These data are consistent with work by Harkins *et al.* (12) showing that murine preadipocytes produce cytokines in response to LPS to a much greater extent than adipocytes using the 3T3-L1 cell line. These data demonstrate that preadipocytes have a greater inflammatory response to LPS than adipocytes in our cultures and are associated with attenuated expression of adiponectin and PPARγ, two markers of insulin sensitivity. Using a second approach, we manipulated the degree of differentiation of the cultures (*e.g.* AD0, AD50, or AD90) before LPS treatment and then measured cytokine/chemokine expression (Fig. 3), glucose uptake (Fig. 4), PPARγ activity and phosphorylation (Fig. 5), and NFκB and MAPK activation (Fig. 6) and signaling (Fig. 7). Both of these approaches gave consistent results, demonstrating that the presence of preadipocytes in the cultures was positively associated with the degree of inflammatory gene expression, implicating preadipocytes as important sources of cytokines/chemokines that adversely affect PPARγ activity and insulin responsiveness involving NFκB and MAPK activation and signaling. Studies are underway to compare the relative abundance of cytokine/chemokine mRNAs from human macrophages, compared with human preadipocytes.

Role of PPARγ in inflammation and insulin resistance

Despite increasing evidence of the casual link between inflammation and insulin resistance, elucidating the precise mechanism by which cytokines/chemokines impair glucose uptake has proved difficult (reviewed in Refs. 21, 22). Our data highlight the importance of preadipocytes in mediating insulin resistance. One possible explanation for this observation is the suppression of adiponectin gene expression by LPS, which is exclusively secreted from adipocytes and positively associated with insulin sensitivity (23). In addition to its role in the modulation of glucose and lipid metabolism, adiponectin has been reported to have potent antisuppressive properties due to its ability to induce the production of antiinflammatory cytokines (*i.e.* IL-10), and inhibit proinflammatory cytokine production (reviewed in Ref. 24). Thus, it seems reasonable to presume that LPS attenuation of insulin responsiveness in AD50 model is due, at least in part, to the suppression of adiponectin expression. Consistent with this notion, LPS administration to cultures containing almost exclusively adipocytes (AD90) did not adversely affect insulin-stimulated glucose uptake (Fig. 4) or adiponectin gene expression (data not shown). Ajuwon and Spurlock (23) reported direct induction of PPARγ by adiponectin, coupled with suppression of NFκB activation, suggesting mutual transcriptional activation of PPARγ and adiponectin may determine adipocyte susceptibility to inflammatory stimuli.

The PPAR subfamily of nuclear receptors controls many different target genes involved in both lipid metabolism and glucose homeostasis. Loss-of-function PPARγ mutations in humans cause insulin resistance (25–27), and activation of PPARγ by thiazolidinediones act as insulin sensitizers (reviewed in Ref. 28). However, detailed mechanisms describing how inflammation suppresses PPARγ activity in human WAT are unclear. In our study, LPS suppressed ligand-induced, but not basal, PPARγ activity. Similarly, the PPARγ antagonist GW9662 decreased ligand-dependent, but not basal, PPARγ activity (our unpublished data), implicating that ligand-inducible PPARγ activity is critical in regulating insulin sensitivity.

One of the putative mechanisms modulating PPARγ activity is phosphorylation. It has been suggested that phosphorylation of PPARγ does one of the following: 1) impairs PPARγ affinity for its ligand (28, 29), 2) controls interactions between PPARs and corepressors and/or coactivators of transcription (30), or 3) alters PPARγ binding to the PPRE (reviewed in 31). ERK1/2 and JNK are two candidate transcription factors reported to phosphorylate PPARγ (32, 33). Thus, LPS-mediated impairment of insulin responsiveness (Fig. 4) and PPARγ activity (Fig. 5A) may be due to changes in PPARγ affinity for its ligand via phosphorylation, as suggested by data in Fig. 5B. We propose that posttranscriptional modification of PPARγ activity through phosphorylation may be one of the mechanisms by which cytokines affect the transcription of genes involved in glucose and lipid metabolism. However, other potential mechanisms (*i.e.* recruitment/dismissal of corepressors/activators or binding affinity to the PPRE) remain to be examined.

