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Journal of Nutritional Biochemistry 23 (2012) 113–122

Journal of Nutritional Biochemistry

Obesity activates toll-like receptor-mediated proinflammatory signaling cascades in the adipose tissue of mice☆

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Received 14 December 2009; received in revised form 8 October 2010; accepted 28 October 2010

Abstract

Obesity is characterized by low-grade and chronic inflammation, a phenomenon explained with a new term, *metaflammation*. Recent studies suggest that adipocytes may play an important role in the physiological regulation of immune responses in fat deposits *via* toll-like receptor (TLR) signaling cascades. This study investigates the role of the visceral as well as subcutaneous adipose tissues in the development of metaflammation by characterizing the tissue-specific expression profiles of TLRs and downstream signaling molecules and explores the differential responsiveness of TLR-mediated proinflammatory signaling cascades to diet-induced obesity (DIO) and obesity induced by a leptin gene deficiency. The obesity that was induced by a high-fat diet or leptin deficiency up-regulated the expression of TLR1–9 and TLR11–13 in murine adipose tissues, a phenomenon linked with downstream nuclear factor κB, interferon regulatory factors, and STAT-1 activation, and up-regulated the expression of cytokines and chemokines via MyD88-dependent and MyD88-independent cascades. The extent of the obesity-induced up-regulation of most TLR genes and related proinflammatory signaling cascades was much greater in the epididymal adipose tissues than in the subcutaneous fat tissues of the mice with DIO. Furthermore, the magnitudes of the obesity-induced up-regulation of the TLR1, TLR4, TLR5, TLR8, TLR9, and TLR12 genes and most of the downstream signaling molecules and target cytokine genes in the visceral adipose tissue were greater in the DIO mice than in the *ob*/*ob* mice. These results suggest that TLRs and related proinflammatory signaling molecules that are overexpressed in enlarged adipose tissues may play an important role in the obesity-associated phenomenon of metaflammation.

Keywords: Toll-like receptors; High-fat diet; *ob*/*ob* mice; Inflammation; Adipose fat depot

1. Introduction

Adipose tissue had been regarded as a silent and inert organ that stores excess energy as triglycerides and releases energy as fatty acid. Beginning with the discovery of leptin in 1995, however, the view of adipose tissue has changed considerably: it is now recognized as an endocrine organ that secretes a wide variety of hormones, cytokines, chemokines, and growth factors that influence metabolism, vascular and endothelial functions, appetite, satiety, immunity, inflammation, tumor growth, and many other physiological processes [1]. In particular, obesity is increasingly being associated with a phenomenon recently termed *metaflammation* (metabolically triggered inflammation), which describes low-grade and chronic activation of the inflammatory response [2]. The physiological mechanisms that link obesity to metaflammation include the production of various adipocytokines (e.g., adiponectin, resistin, visfatin, and leptin) and proinflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-6, and IL-1 by the expanded adipose tissues [3-6]. Furthermore, elevated free fatty acids (FFAs) in the serum and visceral fat tissues in obese humans, as well as in animal models of obesity, have been shown to induce proinflammatory signaling and insulin resistance in peripheral tissues [7,8].

Toll-like receptors (TLRs) are a family of pattern-recognition receptors that play a critical role in the innate immune system by activating proinflammatory signaling pathways in response to microorganisms [9]. Until recently, 10 distinct TLRs have been identified in humans [10], and 13, in mice [11]. Mesenchymal stem cells that were isolated from human adipose tissue were reported to express TLR1 to TLR6 and TLR9 [12], whereas mature adipocytes of human subcutaneous and visceral adipose tissues were reported to express TLR1 to TLR4 and TLR6 [13]. The TLRs recognize the conserved pathogen-associated molecular patterns (PAMPs) of invading microbial pathogens, the structures of which include lipids, carbohydrates, nucleic acid, and various proteins [10, 14, 15]. The TLRs occur as dimers from the PAMPs [16]. For example, TLR1 and TLR2 heterodimerize, and the resulting dimmer recognizes bacterial triacylated lipopeptides, whereas TLR2, which heterodimerizes with TLR6, senses bacterial diacylated lipopeptides. Homodimerized TLRs include TLR4, a receptor for the Gram-negative bacterial product

☆ This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (#A080020) and by the SRC program of the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (#2009-0063409).
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doi:10.1016/j.jnutbio.2010.10.012

lipopolysaccharide; TLR9, a receptor for the unmethylated CpG-containing DNA motifs that occur in bacterial and viral DNAs; TLR3, which senses synthetic and viral double-stranded RNAs and TLR5, which binds flagellin from bacteria [10,14,17-19]. TLR8, which binds viral single-stranded RNAs, heterodimerizes with TLR7 or TLR9. TLR11 responds specifically to uropathogenic bacteria [20,21], whereas the ligands for TLR10, TLR12 and TLR13 have not yet been identified [17].

There are several proofs of the involvement of TLR2 and TLR4 in metabolic functions as well as in innate immune responses in obesity. Shi et al. [22] suggested that TLR4 may be one mechanism by which fatty acids induce inflammation and insulin resistance in conventional insulin-target tissues, such as adipose tissue [22-24] and muscles [25,26] of obese mice and human subjects. Mice with loss-of-function mutation in TLR4 (C3H/HeJ, a TLR4-deficient mouse strain) are protected against the development of diet-induced obesity, inflammation via nuclear factor κB (NFκB) activation and insulin resistance [25]. Recently, three different groups have demonstrated that the activation of a TLR2 signaling pathway is related to the development of insulin resistance in adipocytes or myotubes [27-29]. The adipocytes and preadipocytes that were isolated from the adipose tissues of the *ob*/*ob* and *db*/*db* mice, which are leptin and leptin receptor deficient, respectively, were characterized by more significant up-regulation of TLR1 to TLR9 expression than with wild-type cells [30-32]. Obesity-induced changes, however, in the expression patterns of TLRs and related signaling cascades in the adipose tissues remain widely unclear.

The metabolic and endocrine functions of adipose tissue from various depots differ in a way that may explain the association of visceral but not subcutaneous fat with obesity-related metabolic problems [33]. This study focuses on two aspects. First, it defines the role of the visceral and subcutaneous adipose tissues in the development of metaflammation through the characterization of the tissue-specific expression profiles of TLRs and downstream signaling molecules. Second, the degrees of responsiveness of TLR-mediated proinflammatory signaling cascades to obesity in enlarged adipose tissues between a mouse model with diet-induced obesity (DIO) and a leptin-deficient (*ob*/*ob*) obese mouse were compared.

2. Materials and methods

2.1. Mice and experimental diets

Twenty-five 5-week-old male C57BL/6J mice and five 5-week-old *ob*/*ob* mice (Orient, Gyeonggi-do, Korea) were housed in a room with controlled temperature (21±2.0°C) and humidity (50±5%) and with a 12-hour light/12-hour dark cycle and were fed a commercial diet (Ralston-Purina, St. Louis, MO, USA) for a week. Twenty of the C57BL/6J mice were randomly divided into two groups and fed either the normal diet (ND) or a high-fat diet (HFD) for 12 weeks. The HFD contained 200 g of fat/kg (170 g of lard plus 30 g of corn oil) and 1% cholesterol by weight. The HFD was formulated to provide 40% of the total energy generated by the diet from fat by replacing carbohydrates with lard and corn oil, but it contained the same amount of vitamins and minerals per kJ as were in the ND. The remaining five C57BL/6J mice and five *ob*/*ob* mice were fed standard rodent chow for 12 weeks.

The mice were anesthetized with diethyl ether following overnight fasting. Blood was drawn from their abdominal aorta into a vacuum tube, and the epididymal and subcutaneous fat-pads were removed, weighed, and frozen in liquid nitrogen. All mice were housed in the specific pathogen-free facility of the Yonsei University, Seoul, Korea, and this study was approved by the institutional animal care and use committee of Yonsei University.

