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Study of the Proinflammatory Role of Human Differentiated Omental Adipocytes

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ABSTRACT

Infiltration of monocyte-derived macrophages into adipose tissue has been associated with tissue and systemic inflammation. It has been suggested that macrophage infiltration affects fat expansion through a paracrine action on adipocyte differentiation. Our working hypothesis is that factors released by monocytes/macrophages may also affect mature adipocyte biology. Human differentiated omental adipocytes were incubated with LPS and conditioned media obtained from human macrophage-like cell line THP-1, previously activated or not with LPS. We show that LPS greatly increased the secretion levels of pro-inflammatory adipokines including IL-6, IL-8, GRO, and MCP-1. Macrophage-conditioned medium also upregulated IL-6, IL-8, GRO, and MCP-1 mRNA expression and protein levels and led to the novo secretion of ICAM-1, IL-1β, IP-10, MIP-1α, MIP-1β, VEGF, and TNFα. Human differentiated adipocytes treated by macrophage-conditioned medium displayed marked reduction of adipocyte function as assessed by decreased phosphorylation levels of ERK1, ERK2, and p38α and reduced gene expression of lipogenic markers including PPAR-γ and fatty acid synthase. These data show that macrophage-secreted factors not only inhibit the formation of mature adipocytes but alter their function, suggesting that human differentiated omental adipocytes might also contribute to systemic chronic low-grade inflammation associated with human obesity. J. Cell. Biochem. 107: 1107–1117, 2009.

KEY WORDS: ADIPOCYTOKINES; INFLAMMATION; OBESITY; OMENTAL ADIPOCYTES; MAPK

The increasing prevalence of obesity in recent decades is of particular concern given the various complications associated with this condition. Obesity is recognized as a major risk factor for insulin resistance, and both of these conditions predict the development of type 2 diabetes mellitus (T2DM) and cardiovascular disease [Miranda et al., 2005]. Growing evidence suggest a close association between a state of chronic, low-grade inflammation with insulin resistance, T2DM and cardiovascular diseases [Shoelson et al., 2007].

The adipose tissue is composed of various cell types including adipocytes, preadipocytes, tissue matrix, stromal-vascular cells and macrophages. The large number of macrophages that infiltrated human and rodent adipose tissue correlated positively with BMI, adipocyte size, total amount of body fat [Curat et al., 2004] and insulin resistance [Di Gregorio et al., 2005]. Macrophage infiltration and the expression of inflammatory-related genes in adipose tissue decrease in obese subjects that have lost weight [Cancello et al., 2005].

It is generally assumed that except for leptin and adiponectin, cytokine secretion in adipose tissue is due to non-adipocyte cells that are constituents of the stromal-vascular fraction, preadipocytes and macrophages [Fain et al., 2004a]. It has been widely suggested that adipokines may be involved in the progression of obesity and promote the development of related cardiovascular and metabolic diseases. Increased fat mass leads to dysregulation of adipocyte functions, including oversecretion of inflammatory cytokines and hyposecretion of beneficial ones, such as adiponectin [Guerre-Millo, 2004; Matsuzawa, 2006]. Moreover, the possibility of

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an active role for mature adipocytes in obesity-related inflammation has recently been raised [Lee et al., 2005] but remains to be established.

TNF-α and IL-6 are two extensively studied cytokines with presumed roles in obesity-associated disorders but their role in these disorders remains controversial [Carey et al., 2004]. IL-6 is often grouped together with TNF-α as a so-called "proinflammatory" cytokine and had been implicated as a paracrine regulator of adipose tissue [Sopasakis et al., 2004]. However, recent evidence suggest a wider spectrum of activities for IL-6, which under specific conditions is even anti-inflammatory and opposing TNF-α effects [Starkie et al., 2003; Carey and Febbraio, 2004; Kristiansen and Mandrup-Poulsen, 2005].

The identification of new adipokines, the secretion of which is altered in obese subjects, is a crucial step for understanding the pathogenesis of this disorder and its co-morbidities. So far, the influence of macrophages on human mature adipocytes has been scarcely focused. On the other hand, preferential accumulation of omental rather than subcutaneous fat appears to be stronger risk factor for the adverse health profile linked to obesity [Matsuzawa, 2006]. Yet adipokine secretion by omental fat is still poorly studied in humans.

The aim of the present study was to address the role of human omental mature adipocytes in obesity-related inflammatory conditions and to identify novel adipokines that potentially contribute to the pathogenesis of obesity complications. To this end, we carried out cytokine protein profiling of adipokines secreted by primary cultures of human differentiated omental adipocytes under different inflammatory conditions such us conditioned medium from human macrophage-like cell line and LPS. Representative data were confirmed by RT-PCR gene expression assays. We next examined whether the dysregulation caused by macrophage secreted factors and LPS resulted from impaired MAPK signaling and was related with the loss of adipocyte differentiation.

MATERIALS AND METHODS

MACROPHAGE CELL CULTURE, DIFFERENTIATION, AND STIMULATION

The human monocyte cell line THP-1 (ATCC) was cultured in RPMI 1640 medium containing 10% fetal bovine serum, 5 mM glucose, 2 mM L-glutamine, 50 μg/ml Gentamicin and 20 mM HEPES at 37°C in a humidified 5%CO_2/95°C air atmosphere. The mature macrophage-like state was induced by treating THP-1 cells (1.2×10^6 cells) with 0.162 μM phorbol 12-myristate 13-acetate (PMA, Sigma Chemical Co.) for 24 h. Differentiated, plastic-adherent cells were washed with cold Dulbecco's phosphate-buffered saline (D-PBS, Sigma Chemical Co.) and then incubated with fresh medium without PMA. Differentiated macrophages were treated with fresh medium and fresh medium containing 10 ng/ml LPS (Sigma Chemical Co.) for an additional 24 h. The medium was then collected and centrifuged at 400*g* for 5 min, diluted with OM-AM (Omental adipocyte medium, Zen-Bio, Inc.) (1:1, v/v) and used as macrophage conditioned media (MCM) and LPS-stimulated macrophage conditioned media (MCM LPS), respectively.

ADIPOCYTES CELL CULTURE, DIFFERENTIATION, AND STIMULATION

Human omental preadipocytes from six non-diabetic Caucasian male patients with BMI > 30 and age > 40 (Zen-Bio, Inc.) were cultured with OM-PM (omental preadipocytes medium, Zen-Bio, Inc.) in a humidified 37°C incubator with 5% CO_2. Twenty-four hours after plating, cells were checked for complete confluence and differentiated using OM-DM (omental differentiation medium, Zen-Bio, Inc.) following manufacturer's instructions. Two weeks after the initiation of differentiation, cells appeared rounded with large lipid droplets apparent in the cytoplasm. Cells were then considered mature adipocytes and were incubated with fresh media (control), fresh media containing 10 ng/ml LPS (LPS), macrophage-conditioned medium (MCM), and macrophage-conditioned medium from THP-1 cells previously treated with 10 ng/ml LPS (MCM LPS). In all treatments, RPMI fresh media was diluted with OM-AM (1:1, v/v) to achieve identical conditions. After 24 h, the supernatants were centrifuged at 400*g* for 5 min, the cells harvested, and pellets and supernatants were stored at −80°C for future analysis (Fig. 1).