Role of NFκB and MAPK in inflammation and insulin resistance

In addition to controlling gene expression, PPARγ has been linked to NFκB regulation through physical interactions that block its transcriptional activity (34). Conversely, cytokine-induced NFκB activation suppresses PPARγ DNA binding (35). Consistent with these data, activation of NFκB (34, 36, 37) and MAPK (33, 38, 39) hinders PPARγ DNA binding affinity or transcriptional activation, providing a mechanism by which LPS-induced cytokine production suppresses PPARγ activity. The antiinflammatory role of PPARγ is also demonstrated in this work, showing that the more adipocytes in the culture, and consequently more PPARγ activity, the less robust NFκB signaling observed in Fig. 6. These data support recent findings showing that PPARγ mediates transcriptional repression of NFκB target gene expression (40). Also consistent with data from Berg *et al.* (15) comparing 3T3-L1 preadipocytes to mature adipocytes, LPS activation of NFκB was substantially attenuated in human adipocytes, compared with preadipocytes in our study. However, constitutive NFκB activation found in 3T3-L1 adipocytes was absent in our human adipocyte cultures (15).

The mechanism by which LPS signals to its downstream targets in adipocytes has yet to be clearly established. We showed in Fig. 3D that TLR4 mRNA appears to be constitutively expressed in both preadipocytes (AD0) and adipocytes (AD90). In contrast, TLR2 expression was robustly induced by LPS, particularly in preadipocytes, which could be partially responsible for the higher proinflammatory responsiveness of preadipocytes to LPS (Fig. 3C). In macrophages, LPS signals through TRL4 and TLR2. TRL4 and TLR2 activate at least two downstream pathways, PI3K/AKT and IL-1 receptor-associated kinase 1/TNF receptor-associated factor 6/NFκB-inducing kinase/IKK pathway, which depend on adaptor protein MyD88 (13). Additionally, Covert *et al.* (41) suggested that MyD88-independent, but interferon-regulatory factor 3-dependent, pathways are involved in LPS activation of NFκB. Based on their work in macrophages, we used specific inhibitors to block potential pathways involved LPS signaling. Consistent with these data, activation of the MAPK pathway, including ERK and JNK, and NFκB were critical for LPS-induced cytokine expression in our cultures.

PI3K/AKT pathway appeared to play a minor role judged by TNF-α gene expression, implicating IL-1 receptor-associated kinase 1/TNF receptor-associated factor 6/NFκB-inducing kinase pathway may be the major signaling pathway by which LPS induces cytokine synthesis in human (pre)adipocytes. However, the role of MyD88-indepenent pathways in this study was not examined.

Proposed model

Adipose tissue is a source of mediators of inflammation and insulin resistance. Factors suggested to cause obesity-induced inflammation and insulin resistance include dietary fatty acids (*i.e.* transfats and/or saturated fats), circulating free fatty acids, adipokines, and stress-induced hormones and/or viral/bacterial infection. Currently a chicken-or-egg debate is being waged concerning which factors associated with obesity initiate the inflammatory cascade that promote insulin resistance, *i.e.* do enlarged and/or inflamed adipocytes instigate inflammation that leads to insulin resistance, or do nonadipocytes (*e.g.* immune cells, preadipocytes) initiate the inflammatory cascade that promotes insulin resistance? In either case, we demonstrated by inducing acute inflammation with LPS that human preadipocytes, rather than adipocytes, are the primary source of LPS-induced proinflammatory cytokines/chemokines in these cultures that lack macrophages (Figs. 1–3). Clearly human preadipocytes transmit paracrine signals to neighboring adipocytes that suppress glucose uptake (Fig. 4) and PPARγ activity (Fig. 5) via NFκB and MAPK signaling (Figs. 6 and 7). Based on these data, we propose a working model in Fig. 8 in which LPS initiates proinflammatory signaling through TLRs primarily in preadipocytes, which triggers activation of NFκB, MAPK, and PI3K pathways resulting in cytokine (*i.e.* TNF-α, IL-6) and chemokine (*e.g.* IL-8, MCP-1) production in preadipocytes. These cytokines/chemokines, in turn, activate their cognate cell surface receptors on both adipocytes and preadipocytes, further augmenting cytokine production. In adipocytes, cytokine/chemokine activation of NFκB, MEK/ERK, and JNK leads to decreased PPARγ activity, possibly by increasing PPARγ phosphorylation, thereby attenuating PPARγ target gene expression and insulin-stimulated glucose uptake. We also speculate, given the robust LPS induction of MCP-1 in preadipocytes, that human preadipocytes are involved in the recruitment of monocytes to adipocytes, thereby augmenting the inflammatory cascade.