2.2. Biochemical analysis

The serum glucose concentration was measured using the Express Plus Chemistry Analyzer (Chiron Diagnostics, Emeryville, CA, USA). The serum insulin and leptin levels were measured through radioimmunoassay (Linco Research, St. Louis, MO, USA), and the serum cholesterol, triglyceride and free fatty acid levels were determined using commercially available kits (Bio-Clinical System, Gyeonggi-do, Korea). The hepatic lipids were extracted through the procedure that Folch et al. [34] developed using a chloroform-methanol mixture (2:1 v/v). The dried lipid residues were dissolved in 2 ml ethanol. The hepatic cholesterol, triglyceride and free fatty acid concentrations were analyzed using the same enzymatic kits that were used for the plasma analyses.

2.3. Semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

The total RNA was isolated from the epididymal and subcutaneous adipose tissues of each mouse using Trizol (Invitrogen, Carlsbad, CA, USA), and then pooled for the RT-PCR analysis (n=10). Four micrograms of the total RNA was reverse-transcribed using the Superscript II kit (Invitrogen) according to the manufacturer's recommendations. The PCR was programmed as follows: 10 min at 94°C, 30 cycles of 94°C for 30 s, 55 or 60°C for 30 s, and 72°C for 1 min, and 10-min incubation at 72°C. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as the internal control of equal amounts of cDNA in the PCR reactions. The PCR products were subjected to electrophoresis in 1–2% agarose gel, and the densitometric quantification of the band intensity was analyzed using the Quantity One analysis software (Bio-Rad, Hercules, CA, USA). The primers for the PCR are shown in Table 1.

2.4. Nuclear fraction extraction

The preparation of the nuclear extract (NE) was carried out as described [35]. The epididymal and subcutaneous fat tissues from the ND- or HFD-fed mice (for 12 weeks) were homogenized using a Dounce homogenizer in the lysis buffer, and nuclear proteins were further extracted with buffer C that contained 20 mM of ph 7.9 HEPES-KOH, 1.5 mM of $MgCl_2$, 420 mM of NaCl, 25% glycerol, 0.2 mM of EDTA, 0.5 mM of dithiothreitol (DTT) and 0.5 mM of phenylmethylsulfonyl fluoride (PMSF).

2.5. Western blot analysis

The NE proteins (3 μg/lane) were separated in a 10% sodium dodecyl sulfate polyacrylamide gel and transferred to a nitrocellulose membrane (Amersham, Buckinghamshire, UK). The membranes were then blocked for 2 h in 5% bovine serum albumin (BSA) in a Tris-buffered saline/Tween buffer (20 mM Tris-base, pH 7.5, 150 mM NaCl and 0.2% Tween 20). Then the membranes were probed with a 1:1000 dilution of the primary antibody. After the incubation of membrane with the corresponding secondary antibody, signals were detected using the chemiluminescent detection system (Amersham, Uppsala, Sweden). Antibodies against NFκB p50, p65, Lamin B and horseradish peroxidase-conjugated goat anti-rabbit IgG were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lamin B was used as the internal control of equal amounts of NE protein in Western blot. The ECL chemiluminescent detection reagent was purchased from Amersham (Arlington Heights, IL, USA).

2.6. Electrophoretic mobility shift assay (EMSA)

EMSA was carried out as previously described [35]. For the binding reaction, 2 μg of NE was incubated with the ^{32}P-labeled probe in the binding buffer that contained 10 mM of ph 7.5 Tris, 100 mM of KCl, 1 mM of DTT, 1 mM of EDTA, 0.2 mM of PMSF, 1 mg/ml of BSA and 5% glycerol at room temperature for 30 min. For the competition experiments, a 100-M excess cold probe was added to the reaction mixture. Bound and free DNAs were analyzed *via* electrophoresis through a 6% polyacrylamide gel and exposed to autoradiography. The sequence of the oligonucleotides that were used as probes was as follows: NFκB, 5′-AGTTGAGGGGACTTTCCCAGGC-3′, and STAT-1, 5′-CATGTTATGCATATTCCTGTAAGTG-3′.

2.7. Statistical analyses

The data on the body weight gain and the serum and biochemistries are presented in this study as the means±S.E.M. of 10 mice. The RT-PCR data that are shown are from representative experiments out of three independent experiments in which the RNA samples were pooled from 10 or 5 mice per group. The densitometric quantification of the band intensity is presented here as the mean±S.E.M. of three independent experiments. The statistical significance, denoted as *, ** or ***, was defined as P<.05, P<.01, or P<.001, respectively, and was determined by the Student's t test using the SPSS software.

3. Results

3.1. Phenotypic characteristics of obese mice

The mice that were fed the HFD for 12 weeks had significantly greater final body weight (39% greater, P<.001) and body weight gains (188% greater, P<.001) and heavier relative weights of the visceral fat depots than the mice that were fed the ND. The retroperitoneal, epididymal, mesenteric, and perirenal fat pads were 215%, 151%, 64%,and 186% heavier, respectively, in the mice that were fed the HFD than in those that were fed the ND (P<.001). The HFD-fed mice exhibited significantly higher levels of serum total cholesterol (P<.001), triglycerides (P<.01), free fatty acids (P<.001), glucose (P<.05), insulin (P<.001) and leptin (P<.001) than the ND-fed mice.