CYTOKINE DETERMINATIONS IN CULTURE SUPERNATANTS

A commercially available Custom Human Cytokines Antibody Array for Bionova (Ray Biotech, Inc.) was used to determine the level of 20 cytokines: Adiponectin, E-Selectin (endothelial adhesion molecule 1), growth-regulated oncogene (GRO), herpes virus entry mediator (HVEM), intercellular adhesion molecule-1 (ICAM-1), interleukin-10 (IL-10), interleukin-1alpha (IL-1α), interleukin-1beta (IL-1β), interleukin-6 (IL-6), interleukin 8 (IL-8), interferon gamma inducible protein −10 kDa (IP-10), Leptin, lymphotoxin-like inducible protein that competes with glycoprotein D for herpes virus entry on T cells (LIGHT), monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor (MIF), macrophage inflammatory protein-1alpha (MIP-1α), macrophage inflammatory protein-1beta (MIP-1β), transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), and vascular endothelial growth factor (VEGF). The assay was followed accordingly to manufacturer instructions. In brief, membranes were blocked with a blocking buffer, and then were incubated with 1 ml of the culture supernatants at 4°C for overnight. The membranes were washed and incubated with 1 ml of primary biotin-conjugated antibody at room temperature for 2 h. After washing, 2 ml of HRP-conjugated streptavidin was added and incubated for 30 min at room temperature. The membranes were developed by using detection buffer, exposed to X-ray film, and processed by autoradiography. Detectable spots were scanned and analyzed for densitometry with Scion Image software.

MITOGEN-ACTIVATED PROTEIN KINASES PROFILE

The level of phosphorylation of nine mitogen-activated protein kinases (MAPK) and nine other serine/threonine kinases (ERK1, ERK2, JNK1, JNK2, JNK pan, p38α, p38β, p38γ, p38δ, RSK1, RSK2, GSK3α/β, GSK-3β, Akt1, Akt2, Akt3, Akt pan, MSK2, HSP27, and p70 S6 kinase) were determined in the different experimental conditions by commercially available Proteome Profiler antibody array (Human phospho-MAPK Array Kit, RD Systems), according to the manufacturer instructions. Briefly, 300 μg cell lysates were diluted in Array Buffer and incubated overnight with the Human

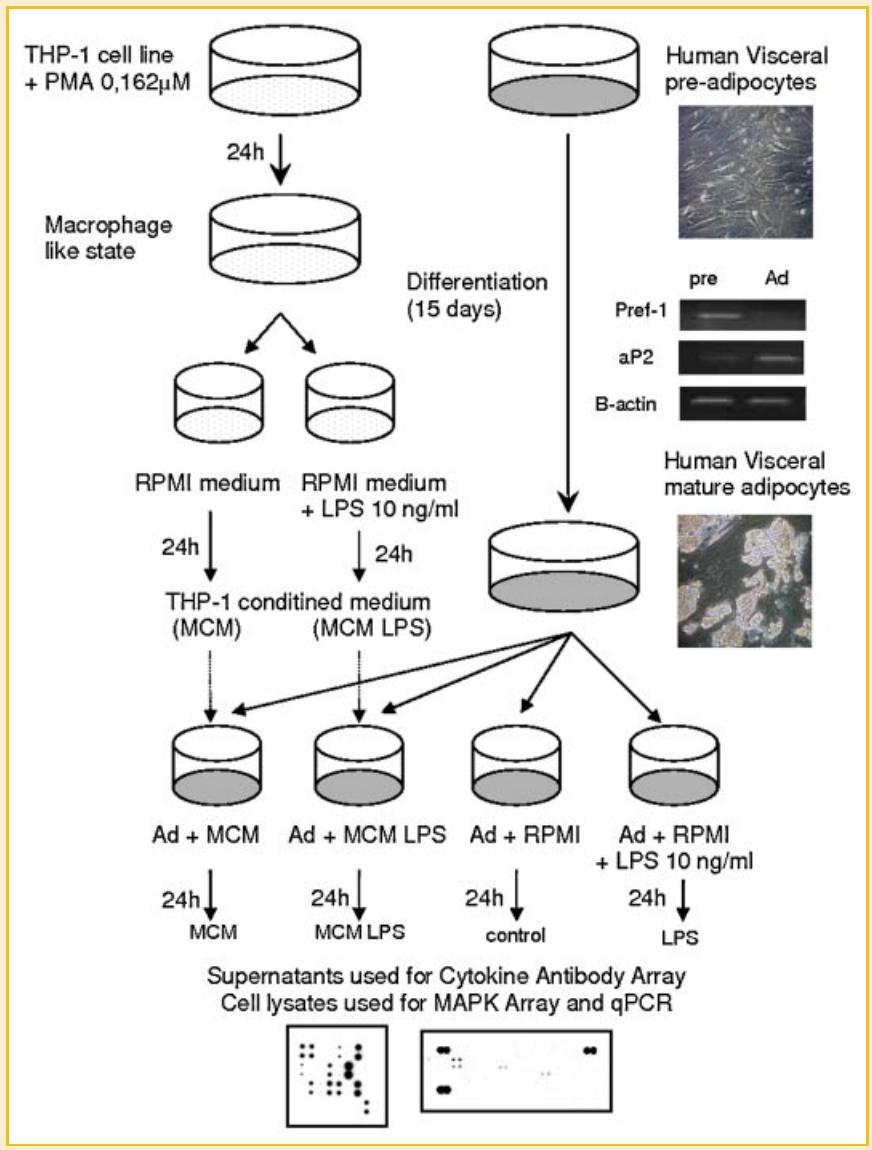

Fig. 1. Omental human adipocytes differentiated in culture during 15 days were incubated for 24 h with fresh media (control) containing 10 ng/ml LPS (LPS), conditioned media from Thp1 macrophage cell line (MCM) and conditioned media from the same cells previously stimulated with LPS (MCM LPS). Cell supernatants were used to measure the level of 20 cytokines with a Human Cytokines Antibody Array, and cell lysates to determine the phosphorylation of several protein kinases related with the MAPK pathway using a Human phospho-MAPK Array. The differentiation process of human adipocytes was assessed by accumulation of lipid droplets, and Pref-1 and aP2 mRNA expression by conventional PCR.

Phospho-MAPK Arrays. After binding and removing the unbound material, a cocktail of phospho-site specific biotinylated antibodies was used to detect phosphorylation via Streptavidin-HRP and chemiluminescence. Phospho-MAPK Array data on developed X-ray film was quantified by using Scion Image software.

WESTERN BLOT ANALYSIS

Cell lysates (prepared according Human Phospho-MAPK Array Kit; R&D Systems) were separated by SDS–PAGE and transferred to nitrocellulose membranes by conventional procedures. Membranes were immunoblotted with anti-human PPAR-γ monoclonal antibody and anti-human Fatty Acid Synthase (FASN) (Santa Cruz Biotech). Anti-mouse IgG coupled to horseradish peroxidase (HRP) was used as secondary antibody. HRP activity was detected by chemiluminescence and quantification of protein expression was performed using Scion Image software.