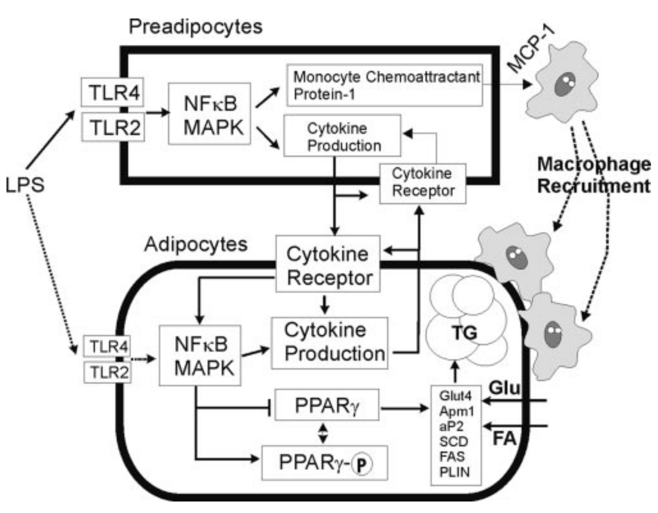

FIG. 8. Working model: LPS-induced inflammation in preadipocytes suppresses PPARγ activity and insulin responsiveness in adipocytes. Our results suggest that preadipocytes are more susceptible targets to LPS than adipocytes, increasing cytokine expression through the NFκB and MAPK pathway. In turn, secreted cytokines activate NFκB and MAPK signaling in adipocytes leading to suppression of the activity and target gene expression, *i.e.* insulin-dependent glucose transporter 4 (Glut4), APM-1, aP2, stearoyl-coA desaturase (SCD), fatty acid synthase (FAS), perilipin (PLIN), of PPARγ and insulin sensitivity. In addition, MCP-1 is induced by NFκB activation in preadipocytes, thereby recruiting monocytes/macrophages to infiltrate adipocytes, further enhancing inflammation and insulin resistance. FA, Fatty acid; TG, triglyceride.

Acknowledgments

We thank Nathan J. Provo and Amanda Troy for their technical help with the fractionation procedure for separating nonadipocytes from adipocytes. We appreciate the critical review of this manuscript by Drs. Susanne Mandrup (University of Southern Denmark) and Phil Pekala (East Carolina University, Greenville, NC). We are grateful for all of the helpful advice and thoughtful discussions with Dr. Ron Morrison (University of North Carolina at Greensboro).

Received April 21, 2006. Accepted July 18, 2006.

Address all correspondence and requests for reprints to: Michael K. McIntosh, Ph.D., R.D., Department of Nutrition, 318 Stone Building, P.O. Box 26170, University of North Carolina at Greensboro, Greensboro, North Carolina 27402-6170. E-mail: mkmcinto@uncg.edu.

This work was supported by grants from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Office of Dietary Supplements (R01DK063070) and the North Carolina Agriculture Research Service (06771) (to M.K.M.).

Disclosure statement: the authors have nothing to disclose.