Table 1
Specific primers used for the RT-PCR

	Primer pair (5′-3′, forward and reverse)	Tm (°C)	Cycles	Size (bp)
TLR1	TCT CTG AAG GCT TTG TCG ATA CA GAC AGA GCC TGT AAG CAT ATT CG	55		426
TLR2	TCT AAA GTC GAT CCG CGA CAT TAC CCA GCT CGC TCA CTA CGT	55		344
TLR3	TTG TCT TCT GCA CGA ACC TG CGC AAC GCA AGG ATT TTA TT	55		217
TLR4	CAA GAA CAT AGA TCT GAG CTT CAA CCC GCTGTC CAA TAG GGA AGC TTT CTA GAG	58		278
TLR5	ACT GAA TTC CTT AAG CGA CGT A AGA AGA TAA AGC CGT GCG AAA	55		401
TLR6	AAC AGG ATA CGG AGC CTT GA CCA GGA AAG TCA GCT TCG TC	58		199
TLR7	TTC CGA TAC GAT GAA TAT GCA CG TGA GTT TGT CCA GAA GCC GTA AT	55		199
TLR8	GGC ACA ACT CCC TTG TGA TT CAT TTG GGT GCT GTT GTT TG	55		412
TLR9	ATG GTT CTC CGT CGA AGG ACT GAG GCT TCA GCT CAC AGG G	55		118
TLR11	TGG AGC ACT TCT TTA TCT G TTC CAC CAA CAAGTA TGA	55		619
TLR12	ATA CCC AAA TAC GGA TGA GC GGC AGC CCA GTG ATA AGG	55		421
TLR13	CAA AGA CGC CTT CAC TCC TGA CTT TAT TGC CAT TCC	58		284
TRIF	ATG GAT AAC CCA GGG CCT T TTC TGG TCA CTG CAG GGG AT	55		528
Tirap	GTG GCC GCT GGA GCA AAG AC TTG CCT CTG CCA TCC ACA TA	58		370
MyD88	AAG AAA GTG AGT CTC CCC TC TCC CAT GAA ACC TCT AAC AC	55		149
TRAF6	GCA CAA GTG CCC AGT TGA CAA TGA AGT GTC GTG CCA AGT GAT TCC TCT	56		479
IRF1	AGC TGT GTG CAG ATG TTA GCC CGT GAA GAC ATG TTG TAT GCC	55		478
IRF3	ACA TCT CCA ACA GCC AGC CTA T AGT CCA TGT CCT CCA CCA AGT C	56	30	80
IRF5	AAT ACC CCA CCA CCT TTT GA TTG AGA TCC GGG TTT GAG AT	58		191
IRF7	CAG CGA GTG CTG TTT GGA GAC AAG TTC GTA CAC CTT ATG CGG	58		350
TNFα	TGT CTC AGC CTC TTC TCA TT AGA TGA TCT GAG TGT GAG GG	58		156
IL-6	ATG AAG TTC CTC TCT GCA AGA GAC T CAC TAG GTT TGC CGA GTA GAT CTC	55		638
IFNα	ATG GCT AG(G/A) CTC TGT GCT TTC CT GGG CTC TCC AGA (T/C)TT CTG CTC TG	58		500
IFNβ	CCA CAG CCC TCT CCA TCA ACT ATA AGC AGC TCT TCA ACT GGA GAG CAG TTG AGG	56		372
IL-12-p45	ATC GTT TTG CTG GTG TCT CC CTT TGT GGC AGG TGT ACT GG	58		321
CXCL2	GTG CCT CGC TGT CTG AGA GTT AGT TCC CAA CTC ACC CTC TCC	58		277
IκBξ	ATC CGA AGC AAC AAG CAG AA CAC GAA GTG AGA AGG CAA CA	58		186
F4/80	TTTCCTCGCCTGCTTCTTC CCCCGTCTCTGTATTCAACC	60		256
CD68	AGGGTGGAAGAAAGGTAAAGC AGAGCAGGTCAAGGTGAACAG	60		382
CD14	GGCTTGTTGCTGTTGCTTC CAGGGCTCCGAATAGAATCC	60		325
NFκB (p50)	CGCCAAAGTATAAGGATGTC GTAGAGAAAGGGTTTCGGTT	55		163
NFκB (p650)	AGCACAGATACCACCAAGAC TCAGCCTCATAGTAGCCATC	55		189
STAT-1	GGAGGTGAACCTGACTTCCA TCTGGTGCTTCCTTTGGTCT	60		286
SOCS-1	GAG GTC TCC AGC CAG AAG TG CTT AAC CCG GTA CTC CGT GA	59		235
GAPDH	AGA ACA TCA TCC CTG CAT CC TCC ACC ACC CTG TTG CTG TA	55		367

The feeding of the mice with the HFD led to more significant increases in the relative weight of their liver (87% increase) and in the hepatic levels of their cholesterol (150% increase), triglycerides (86% greater), and free fatty acids (344% greater) than in the ND mice ($P<.001$) (Table 2). Compared to the values observed in the DIO mice, final body weight (53% greater), body weight gain (114% greater) and visceral fat-pad weights (69% greater) were greater in the age-matching *ob*/*ob* mice that were raised on a regular diet for the same period (Table 2).

The fat cell size was compared in the epididymal and subcutaneous fat depots of ND and HFD groups. The HFD-fed mice had 98% and 166% increased epididymal and subcutaneous fat-cell sizes, respectively, relative to the ND-fed mice (Fig. 1A and B). Therefore, the magnitude of HFD-induced enlargement of adipocyte volume was greater in the subcutaneous fat depot than in the epididymal fat depot. To quantify macrophage infiltration into different depots of adipose tissues, we analyzed the mRNA expression of macrophage markers, such as F4/80, CD68 and CD14 in both visceral and subcutaneous adipose tissues of mice. The densitometric analyses of these gene bands in the epididymal adipose tissue indicated that the HFD up-regulated the expression of F4/80 (190% increase), CD68 (280% increase) and CD14 (110% increase) as compared with the values for the ND mice. These HFD-induced up-regulations of macrophage marker genes were also observed in the subcutaneous adipose tissue of mice, but to a lesser extent (for F4/80, 160% increase; CD68, 20% increase; CD14, 90% increase) than in the epididymal adipose tissue (Fig. 1C and D).

3.2. HFD-induced changes in the expression of TLRs and their adaptor molecules

The expression patterns of the TLR genes in the visceral and subcutaneous adipose tissues of the mice that were fed HFD or ND were determined through RT-PCR analyses. Stronger signals for all 12 TLR genes (TLR1 to TLR9, and TLR11 to TLR13) were detected in the epididymal and subcutaneous adipose tissues of the HFD-fed mice than in the respective adipose tissues that were obtained from the ND mice (Fig. 2A). The densitometric analyses of the TLR gene bands from

Table 2
Body weight, visceral fat-pad weights, serum and hepatic biochemistries

	ND	HFD	Lean	ob/ob
Final body weight (g)	26.3±0.8	36.5±1.2***	23.8±0.5	55.9±1.3***
Body weight gain (g/12 weeks)	5.8±0.3	16.7±0.6***	4.9±0.1	35.8±0.6***
Visceral fat-pad weight (mg)				
Retroperitoneal	139±10	605±32***	92.8±7.1	1123±156*
Epididymal	581±34	2025±62***	416±35	3303±83***
Mesenteric	310±21	708±36***	268±38	1173±44**
Perirenal	73±5.2	288±7.3***	26.2±2.4	525±61**
Total	1101±57	3628±120***	806±102	6121±190***
Serum				
Total cholesterol (mmol/L)	2.7±0.1	5.1±0.2***	ND	ND
Triglyceride (mmol/L)	0.5±0.01	0.6±0.02**	ND	ND
Free fatty acid (μEq/L)	460±13	866±14***	ND	ND
Glucose (mmol/L)	9.0±0.2	12.6±0.5*	ND	ND
Insulin (pmol/L)	28.0±5.8	83.6±10.6***	ND	ND
Leptin (ng/ml)	6.7±0.7	22.2±1.4***	ND	ND
Liver				
Weight (mg/100 g body weight)	3.4±0.1	6.4±0.2***	ND	ND
Cholesterol (μmol/g liver)	9.9±0.2	24.8±0.7***	ND	ND
Triglyceride (μmol/g liver)	24.5±3.0	45.6±2.2***	ND	ND
Free fatty acid (μEq/g liver)	2.0±0.2\	8.7±0.4***	ND	ND

Each value represents the mean±S.E.M., $n=10$.
* $P<.05$.
** $P<.01$.
*** $P<.001$.