RNA EXTRACTION AND PCR

Total RNA from cell cultures was isolated using RNeasy Lipid Mini Kit (Quiagen) according to manufacturer's instructions. First strand cDNA was synthesized from 1 μg total RNA with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Eighty nanograms of cDNA was used to characterize Pref-1, (preadipocyte factor-1), and aP2 (fatty acid binding protein), using forward 5′-TACGAGTGTCTGTGCAAGC-3́ and reverse 5′-ACACAAGAGA-TAGCGAACACC-3́ oligonucleotides and 37 cycles (95°C for 30 s, 56°C for 30 s, and 72°C for 30 s) and forward 5′-AACCTTAGATG-GGGGTGTCCTG-3́ and reverse 5́-TCGTGGAAGTGACGCCTTTC-

3'oligonucleotides and 35 cycles (94°C for 1 min, 58°C for 1 min, and 72°C for 1 min), respectively. Actin was used as control, using forward 5′-TACATGGCTGGGGTGTTGAA-3′ and reverse 5′-AAGAGAGGCATCCTCACCCT-3′ oligonucleotides and 35 cycles (94°C for 45 s, 64°C for 45 s, and 72°C for 45 s).

REAL-TIME PCR

Quantitative real-time PCR was performed with commercial predesigned primers for IL-6, IL-8, MCP-1, adiponectin, PPAR-γ, and FASN (Applied Biosystems). The analysis was run on an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using the following assays, Hs00174103m1 for IL-8, Hs00234140m1 for MCP-1, Hs00985639m1 for IL-6, Hs00605917m1 for adiponectin, Hs00234592m1 for PPAR-γ, Hs00188012m1 for FASN, and Hs99999904m1 for Cyclophilin A. As endogenous control gene human cyclophillin was used. The relative expression was determined by the comparative threshold method.

STATISTICAL ANALYSIS

Data are expressed as the mean ± SEM. The experiments were performed at least three times, each using adipocytes from different human subjects. Differences between the groups were evaluated with an unpaired Student's *t*-test with Bonferroni correction for multiple comparisons.

RESULTS

PRIMARY CULTURES OF DIFFERENTIATED ADIPOCYTES

Human omental preadipocytes from non-diabetic male patients with BMI > 30 kg/m^2 and aged > 40 years were cultured and differentiated. Adipocyte differentiation was assessed microscopically by accumulation of lipid droplets in the cytoplasm of 80% of the cells (Fig. 1). Additionally, a PCR assay was used to demonstrate that preadipocytes were differentiated to adipocytes, by detecting Pref-1 and aP2 mRNA, specific markers for preadipocytes and adipocytes respectively. As expected, a low level of Pref-1 expression and an increment of aP2 expression in differentiated adipocytes were observed (Fig. 1).

LPS-INDUCED CYTOKINE RELEASE BY MACROPHAGES AND ADIPOCYTES

Differentiated THP-1 cells secreted a large amount of different chemokines such as GRO, HVEM, ICAM-1, IL-8, MCP-1, MIP-1α, MIP-1β, IP-10, MIF, in addition to the cytokines TNF-α TGF-β, IL-6, and IL-1β (Figs. 2C and 3). When THP-1 cells were stimulated with LPS, the level of TNF-α, MIF, MCP-1 and ICAM-1 increased significantly, and IL-1α appeared de novo (Figs. 2D and 3).

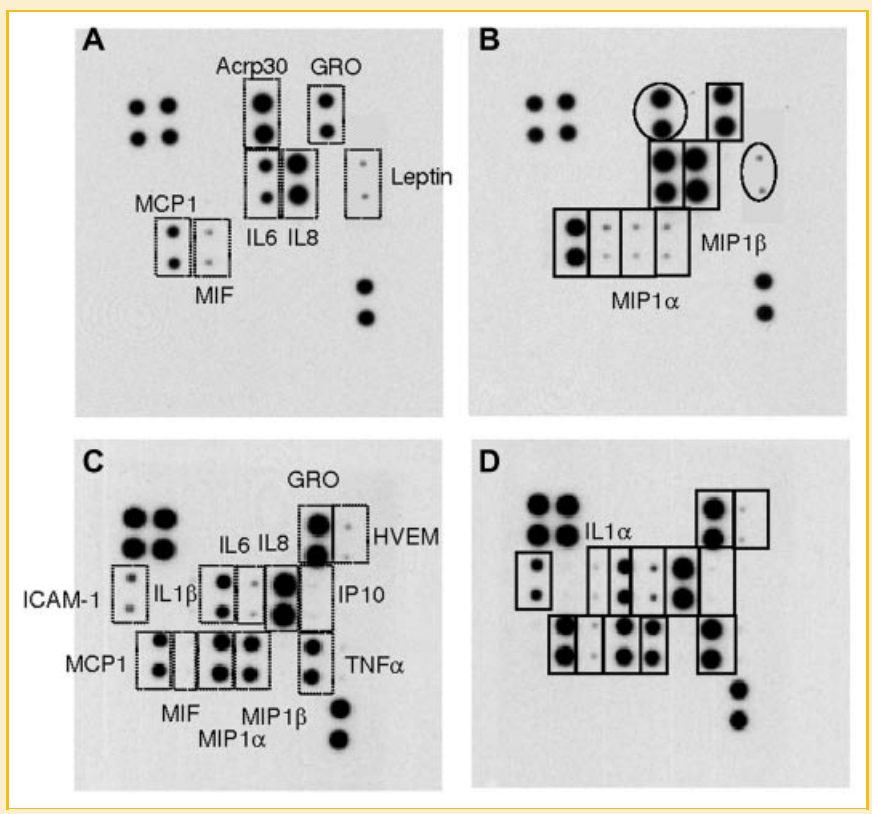

Fig. 2. Human cytokines antibody array were used to detect adipokines secreted by omental human differentiated adipocytes (A,B) and THP1 macrophage cells (C,D) stimulated with LPS (B,D) or control media (A,C) for 24 h. The relative cytokine level was determined by chemiluminescence detection and autoradiography. Positive controls were located at the upper left-hand corner (four spots) and at the lower right-hand corner (two spots) in each membrane. Pairs of spots identified by squares correspond to cytokines whose levels were increased and circles are cytokines whose levels were reduced. The arrays shown are representative of three independent experiments.