References

1. **Wellen KE, Hotamisligil GS** 2003 Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 112:1785–1788
2. **Trayhurn P** 2005 Endocrine and signaling role of adipose tissue: new perspective on fat. Acta Physiol Scand 184:285–293
3. **Hauner H** 2005 Secretory factors from human adipose tissue and their functional role. Proc Nutr Soc 64:163–169
4. **Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW** 2004a Comparison of the release of cytokines by adipose tissue, adipose tissue matrix, and adipocytes form visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 145:2273–2282
5. **Fain JN, Bahouth SW, Madan AK** 2004b TNF-α release by the nonfat cells of human adipose tissue. Int J Obes 28:616–623
6. **Cousin B, Munoz O, Andre M** 1999 A role for preadipocytes as macrophage-like cells. FASEB J 13:305–312
7. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia, LA, Chen H** 2003 Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112: 1821–1830
8. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW** 2003 Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
9. **Charrière G, Cousin B, Arnaud E, André M, Bacou F, Pénicaud L, Casteilla L** 2003 Preadipocyte conversion to macrophage. J Biol Chem 278:9850–98555
10. **Brown JM, Boysen MS, Chung S, Fabiyi O, Morrison RF, Mandrup S, McIntosh MK** 2004 Conjugated linoleic acid induces human adipocyte delipidation: autocrine/paracrine regulation by MEK/ERK signaling by adipocytokines. J Biol Chem 279:26735–26747
11. **Chung S, Brown JM, Provo NJ, Hopkins R, McIntosh MK** 2005 Conjugated linoleic acid promotes human adipocyte insulin resistance through NFκB-dependent cytokine production. J Biol Chem 280:38445–38456
12. **Harkins JM, Moustaid-Moussa N, Chung Y, Penner KM, Pestka JJ, North CM, Claycombe KJ** 2004 Expression of interleukin-6 is greater in preadipocytes than in adipocytes of 3T3–L1 cells and C57BL/6J and ob/ob mice. J Nutr 134:2673–2677
13. **Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, Sizemore N, Hwang DH** 2003 Reciprocal modulation of toll-like receptor-4 signaling pathways involving

MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem 278:37041–37051
14. **Lin Y, Lee H, Berg AH, Lisanti MP, Shapiro L, Scherer PE** 2000 The lipopolysaccharide-activated toll-like receptor (TRL)-4 induces synthesis of the closely related receptor TRL-2 in adipocytes. J Biol Chem 275:24255–24263
15. **Berg AH, Lin Y, Lisanti MP, Scherer PE** 2004 Adipocyte differentiation induces dynamic changes in NFκB expression and activity. Am J Physiol Endocrinol Metab 287:E1178–E1188
16. **Kliewer S, Umesono K, Noonan D, Heyman R, Evans R** 1992 Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature 358:771–774
17. **Guha M, Mackman N** 2002 The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. J Biol Chem 277:32124–32132
18. **Fang H, Pengal RA, Cao X, Ganesan LP, Wewers MD, Marsh CB, Tridandapani S** 2004 Lipopolysaccharide-induced macrophage inflammatory response is regulated by SHIP. J Immunol 173:360–366
19. **Granneman JG, Li P, Lu Y, Tilak J** 2004 Seeing the trees in the forest: selective electroporation of adipocytes within adipose tissue. Am J Physiol Endocrinol Metab 287:E574–E582
20. **Skurk T, Herder C, Kraft I, Muller-Scholze S, Hauner H, Kolb H** 2005 Production and release of macrophage migration inhibitory factor from human adipocytes. Endocrinology 146:1006–1011
21. **Rajala M, Scherer PE** 2003 Minireview: the adipocyte-at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144: 3765–3773
22. **Wellen KE, Hotamisligil GS** 2005 Inflammation, stress and diabetes. J Clin Invest 115:1111–1119
23. **Ajuwon KM, Spurlock ME** 2005 Adiponectin inhibits LPS-induced NFκB activation and IL-6 production and increases PPARγ2 expression in adipocytes. Am J Physiol Regul Integr Comp Physiol 288:R1220–R1225
24. **Gil-Campos M, Cañete R, Gil A** 2004 Adiponectin, the missing link in insulin resistance and obesity. Clin Nutr 23:963–974
25. **Zhang J, Fu M, Cui T, Xiong C, Xu K, Zhong W, Xiao Y, Floyd D, Liang J, Li E, Song Q, Chen YE** 2004 Selective disruption of PPARγ2 impairs the development of adipose tissue and insulin sensitivity. Proc Natl Acad Sci USA 101:10703–10708
26. **Freedman BD, Lee E, Park Y, Jameson L** 2005 A dominant negative peroxisome proliferator-activated receptor γ knock-in mouse exhibits features of the metabolic syndrome. J Biol Chem 280:17118–17125
27. **Hegele RA** 2005 Lessons from human mutations in PPARγ. Int J Obes 29: S31–S35
28. **Yki-Jarvinen H** 2004 Thiazolidinediones. N Engl J Med 351:1106–1118
29. **Shao D, Rangwala SM, Bailey ST, Krakow SL, Reginato MJ, Lazar MA** 1998 Interdomain communication regulating ligand binding by PPAR-γ. Nature 396:377–380
30. **Guan H, Ishizuka T, Chui PC, Lehrke M, Lazar MA** 2005 Corepressors selectively control the transcriptional activity of PPARγ in adipocytes. Genes Dev 19:453–461
31. **Diradourian C, Girard J, Pegorier J** 2005 Phosphorylation of PPARs: from molecular characterization to physiological relevance. Biochemie 87:33–38
32. **Hu E, Kim JB, Sarraf P, Spiegelman BM** 1996 Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARγ. Science 274:2100–2103
33. **Adams M, Reginato M, Shao D, Lazar M, Chatterjee VK** 1997 Transcriptional activation by peroxisome proliferators-activated receptor γ is inhibited by phosphorylation at a consensus mitogen-activated protein kinase site. J Biol Chem 272:5128–5132
34. **Ruan H, Pownall H, Lodish H** 2003 Troglitazone antagonizes tumor necrosis factor-a induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NFκB. J Biol Chem 278:28181–28192
35. **Suzawa M, Takada I, Yanagisawa J, Ohtake F, Ogawa S, Yamauchi T, Kadowaki T, Takeudhi Y, Shibuya H, Gotoh Y, Matsumoto K, Kato S** 2003 Cytokines suppress adipogenesis and PPARγ function through the TAK1/TAB1/NIK cascade. Nat Cell Biol 5:224–230
36. **Ruan H, Miles PD, Ladd CM, Ross K, Golub TR, Olefsky JM, Lodish HF** 2002 Profiling gene transcription in vivo reveals adipose tissue as an intermediate target for tumor necrosis alpha: implications for insulin resistance. Diabetes 51:1376–3188
37. **Bailey ST, Ghosh S** 2005 'PPAR'ting ways with inflammation. Nat Immunol 6:966–967
38. **de Mora J, Porras A, Ahn N, Santos E** 1997 Mitogen-activated protein kinase activation is not necessary for, but antagonizes, 3T3-L1 adipocytic differentiation. Mol Cell Biol 17:6068–6075
39. **Camp H, Tafuri S** 1997 Regulation of peroxisome proliferator-activated receptor activity by mitogen activated protein kinase. J Biol Chem 272:10811–10816
40. **Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson, TM, Rosenfeld MG, Glass CK** 2005 A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ. Nature 437:759–763
41. **Covert MW, Leung TH, Gastpm JE, Baltimore D** 2005 Achieving stability of lipopolysaccharide-induced NFκB activation. Science 309:1854–1857
42. **Brown JM, Boysen MS, Jensen SS, Morrison RF, Storkson J, Lea-Currie R, Pariza M, Mandrup S, McIntosh MK** 2003 Isomer-specific regulation of metabolism and PPARγ signaling by CLA in human preadipocytes. J Lipid Res 44:1287–1300

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**