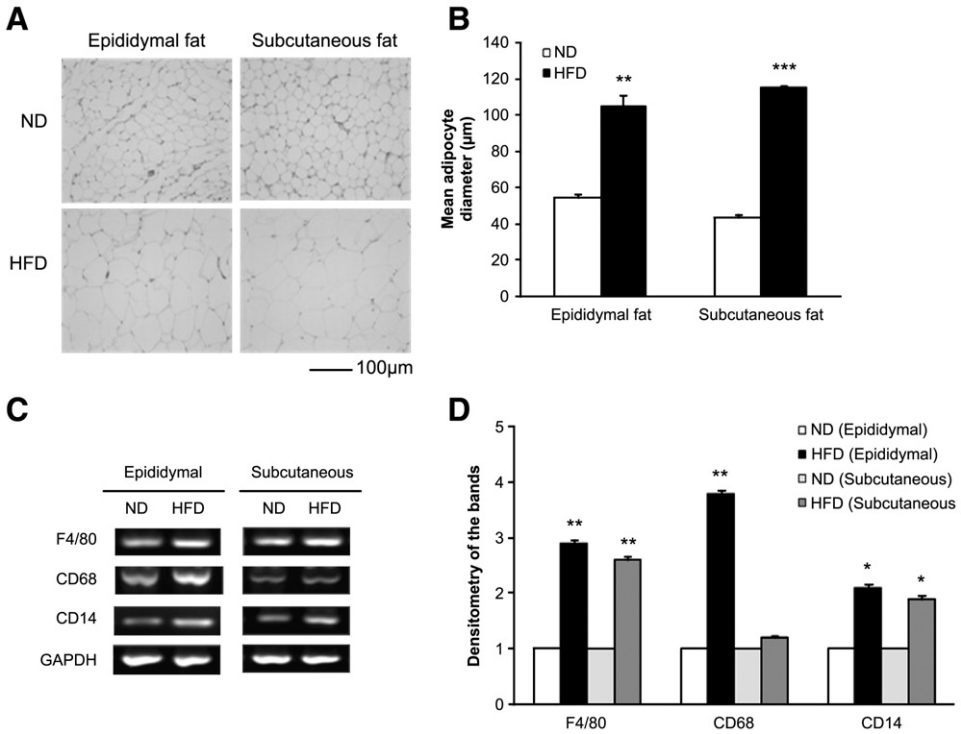

Fig. 1. Adipocyte hypertrophy and macrophage infiltration. (A and B) Histology and mean adipocyte diameter of epididymal and subcutaneous fat-pads of mice. (C) Representative RT-PCR results of the detection of F4/80, CD68, and CD14 gene expression. (D) Densitometry of the F4/80, CD68, CD14 and GAPDH gene bands for a semi-quantitative comparison of their expression levels. The data are the mean±S.E.M. for three independent experiments using RNA samples pooled from 10 mice per group. *P<.05; **P<.01; ***P<.001.

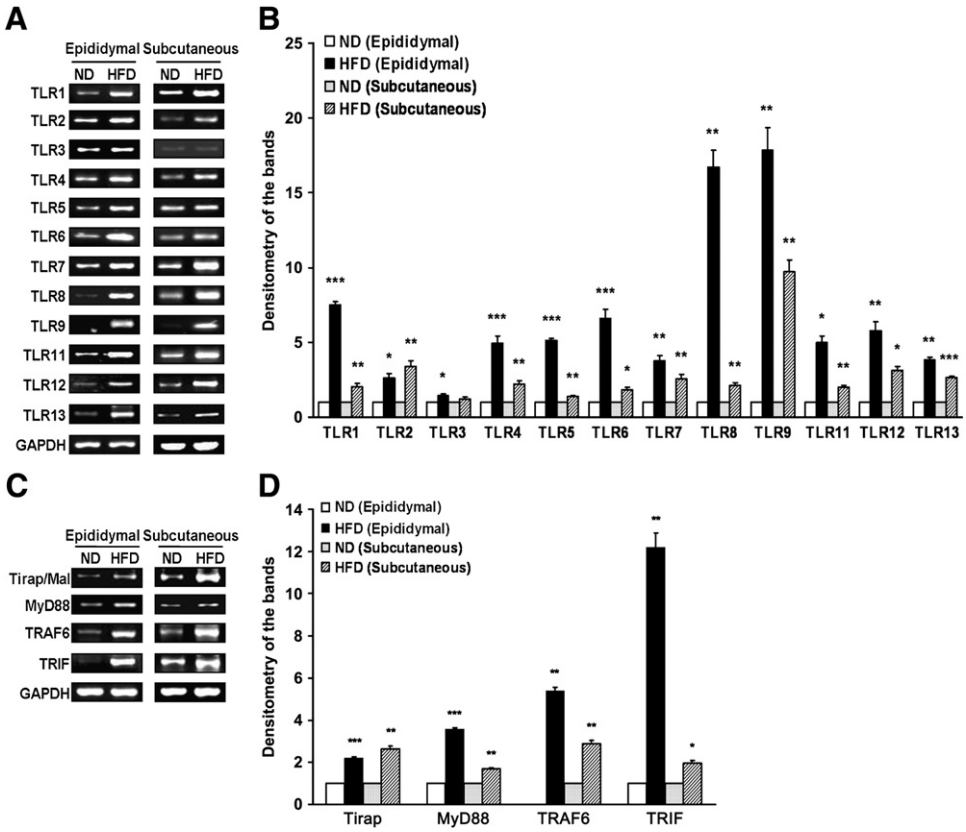

Fig. 2. Expression pattern of TLRs and their adaptor molecules in the epididymal and subcutaneous adipose tissues of the mice. (A) and (C) Representative images of the RT-PCR for the detection of the TLRs and their adaptor molecules. (B) and (D) The densitometry of the TLRs, adaptor molecules and GAPDH gene bands was presented for a semi-quantitative comparison of their expression levels. The data are the mean±S.E.M. for three independent experiments using RNA samples pooled from 10 mice per group. *P<.05; **P<.01; ***P<.001.

three independent experiments indicated that the HFD up-regulated the expression of TLR1 (653% increase), TLR2 (162% increase), TLR3 (45% increase), TLR4 (394% increase), TLR5 (410% increase), TLR6 (559% increase), TLR7 (276% increase), TLR8 (1572% increase), TLR9 (1683% increase), TLR11 (397% increase), TLR12 (478% increase) and TLR13 (281% increase) genes in the epididymal adipose tissue of the mice compared with those of ND (Fig. 1B). The HFD-induced up-regulation of the TLR genes was also observed in the subcutaneous adipose tissue but to a lesser extent than in the epididymal adipose tissue for TLR1 (105% increase), TLR3 (21% increase), TLR4 (121% increase), TLR5 (39% increase), TLR6 (82% increase), TLR7 (157% increase), TLR8 (113% increase), TLR9 (872% increase), TLR11 (100% increase), TLR12 (212% increase) and TLR13 (164% increase) genes, with the exception of the TLR2 (237% increase) gene, which was up-regulated *via* HFD feeding to a greater extent in the subcutaneous adipose tissue than in the epididymal adipose tissue (Fig. 2B).

The HFD-induced changes in the expression of TLR-related adaptor molecules in the epididymal and subcutaneous adipose tissues of the mice that were fed the HFD or ND were also evaluated. Compared to the ND, the HFD resulted in significant increases in the expression of MyD88-dependent adaptor molecules, such as the Toll/IL-1 receptor (TIR)-associated protein (Tirap), MyD88 and the TNF receptor-associated factor 6 (TRAF6), in the epididymal (116%, 259% and 440% increases, respectively) and subcutaneous (164%, 69% and 188% increases, respectively) adipose tissues of the mice. TIR-domain-containing and adaptor-inducing interferon (IFN) (TRIF), a MyD88-independent adaptor molecule, was also significantly up-regulated in the epididymal (1121% increase) and subcutaneous (94% increase) adipose tissues of the HFD-fed mice (Fig. 2C and D).

3.3. HFD-induced changes in TLR-mediated transcription factors

To determine whether the HFD activated the NFκB translocation into the nucleus, EMSA and Western blot analyses were performed using a nuclear fraction extract that was prepared from the epididymal and subcutaneous adipose tissues of mice that were fed either the ND or HFD. For the competition experiments, a 100-M-excess cold probe was added to the reaction mixture using the nuclear fraction extract from the epididymal adipose tissues of the HFD-fed mice. The results showed that the HFD induced NFκB activation in the epididymal adipose tissue of the mice. HFD-induced NFκB activation was also detected in the subcutaneous adipose tissues of the mice but to a lesser degree than in the epididymal adipose tissue (Fig. 3A). Nuclear NFκB subunits, p65 and p50, were released from cytoplasmic complexes with the inhibitor of the NFκB (IκB) α and β protein following IκB kinase complex (IKK)-mediated phosphorylation, ubiquitylation, and proteolytic degradation [36]. Consistent with the activation of NFκB in the adipose tissues of the HFD-fed mice, the Western blot analysis revealed higher increased levels of both NFκB sub-units, p65 and p50, in the nucleus of the epididymal adipose tissues of the HFD group than with the ND mice, whereas NFκB p65 and p50 were not detected in the nucleus of the subcutaneous adipose tissues (Fig. 3B).