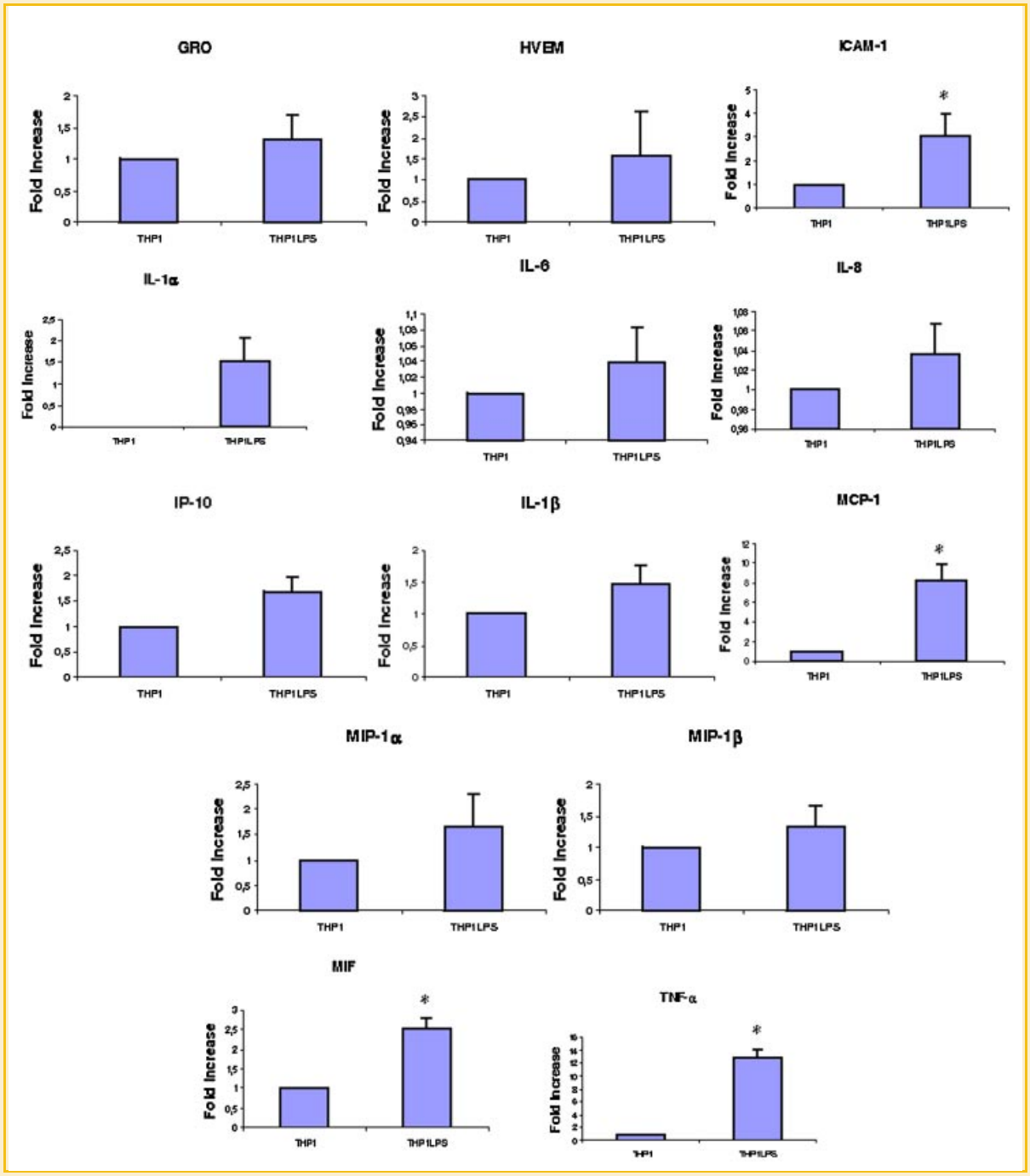

Fig. 3. Fold increase of cytokines secreted by THP-1 cells in macrophage-like state stimulated with 10 ng/ml LPS (THP-1 LPS) or control media (THP-1), measured by densitometry from the human cytokines antibody array (shown in Fig. 2). Cell-free supernatants of THP-1 cells were normalized to 1-fold, and cell-free supernatants of THP-1 stimulated with LPS were calculated accordingly. Data are the mean of three independent experiments. $^{*}P < 0.05$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Of the 20 cytokines measured in the culture supernatants, 7 were spontaneously secreted by the human omental differentiated adipocytes (Figs. 2A and 4). These proteins included GRO, IL-8, MCP-1, IL-6, and MIF, in addition to adiponectin and leptin. When adipocytes were stimulated with LPS, there was a significant increase of GRO, IL-6, IL-8 and MCP-1 (Figs. 2B and 4).

EFFECT OF MACROPHAGE SECRETED FACTORS ON CYTOKINE PRODUCTION BY HUMAN MATURE ADIPOCYTES

Because both adipocytes and macrophages are capable of secreting a wide number of cytokines and communicate via paracrine mechanisms, we have incubated human omental adipocytes with macrophage conditioned media from human differentiated

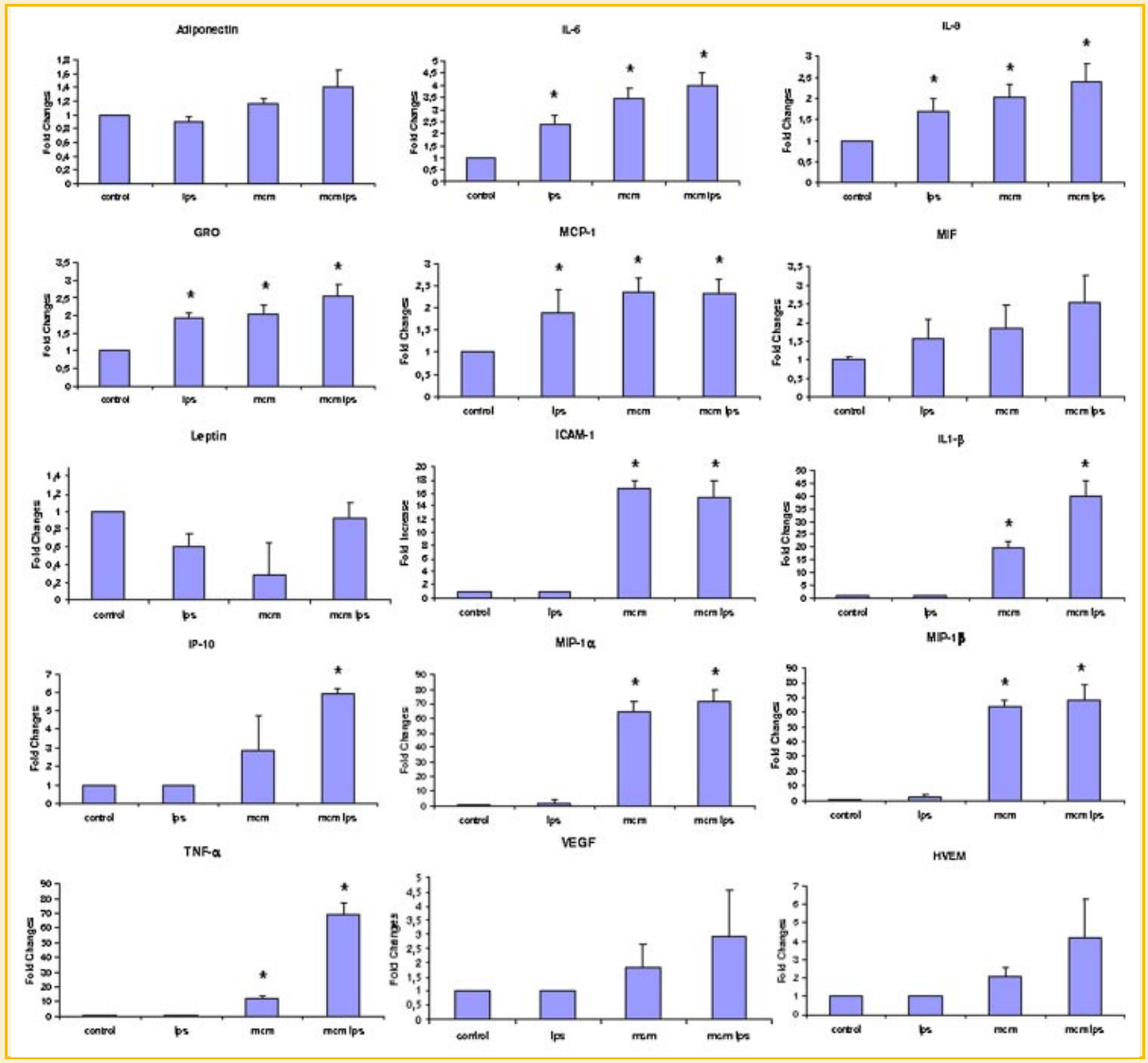

Fig. 4. Fold increase of cytokines secreted by omental differentiated adipocytes under different inflammatory conditions. It was measured by densitometry from the human cytokines antibody array (like that shown in Fig. 2). Cell-free supernatants of human omental adipocytes cultured in fresh media without additives were normalized to 1-fold, and cell-free supernatants of the different experimental conditions were calculated accordingly. Results are the average of three experiments. $^{*}P < 0.05$ compared to control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

THP-1 macrophages (MCM) and with conditioned media from THP-1 macrophages stimulated with LPS for 24 h (MCM LPS). Using this approach, we could infer the cytokines released by adipocytes under the physiologic mixture of macrophage secreted factors.

Macrophage secreted factors, activated or not with LPS, stimulated the newly secretion of several inflammation-related proteins with diverse proinflammatory functions such as ICAM-1, IL-1β, IP-10, MIP-1α, MIP-1β, HVEM, VEGF, and TNFα. Of note, the conditioned medium from macrophages led to a significant increased of IL-6, IL-8, GRO, and MCP-1 levels (Figs. 4 and 5).

The production of some of these cytokines was also studied by real time PCR. As shown in Figure 6, the mRNAs of IL-6, IL-8, and MCP-1 were up-regulated by macrophage secreted factors after 24 h co-incubation, and adiponectin gene expression was down-regulated.

DECREASE OF ADIPOCYTE-SPECIFIC MARKERS BY MACROPHAGE SECRETED FACTORS

It is known that mature adipocytes express high levels of fatty acid synthase (FASN) and PPAR-γ. Both FASN and PPAR-γ gene expression and protein levels were significantly down-regulated when co-incubated with macrophage secreted factors. LPS treatment had no effect on these proteins (Fig. 7).

MAPK PATHWAYS ARE INHIBITED BY MACROPHAGE SECRETED FACTORS IN ADIPOCYTES

Differentiated adipocytes showed baseline phosphorylation of ERK1, ERK2, p38α, and a slight phosphorylation of HSP27 when these cells were co-incubated with fresh media without additives for 24 h. None of the MAPKs studied in the present report were activated

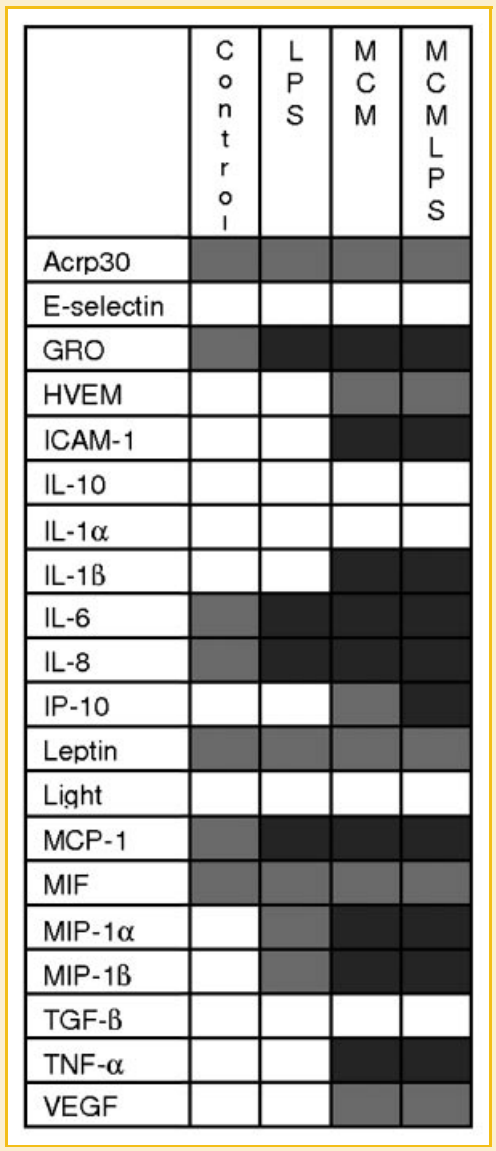

Fig. 5. Diagram showing the changes of cytokine secretion by human omental differentiated adipocytes under the experimental conditions, measured by densitometry from the Human Cytokines Antibody Array. Proteins present on cell-free supernatants of omental adipocytes are shown in grey, and proteins whose secretion was not detected are in white. Proteins marked in black correspond to cytokines whose levels were increased compared to control. The results are the average of three independent experiments.

by the inflammatory conditions used. On the contrary, their phosphorylation levels were reduced. This effect was even higher when the adipocytes were co-incubated with macrophage secreted factors (Fig. 8).

DISCUSSION

It is widely known that macrophage-secreted factors may contribute significantly to the systemic inflammation and insulin resistance associated with obesity [Permana et al., 2006]. This function has been mainly attributed to preadipocytes, which secreted higher levels of inflammatory signals (i.e., cytokines and chemokines) and expressed higher levels of proinflammatory genes than adipocytes. Several authors have studied the effect of macrophage-like cells secretion on 3T3-L1 and human preadipocytes, and the regulation of inflammation-related genes [Suganami et al., 2005; Permana et al., 2006]. However, the effects of these factors on human differentiated adipocytes are still unclear. We here evaluated the effect of LPS and conditioned media obtained from human macrophage-like cell line THP-1, previously activated or not with LPS, on human differentiated omental adipocytes. We studied the secretion of a wide number of cytokines, the expression of several adipogenic-related genes and the possible role of MAPKs in this context.

Usually, non-adipocyte cells or SV cells are considered to be the main source of proinflammatory adipokine release by adipose tissue [Fain et al., 2004a]. Moreover, the secretion of a wide number of cytokines is known to decrease following adipocyte differentiation [Gerhardt et al., 2001; Chung et al., 2006]. Here, we showed that human omental differentiated adipocytes spontaneously released the proinflammatory cytokines IL-6 and MIF, and the chemokines IL-8, GRO, and MCP-1. Other proteins such as IL-1β, ICAM-1, MIF, MIP1α, MIP1β, and VEGF were not detected when adipocytes were incubated with control media. Although TNF-α was reported to be expressed in adipocytes [Sopasakis et al., 2005], its secretion was also undetectable in our cultures. In fact, other authors have demonstrated that TNF-α protein release from human adipose tissue was mainly due to non-fat cells [Fain et al., 2004b].