The HFD-induced changes in the expression of transcription factors such as the interferon regulatory factor (IRF) 1, 3, 5 and 7 were determined. The HFD resulted in a down-regulation of IRF1 expression in the epididymal (84% reduction) and subcutaneous (43% reduction) adipose tissues of the mice, whereas it led to an up-regulation of IRF5 gene expression in the epididymal (223% increase)

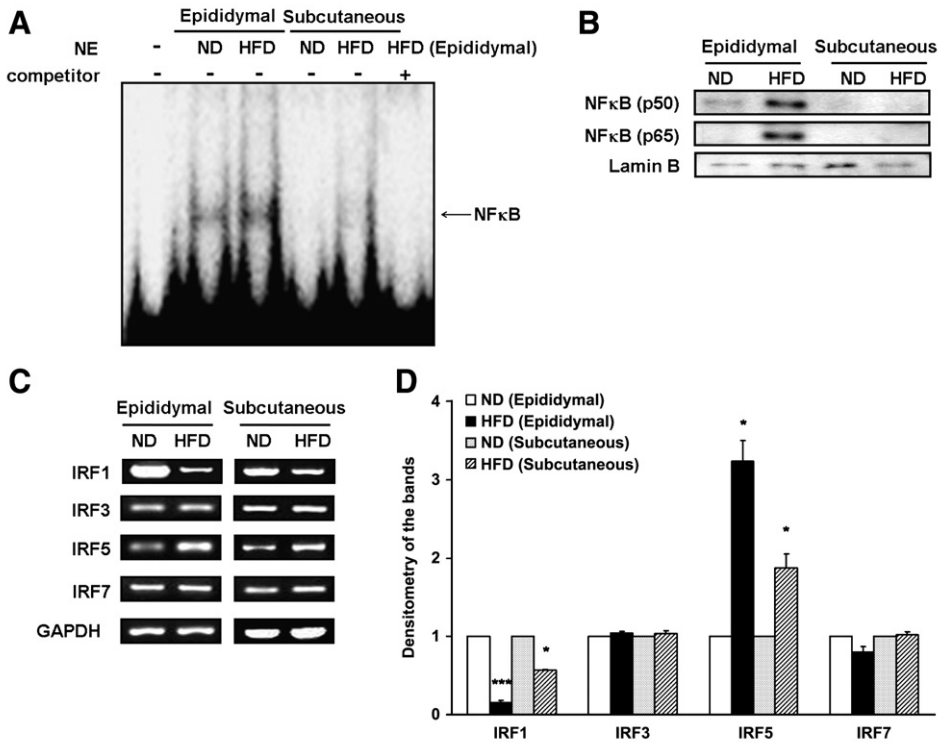

Fig. 3. Activation and expression of TLR-mediated transcription factors in the epididymal and subcutaneous adipose tissues of the mice. (A) Comparison of the NFκB activation in the epididymal and subcutaneous adipose tissues of the HFD- and ND-fed mice. The nuclear extracts were prepared and used in the electrophoretic mobility shift assays. A cold probe was used for competition control. (B) Representative images of Western blotting for the translocation of NFκB p50/p65 into the nucleus at protein levels. Western blots of the nuclear extract from the HFD- and ND-fed mice were analyzed using antibodies against NFκB p50 and p65. The EMSA represent at least three independent experiments performed per condition. (C) Representative images of the RT-PCR for the detection of the IRFs. (D) Densitometry of the IRFs and the GAPDH gene bands for a semi-quantitative comparison of their expression levels. The data are the mean±S.E.M. for three independent experiments using RNA samples pooled from 10 mice per group. *P<.05; **P<.01; and ***P<.001.

and subcutaneous (87% increases) adipose tissues of the mice. The HFD-induced obesity did not affect the expression levels of the IRF3 and IRF7 genes in both the visceral and subcutaneous adipose tissues (Fig. 3C and D).

3.4. HFD-induced changes in the expression of proinflammatory cytokine genes

To confirm the regulation of the downstream target genes the expressions of which were induced by NFκB or IRFs nuclear transcription factors in the adipose tissues of the mice, a semiquantitative RT-PCR analysis was performed. The results demonstrated that the expressions of the TNFα (2190% increase), IL-6 (115% increase), IFNα (2355% increase), IFNβ (1439% increase), IL-12-p40 (509% increase), and chemokine (C-X-C motif) ligand 2 (CXCL2) (248%) genes were more significantly up-regulated in the epididymal adipose tissue of the HFD-fed mice than of the ND-fed mice. The HFD-induced up-regulation of these proinflammatory cytokine genes, such as TNFα (247% increase), IFNα (836% increase), IFNβ (527% increase) and IL-12-p40 (403% increase), were also observed in the subcutaneous adipose tissue, but to a lesser extent than those observed in the epididymal adipose tissue. The extent of the HFD-induced overexpression of the CXCL2 (392% increase) gene was greater in the subcutaneous adipose tissue than in the visceral fat tissues, whereas the HFD induced the IL-6 gene to a similar extent in the subcutaneous and visceral adipose tissues. The HFD did not affect the expression of IκBξ gene in the visceral and subcutaneous adipose tissues of the mice (Fig. 4A and B).

The results of the EMSA using the nuclear extracts that were prepared from the adipose tissues of the mice indicate that the activity of signal transducers and the activator of transcription-1 (STAT-1) significantly increased in the epididymal adipose tissue of the HFD-fed mice than of the ND-fed mice, whereas the HFD did not affect the STAT-1 activity in the subcutaneous adipose tissues (Fig. 4C). Similarly, the immunoblot results indicate that the protein level of STAT-1 more significantly increased in the epididymal adipose tissue (but not in the subcutaneous adipose tissue) of the HFD-fed mice than of the ND-fed mice (Fig. 4D).

3.5. Expression of TLRs and their adaptor molecules in the ob/ob mice

The expression patterns of the TLRs and their adaptor molecules in the epididymal and subcutaneous adipose tissues of the leptin-deficient obesity model, such as the *ob/ob* mice, were compared with those of their age-matching lean controls. Compared with the lean control, the *ob/ob* mice exhibited significantly increased expression levels of a wide spectrum of TLR genes (TLR 1–9, and TLR 11–13) and their adaptor molecules in both the visceral and subcutaneous adipose tissues. The semiquantitative RT-PCR results demonstrated that the TLR1 (186% increase), TLR2 (462% increase), TLR3 (241% increase), TLR4 (79% increase), TLR5 (172% increase), TLR6 (582% increase), TLR7 (357% increase), TLR8 (664% increase), TLR9 (625% increase), TLR11 (829% increase), TLR12 (159% increase) and TLR13 (1720% increase) genes were more significantly up-regulated in the epididymal adipose tissue of the *ob/ob* mice than of the lean control. Most of the TLR genes, including TLR3, TLR4, TLR5, TLR7, TLR8 and