In agreement with previous reports [Chung et al., 2006; Hoch et al., 2008] the present work with human differentiated adipocytes demonstrated that substantial amounts of proinflammatory cytokines and chemokines were induced by LPS. We found that LPS-treated adipocytes caused a marked induction of IL-6, IL-8, MCP-1 and GRO secretion in omental adipocytes. It is well known that adipocytes express toll-like receptor 4 (TLR4) through which LPS activate intracellular inflammation pathways [Lin et al., 2000]. TLR4 was recently shown to play an important role in obesity-related inflammation [Shi et al., 2006] and elevated circulating LPS levels were recently reported in obese subjects [Creely et al., 2007]. Macrophages also express TLR4 [Shi et al., 2006], and its activation leads to secretion of proinflammatory products [Aderem and Ulevitch, 2000]. We here confirm that LPS increase the secretion of TNF-α, ICAM-1, MCP-1, and MIF in differentiated THP-1 cells [Harrison et al., 2005; Chung et al., 2006].

The stimulation with macrophage secreted factors, activated or not with LPS, resulted in increased mRNA expression and increased secretion of IL-6, IL-8, GRO, and MCP-1, as well as to decreased adiponectin gene expression. The new secretion of several inflammation-related proteins with diverse proinflammatory functions such as ICAM-1, IL-1β, IP-10, MIP-1α MIP-1β, and TNF-α was also observed. Our results were in general agreement with previous studies with 3T3-L1 and human preadipocytes incubated with conditioned media by macrophages. These studies reported a marked up-regulation of several inflammation-related genes that encode proteins with diverse proinflammatory functions (TNF-α MCP-1, MIP-1α, MIP-1β, ICAM-1, E-selectin, IL-1β, IL-6, and IL-8) and the down-regulation of the anti-inflammatory adipokine adiponectin [Suganami et al., 2005; Permana et al.,

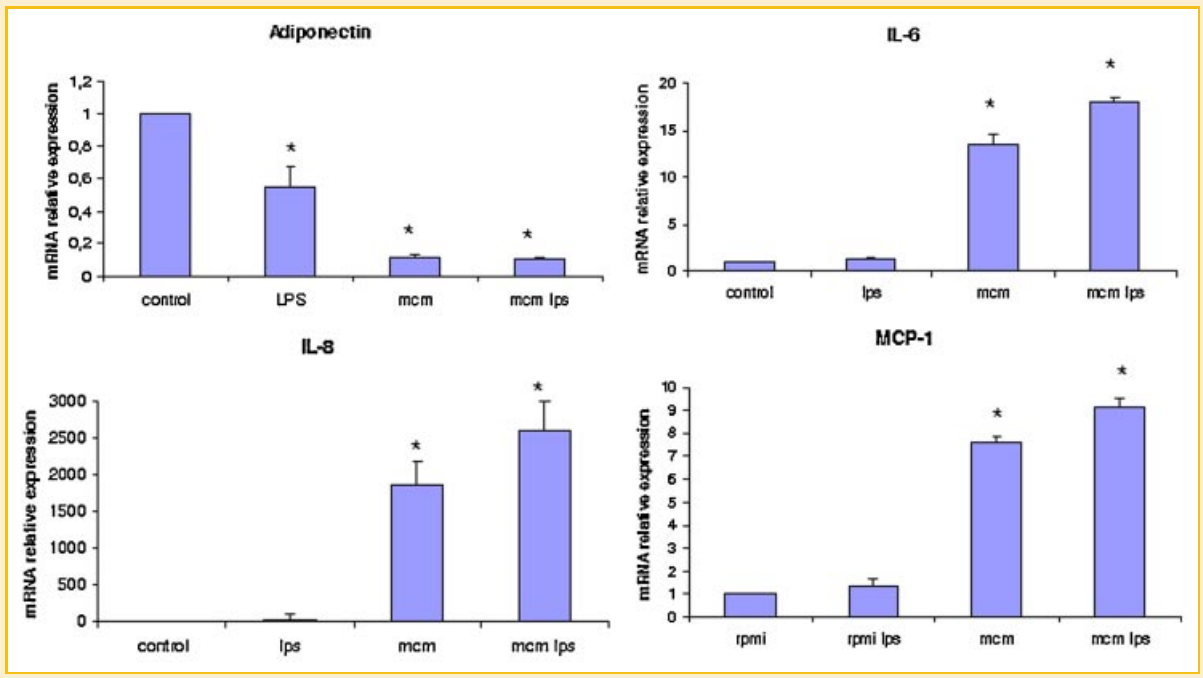

Fig. 6. Real time PCR of genes coding for Adiponectin, IL-6, IL-8, and MCP-1 on human differentiated adipocytes treated for 24 h with LPS and macrophage secreted factors activated or not with LPS. Relative mRNA levels were normalized to the levels of Cyclophilin A. Results are the average of three experiments. $^{*}P < 0.05$ compared with control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

2006; Lacasa et al., 2007]. Macrophage-secreted factors also induced preadipocyte proliferation and impaired adipogenesis [Lacasa et al., 2007]. In fact, increased lipolysis and reduced insulin sensitivity were also observed in these adipocytes [Permana et al., 2006]. These findings point to soluble molecules, present in the macrophage secreted media, to be responsible for the up-regulation of proinflammatory cytokines. Future investigations will be aimed at determining which of the macrophage secreted cytokines are responsible for this regulation. Among the adipokines secreted by differentiated omental adipocytes, IL-8, MCP-1, GRO, and MIP-1β have already been described as involved in cardiovascular diseases such as atherosclerosis mainly by modulating the inflammatory recruitment of monoyctes/macrophages [Ito and Ikeda, 2003; Boisvert, 2004]. This finding sheds light on the crosstalk between inflammation and adiposity.

According to previous works in murine cell lines, the up-regulation of proinflammatory cytokines by macrophage-secreted factors was significantly abolished by inhibiting MAP kinases, suggesting that the activation of MAP kinases was important for the induction of inflammatory changes in adipocytes and macrophages [Suganami et al., 2005; Permana et al., 2006]. In this study, we also have focused our attention in the MAPK pathway. Members of the MAPK family enable cells to transducer extracellular signals into an intracellular response [Chang and Karin, 2001]. In mammalian cells, three parallel MAPK pathways have been identified which include the extracellular signal-regulated kinases (ERKs), p44 MAPK (ERK1), and p42 MAPK (ERK2), c-Jun-NH2-terminal kinases (JNK), and the p38MAPK. ERKs are activated by growth factors and are involved in both cell proliferation and differentiation [Chang and Karin, 2001]. JNK and p38MAPK are preferentially activated in response to proinflammatory cytokines and environmental stress [Chang and Karin, 2001]. We have observed that human omental adipocytes spontaneously expressed activated ERK1, ERK2, and low levels of p38α. The levels of phosphorylation of these kinases decreased when incubated with LPS, and this reduction was accentuated when co-incubated with MCM and MCM LPS for 24 h, suggesting that no activation of MAPK proinflammatory pathways occurred in this model. Even though, it has already been described that LPS-induced proinflammatory cytokine/chemokine expression, MAPK signaling processes and NF-kB activity were down-regulated during human adipocyte differentiation [Chung et al., 2006]. This suggests that differentiated adipocytes do not respond properly to cytokine-induced MAPK signaling, and proinflammatory signaling may differ and be unique in adipocytes. Macrophage conditioned media may also contain inhibitors of these mitogen activated kinases.

Overproduction of adipokines by adipocytes or SV cells in obesity may reflect altered properties of the cells. Changes in intrinsic properties of hypertrophied adipocytes are in agreement with the increase in constitutive activity of the proinflammatory transcription factor NF-kB and the upregulation of adipokine expression during fat cell differentiation [Berg et al., 2004]. The downregulation of PPAR-γ and FASN adipogenic gene, as well as the inhibition of ERK signaling, suggests that some of the macrophage secreted factors may enhance these alterations in mature adipocytes.

To summarize, current results suggest that human mature adipocytes may also contribute to the systemic inflammation of adipose tissue by the secretion of several adipokines, in response to

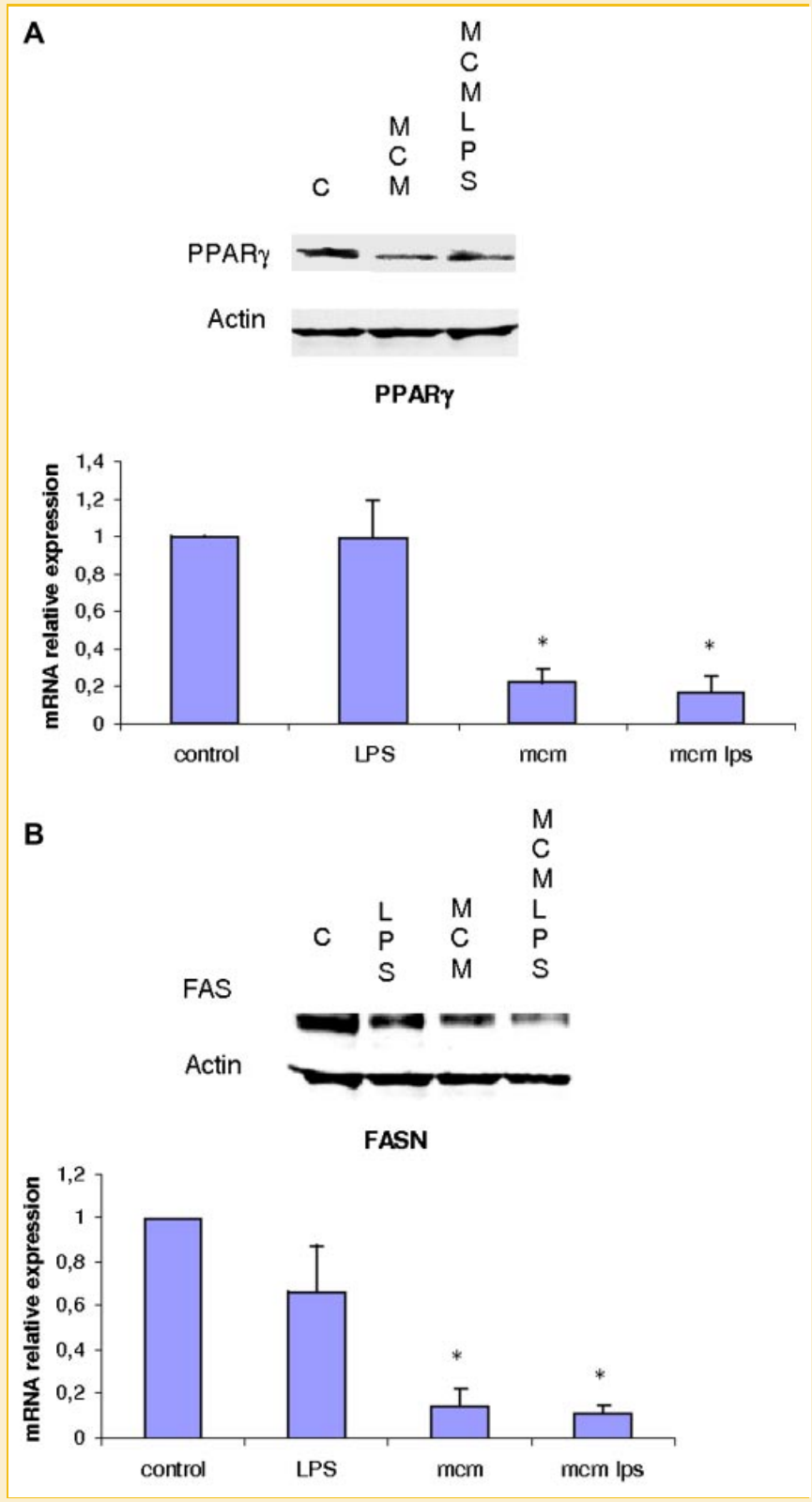

Fig. 7. Western blot and RT-qPCR analysis of PPAR-γ (A) and FASN (B) in omental human differentiated adipocytes cultured with LPS and macrophage secreted factors activated or not with LPS. Data are the mean of three independent experiments. $^{*}P < 0.05$ compared with control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