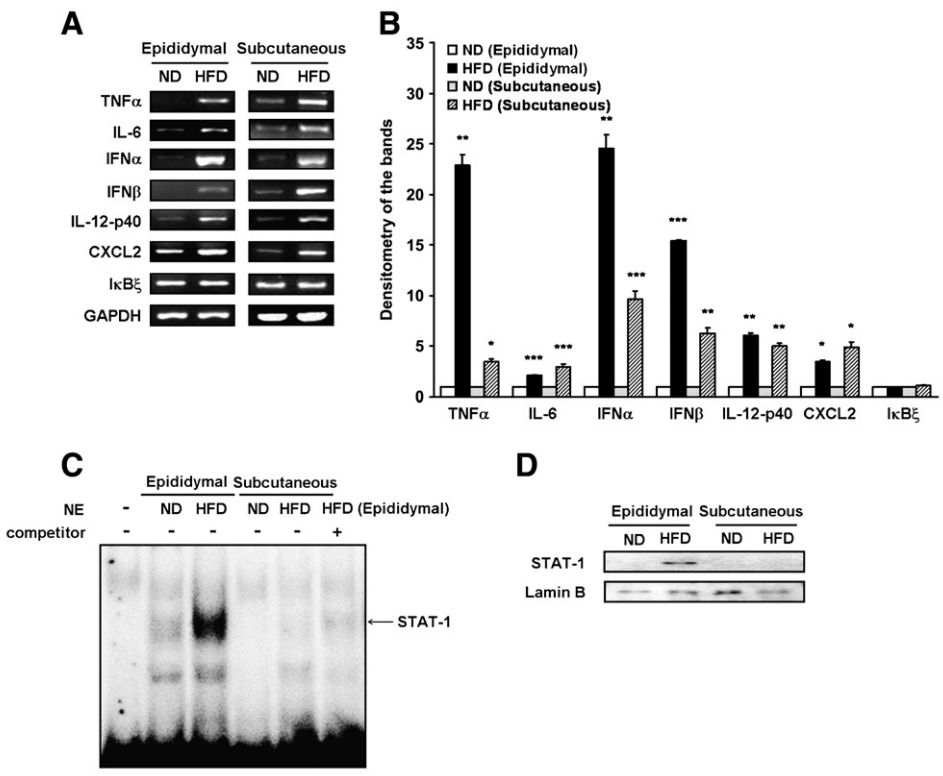

Fig. 4. Expression of TLR-mediated proinflammatory cytokine and chemokine genes and activation of STAT-1 in the epididymal and subcutaneous adipose tissues of the mice. (A) Representative images of the RT-PCR for the detection of proinflammatory cytokine and chemokine genes expression. (B) Densitometry of the TNFα, IL-6, IFNα, IFNβ, IL-12-p40, CXCL2, IκBξ and GAPDH gene bands for a semi-quantitative comparison of their expression levels. (C) Comparison of the STAT-1 activation in the epididymal and subcutaneous adipose tissues of the HFD- and ND-fed mice. The nuclear extracts were prepared and used in the EMSAs. A cold probe was used for competition control. The EMSAs represent at least three independent experiments performed per condition. (D) Representative images of Western blotting for the translocation of STAT-1 into the nucleus at protein levels. Western blots of the nuclear extract from the HFD- and ND-fed mice were analyzed using antibodies against STAT-1. The RNA and NE protein samples were pooled from 10 mice per group. The data are the mean±S.E.M. for three independent experiments. *$P<.05$; **$P<.01$; ***$P<.001$.

TLR9, were also more significantly up-regulated in the subcutaneous adipose tissue of the *ob*/*ob* mice than of their lean control, and the magnitudes of these obesity-induced over-expressions of the TLR genes were similar to those observed in their epididymal adipose tissues. Interestingly, the TLR2 (221% increase), TLR6 (204% increase), and TLR11 (414% increase) genes were up-regulated to a lesser extent, whereas the TLR1 (313% increase), TLR12 (471% increase), and TLR13 (2176% increase) genes were up-regulated to a greater extent, in the subcutaneous adipose tissue than in the epididymal adipose tissue of the *ob*/*ob* mice (Fig. 5A and B).

The expression levels of the TLR-related adaptor molecules in the epididymal and subcutaneous adipose tissues of the *ob*/*ob* mice were compared with those of their age-matching lean controls. The *ob*/*ob* mice exhibited more significant up-regulation of the *Tirap*, *MyD88* and *TRAF6* genes in their epididymal (124%, 67% and 232% increases, respectively) and subcutaneous (341%, 91%, and 273% increases, respectively) adipose tissues than the lean controls. The TRIF gene was also more significantly up-regulated in the epididymal (1155% increase) and subcutaneous (380% increase) adipose tissues of the *ob*/*ob* mice than of the lean control (Fig. 5C and D).

3.6. Expression of IRFs and proinflammatory cytokine genes in the ob/ob mice

The *ob*/*ob* mice exhibited more significantly increased mRNA levels of IRF1, 5 and 7 in their epididymal (358%, 130% and 51% increases, respectively) and subcutaneous (1512%, 105% and 4.6% increases, respectively) adipose tissues than their lean controls. The expression levels of IRF3 in the visceral and subcutaneous adipose tissues of the *ob*/*ob* mice and the lean mice did not differ (Fig. 6A and B).

The RT-PCR results indicated that the expressions of the TLR-mediated proinflammatory cytokine genes, such as TNFα (1845% increase), IL-6 (441% increase), IL-12-p40 (466% increase), CXCL2 (395% increase) and IκBξ (244% increase), were more significantly up-regulated in the epididymal adipose tissue of the *ob*/*ob* mice than of their lean controls (Fig. 5D and E). The obesity-induced up-regulation of the TNFα (1606% increase), IL-12-p40 (63% increase), CXCL2 (76% increase), and IκBξ (151% increase) genes was less evident, whereas the over-expression of IL-6 (1126 % increase) was more evident, in the subcutaneous adipose tissue than in the epididymal adipose tissue of the *ob*/*ob* mice. In contrast, the expression levels of the adipose tissue IFNα and β genes of the *ob*/*ob* and lean mice did not differ (Fig. 6C and D). Leptin deficiency led to a significant up-regulation of the NFκB (p65 and p50) and down-regulations of STAT-1 and suppressor of cytokine signaling 1 (SOCS-1) in both visceral and subcutaneous fat tissues (Fig. 6E, F).

4. Discussion

TLRs play a crucial role in host defense against invading pathogens by mediating innate and adaptive immunities. TLR signaling pathways are initiated by a conserved cytosolic domain called the TIR domain, which associates with intracellular TIR-domain-containing adaptors including MyD88, Tirap, TRIF and the TRIF-related adaptor molecule (TRAM) [17-19,37]. Different TLRs trigger signals *via* different combinations of adaptors. The TLR4 activation recruits four major adaptors, MyD88, Tirap, TRIF, and TRAM. The TLR3 signaling depends mostly on TRIF, whereas TLR2 requires MyD88 and Tirap. The MyD88-dependent pathway includes the association and phosphorylation of IL-1 receptor-associated kinase (IRAK) 4 (IRAK4) and IRAK1. TRAF6 is recruited to these receptor complexes, which results in the activation of the IKK complex and the phosphorylation of IκB. The latter leads to the translocation of NFκB into the nucleus and eventually leads to increased expression of its target genes. The activation of downstream signaling pathways of TLR5, TLR7 or TLR9