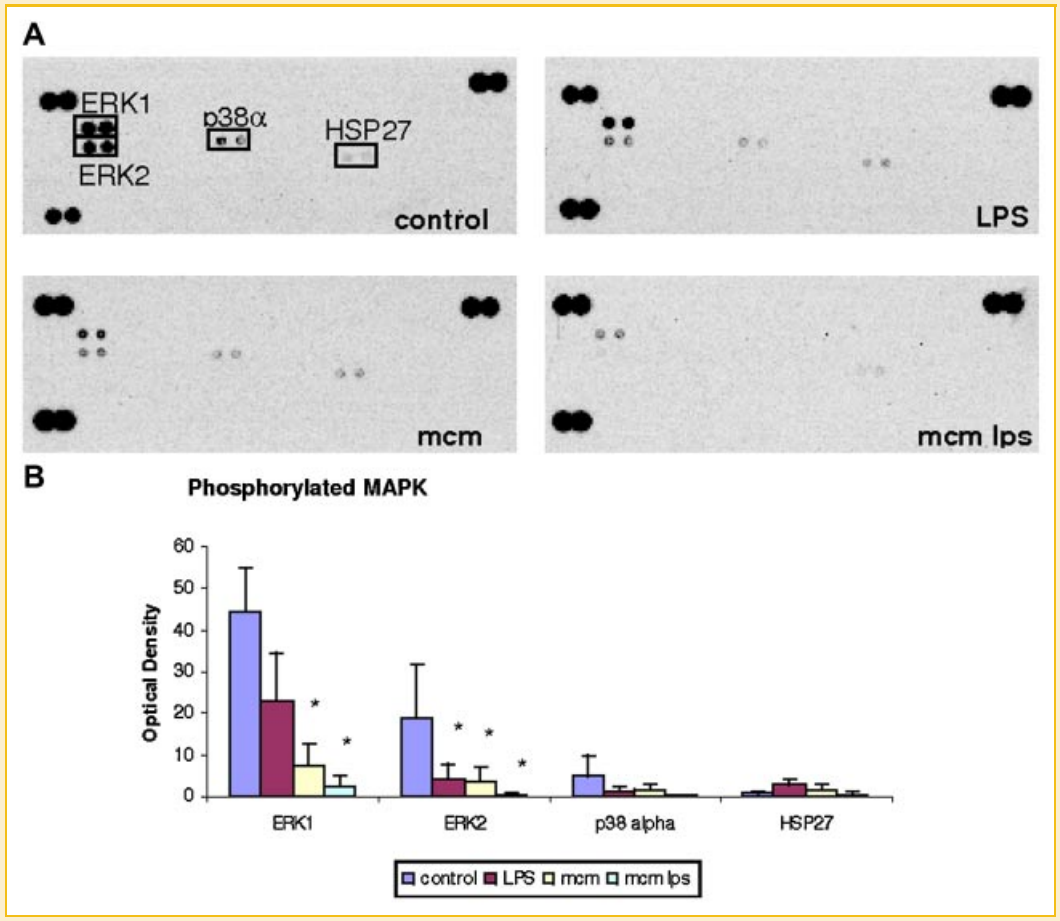

Fig. 8. Effect of LPS, MCM, and MCM LPS-stimulated on the phosphorylation of several protein kinases related with the MAPK pathway, measured with densitometry from the Human Phospho-MAPK Array kit. A: Human Phospho-MAPK array membranes representative of three independent experiments. B: Densitometry data obtained from scanned detectable spots. $^{*}P < 0.05$ compared with control. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

activated conditioned media (LPS and macrophage secreted factors). These cytokines may potentially contribute to the pathogenesis of obesity and its comorbidities. It is tempting to hypothesize that a vicious cycle involving macrophages and adipocytes aggravates inflammatory changes in obese adipose tissue. We have identified four adipokines (IL-6, IL-8, GRO, and MCP-1) oversecreted and overexpressed by human mature adipocytes under LPS and macrophage secreted factors, and six cytokines whose secretion was newly induced by macrophage secreted factors (ICAM, IL-1β, IP-10, MIP-1α, MIP-1β, and TNFα). Macrophage secreted factors alter adipocyte function, as assessed by the inhibition of the MAPK pathway and the down-regulation of adipogenic markers. Thus, these data strengthen the concept that omental differentiated adipocytes are a source of proinflammatory molecules that are involved in obesity-associated complications and chronic low-grade inflammation.

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REFERENCES

Aderem A, Ulevitch RJ. 2000. Toll-like receptors in the induction of the innate immune response. Nature 406: 782–787.

Berg AH, Lin Y, Lisanti MP, Scherer PE. 2004. Adipocyte differentiation induces dynamic changes in NF-kappaB expression and activity. Am J Physiol Endocrinol Metab 287: E1178–1188.

Boisvert WA. 2004. Modulation of atherogenesis by chemokines. Trends Cardiovasc Med 14: 161–165.

Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, Bouloumié A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clément K. 2005. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 54: 2277–2286.

Carey AL, Febbraio MA. 2004. Interleukin-6 and insulin sensitivity: Friend or foe? Diabetologia 47: 1135–1142.

Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olsen DB, Saltin B, Hawley JA, Febbraio MA. 2004. Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with Type 2 diabetes: Evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. Diabetologia 47: 1029–1037.

Chang L, Karin M. 2001. Mammalian MAP kinase signalling cascades. Nature 410: 37–40.

Chung S, Lapoint K, Martinez K, Kennedy A, Boysen Sandberg M, McIntosh MK. 2006. Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. Endocrinology 147: 5340–5351.

Creely SJ, McTernan PG, Kusminski CM, Fisher M, Da Silva NF, Khanolkar M, Evans M, Harte AL, Kumar S. 2007. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 292: E740–747.

Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, Bouloumie A. 2004. From blood monocytes to adipose tissue-resident macrophages: Induction of diapedesis by human mature adipocytes. Diabetes 53: 1285–1292.

Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA. 2005. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: Association with cytokine expression, insulin resistance, and reduction by pioglitazone. Diabetes 54: 2305–2313.

Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. 2004a. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 145: 2273–2282.

Fain JN, Bahouth SW, Madan AK. 2004b. TNFalpha release by the nonfat cells of human adipose tissue. Int J Obes Relat Metab Disord 28: 616–622.

Gerhardt CC, Romero IA, Cancello R, Camoin L, Strosberg AD. 2001. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Mol Cell Endocrinol 175: 81–92.

Guerre-Millo M. 2004. Adipose tissue and adipokines: For better or worse. Diabetes Metab 30: 13–19.

Harrison LM, van den Hoogen C, van Haaften WC, Tesh VL. 2005. Chemokine expression in the monocytic cell line THP-1 in response to purified shiga toxin 1 and/or lipopolysaccharides. Infect Immun 73: 403–412.

Hoch M, Eberle AN, Peterli R, Peters T, Seboek D, Keller U, Muller B, Linscheid P. 2008. LPS induces interleukin-6 and interleukin-8 but not tumor necrosis factor-alpha in human adipocytes. Cytokine 41: 29–37.

Ito T, Ikeda U. 2003. Inflammatory cytokines and cardiovascular disease. Curr Drug Targets Inflamm Allergy 2: 257–265.

Kristiansen OP, Mandrup-Poulsen T. 2005. Interleukin-6 and diabetes: The good, the bad, or the indifferent? Diabetes 54 (Suppl 2): S114–S124.

Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K. 2007. Macrophage-secreted factors impair human adipogenesis: Involvement of proinflammatory state in preadipocytes. Endocrinology 148: 868–877.

Lee YH, Nair S, Rousseau E, Allison DB, Page GP, Tataranni PA, Bogardus C, Permana PA. 2005. Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. Diabetologia 48: 1776–1783.

Lin Y, Lee H, Berg AH, Lisanti MP, Shapiro L, Scherer PE. 2000. The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. J Biol Chem 275: 24255–24263.

Matsuzawa Y. 2006. Therapy Insight: Adipocytokines in metabolic syndrome and related cardiovascular disease. Nat Clin Pract Cardiovasc Med 3: 35–42.

Miranda PJ, DeFronzo RA, Califf RM, Guyton JR. 2005. Metabolic syndrome: Definition, pathophysiology, and mechanisms. Am Heart J 149: 33–45.

Permana PA, Menge C, Reaven PD. 2006. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochem Biophys Res Commun 341: 507–514.

Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. 2006. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 116: 3015–3025.

Shoelson SE, Herrero L, Naaz A. 2007. Obesity, inflammation, and insulin resistance. Gastroenterology 132: 2169–2180.

Sopasakis VR, Sandqvist M, Gustafson B, Hammarstedt A, Schmelz M, Yang X, Jansson PA, Smith U. 2004. High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. Obes Res 12: 454–460.

Sopasakis VR, Nagaev I, Smith U. 2005. Cytokine release from adipose tissue of nonobese individuals. Int J Obes (Lond) 29: 1144–1147.

Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. 2003. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J 7: 884–886.

Suganami T, Nishida J, Ogawa Y. 2005. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: Role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol 25: 2062–2068.