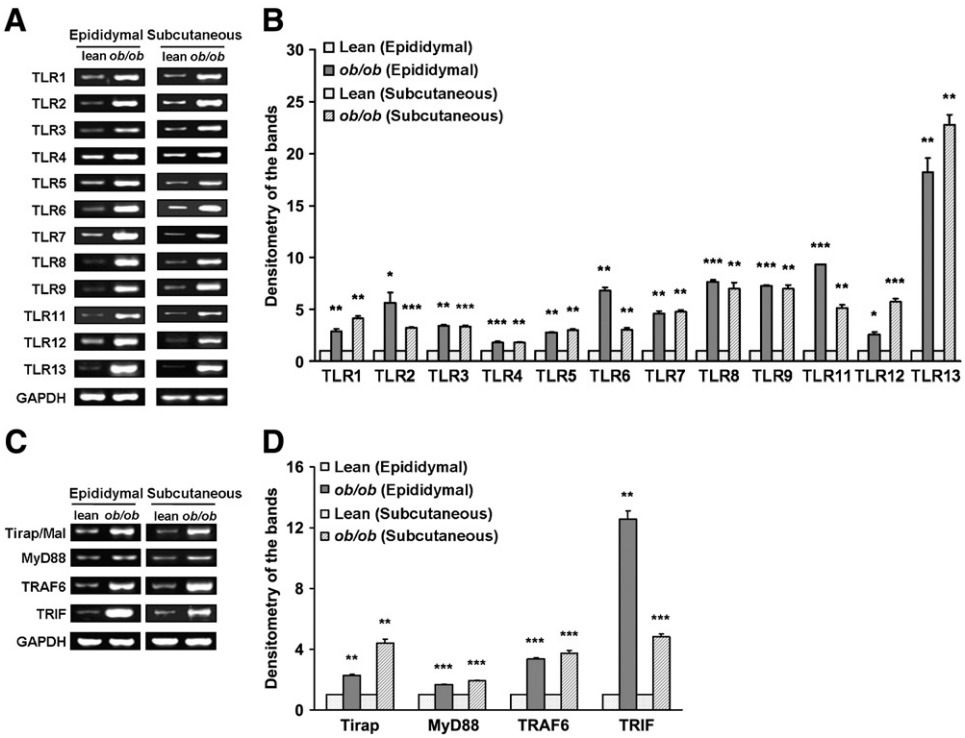

Fig. 5. Comparison of the expression of the TLRs and their adaptor molecules in the epididymal and subcutaneous adipose tissues of the *ob*/*ob* mice and of the lean control. (A) and (C) Representative image of the RT-PCR for the detection of the TLRs and their adaptor molecules. (B) and (D) Densitometry of the TLRs, adaptor molecules, and GAPDH gene bands for a semiquantitative comparison of their expression levels in the subcutaneous adipose tissues of the *ob*/*ob* and lean groups. The data are the mean±S.E.M. for three independent experiments using RNA samples pooled from five mice per group. **P*<.05; ***P*<.01; and ****P*<.001.

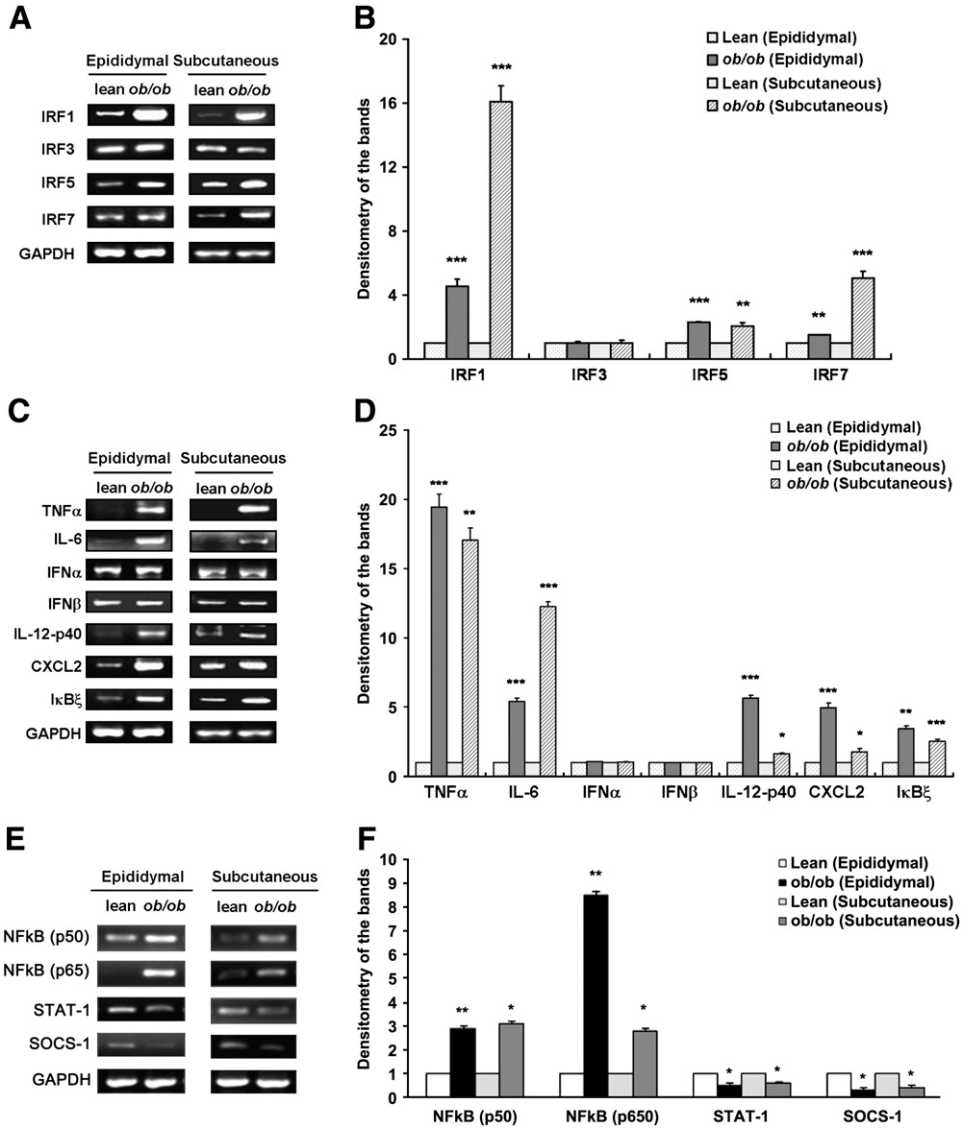

Fig. 6. Comparison of the expression of IRFs and TLR-mediated proinflammatory cytokine and chemokine genes in the epididymal and subcutaneous adipose tissues of the *ob/ob* and lean control mice. (A, C and E) Representative RT-PCR results of the detection of IRF, proinflammatory cytokine and chemokine, NFκB (p50, p65), STAT-1 and SOCS-1 gene expression. (B, D and F) Densitometry of the IRFs, TNFα, IL-6, IFNα, IFNβ, IL-12-p40, CXCL2, IκBξ, NFκB (p50, p65), STAT-1, SOCS-1 and GAPDH gene bands for a semi-quantitative comparison of their expression levels. The data are the mean±S.E.M. for three independent experiments using RNA samples pooled from five mice per group. **P*<.05; ***P*<.01; ****P*<.001.

also depends on MyD88. TLR7 and TLR9 induce the expression of Type I IFNs through the MyD88-IRF7 or -IRF1 pathway depending on the type of cell and/or ligand [17-19,37-43]. In contrast, the TRIF pathway is essential for the MyD88-independent pathway of the TLR-mediated signaling cascade. IKKε and the TRAF family member-associated NFκB activator-binding kinase 1 are the downstream kinases of TRIF, which lead to the activation of IRF3 and the subsequent expression of Type I IFNs and chemokines.

In addition to mature adipocytes, the adipose tissue is composed of various cell types; the remaining stromal vascular fraction includes preadipocytes, endothelial cells, fibroblasts and immune cells. In the advanced stages of obesity, there are various kinds of stromal immune cells such as neutrophils, T lymphocytes, and macrophages, which infiltrate into obese adipose tissue and thus, enhance the inflammatory changes through the crosstalk with parenchymal adipocytes [44,45]. The presence of TLRs was originally discovered in innate immune cells. Latest research, however, indicates that TLR expression is not restricted to innate immune cells and TLRs can be detected in adaptive immune cells as well as in parenchymal cells [46-48]. Thus, the dynamic change, such as increased TLR expression, seen in obese adipose tissues in the present study can be referred to as adipose tissue remodeling during the course of obesity, in which stromal cells change dramatically in number and cell type and/or to as activated TLR-mediated proinflammatory signaling cascades within a specific cell type.

Interestingly, this study indicates that the extent of the obesity-induced up-regulation of most TLR genes and related proinflammatory signaling cascades was much greater in the epididymal adipose tissues than in the subcutaneous fat tissues of the mice with DIO. It is possible that fat cells in various regions have different origins and, because of this, express different genes. For example, the expression levels of the *angiotensinogen* [33], *carboxypeptidase E*, *thrombospondin-1*, *calcyclin*, *ras* and *adipsin* genes [49,50] were greater in the visceral adipose tissues than in the subcutaneous adipose tissues of obese humans. It is well established that accumulation of visceral fat is associated with a higher risk of the development of obesity-related diseases such as Type 2 diabetes, cardiovascular disease, hypertension and hyperlipidemia in humans [51]. A recent study on mice reported

that enlarged visceral adipose tissues activate $CD8^+$ T cells, which, in turn, initiate and propagate inflammatory cascades, including the recruitment of monocytes and macrophages and their subsequent differentiation and activation [52]. In contrast, no significant changes were found in the numbers of the $CD8^+$ and $CD4^+$ T cells in the subcutaneous fat-pads of the obese mice, unlike in the lean controls [52]. These results seem to show that the visceral adipose tissue plays a more significant role in the development of metaflammation induced by an HFD than does the subcutaneous fat tissue.

A recent study suggested that saturated fatty acids activate both MyD88-dependent and -independent pathways in RAW264.7 cells [53] and modulate the activation of TLR4 and its downstream signaling pathways involving MyD88/IRAK/TRAF6 in macrophages (RAW264.7) and 293T cells transfected with TLR4 and myeloid differentiation-2 [54]. Creely et al. demonstrated that the expression of key components of innate immune cascades, including TLR2, MyD88 and TRAF6, increased more significantly in the subcutaneous adipose tissue of obese subjects and Type 2 diabetic patients than in the lean controls and non-diabetic subjects, respectively [29]. In this study, proof is given that obesity induced by diet or leptin gene mutation leads to significant induction of Triap, MyD88, TRAF6 and TRIF at their mRNA levels in both types of adipose tissues. These results suggest the first line of evidence that, in the enlarged adipose tissues of mice, TLR-mediated inflammatory signaling cascades activate both MyD88-dependent and MyD88-independent signaling molecules.

The capacity of TLRs to trigger the induction of tailored profiles of genes is underpinned by their ability to activate various transcription factors. MyD88-dependent and MyD88-independent pathways of TLR signaling couple to downstream protein kinases, which ultimately leads to the activation of transcription factors, such as NFκB, the activator protein-1 (AP-1), and the IRF family [17-19]. Much interest has been focused on NFκB, a transcription factor that is activated by all TLRs. Correlated with the HFD-induced changes in the TLR gene expression patterns, the extents of the obesity-induced activation and translocation of NFκB were much greater in the epididymal adipose tissue than in the subcutaneous adipose tissue of the HFD-fed mice, whereas the HFD did not induce the activation and translocation of AP-1 in the visceral and subcutaneous adipose tissues of the mice (data not shown). Therefore, the visceral adipose tissue, rather than the subcutaneous fat tissue, appears to play a critical role in TLR-mediated metaflammation in mice with DIO through the activation of NFκB.

Recent studies have highlighted specific members of the IRF family as crucial factors in the induction of TLR-responsive genes [41]. In this study, it was demonstrated that IRF5 expression was more significantly up-regulated in both types of adipose tissues of the DIO and *ob*/*ob* mice than in their lean controls. A recent publication [53] stated that FFAs lead to TLR4-mediated activation of IRF3, which results in the induction of the IFNβ expression and the subsequent autocrine/paracrine action of IFNβ, including STAT-1 phosphorylation and induction of proinflammatory genes in the macrophage [53]. The IRF1 mRNA expression was reported to be down-regulated by the SOCS-1 expression which is regulated by the STAT-1 [55]. Therefore, increased STAT-1 activation observed in the adipose tissue of DIO mice appears to play a role in down-regulating the IRF1 expression. In this study, the extents of the obesity-induced activation and translocation of STAT-1 were much greater in the epididymal adipose tissue than in the subcutaneous adipose tissue of the mice with DIO. In contrast, the *ob*/*ob* mice exhibited significantly decreased mRNA level of STAT-1 in their epididymal and subcutaneous adipose tissues compared with the lean controls. Leptin is known to activate JAK-STAT signaling [56], and as a result, STAT-1 expression and phosphorylation were reduced in the lungs of *ob*/*ob* vs. wild-type mice [57].

Adipose tissues or adipocytes release proinflammatory cytokines, including TNFα, IL-1 and IL-6, and chemokines such as monocyte chemoattractant protein-1, chemokine (C-C motif) ligands, and CXCLs via TLR-mediated NFκB activation cascades [13,22,24,28,29,32]. The TLR-dependent release of cytokines and chemokines from adipose tissues could directly mediate severe inflammatory responses and insulin resistance. The DIO and *ob*/*ob* mice exhibited significantly increased mRNA levels of various proinflammatory cytokine and chemokine genes such as TNFα, IL-6, IL-12-p40 and CXCL2 in their visceral and subcutaneous adipose tissues.

The magnitudes of the obesity-induced up-regulation of the TLR1, TLR4, TLR5, TLR8, TLR9 and TLR12 genes in the visceral adipose tissue were greater in the DIO mice than in the *ob*/*ob* mice. Similarly, the expression of the IFNα and IFNβ genes significantly increased in the adipose tissues of the DIO mice but did not change in the adipose tissues of the *ob*/*ob* mice. Therefore, in general, visceral and subcutaneous adipose tissues appear to have equal contributions, based on their regulation of relevant genes, to the obesity-induced activation of inflammatory responses mediated through TLRs and downstream signaling molecules. Nevertheless, the magnitudes of the obesity-induced up-regulation of IκBξ, IRF1 and IRF7 genes in the visceral and subcutaneous adipose tissues were greater in the *ob*/*ob* mice than in the DIO mice. These different degrees of responsiveness of TLR-mediated proinflammatory signaling cascades seen in the DIO and *ob*/*ob* mice could possibly be attributed to the presence and absence of leptin. Recent data from literature indicate that adipokines can actively participate in the regulation of the immune system. This is especially so for leptin, a hormone that can be structurally and functionally classified as a cytokine [58,59]. Leptin-deficient *ob*/*ob* mice are protected in multiple models of inflammation such as autoimmune encephalomyelitis, collagen-induced arthritis, delayed-type hypersensitivity and dextran sulfate sodium- as well as trinitrobenzene sulfonic acid-induced colitis [60-64]. Furthermore, a recent study showed that leptin strongly regulated the expression and responsiveness of TLR1–9 in murine preadipocytes as well as adipocytes [32]. Further investigation is needed to explain the role of leptin in association with TLR-mediated proinflammatory signaling cascades.

In summary, this study showed that obesity that was induced by an HFD or a leptin deficiency up-regulated the expression of TLR1 to 9 and TLR11 to 13 in murine adipose tissues. These up-regulated expressions of TLRs in the expanded adipose tissues of obese animals are linked with downstream NFκB, IRFs and STAT-1 activation and up-regulated cytokines and chemokines expression via MyD88-dependent and -independent cascades in the adipose tissues of the obese mice. The visceral adipose tissues were much more susceptible to DIO than the subcutaneous adipose tissues in terms of the induction and/or activation of TLR genes and related proinflammatory signaling cascades. Furthermore, the magnitudes of the obesity-induced up-regulation of most of the TLR genes, downstream signaling molecules and target cytokines in the visceral adipose tissue were greater in the DIO mice than in the *ob*/*ob* mice. It is suggested that TLRs and related proinflammatory signaling molecules that are over-expressed in the enlarged adipose tissues of mice with DIO or *ob*/*ob* mice may play an important role in the obesity-associated phenomenon of metaflammation.

References

[1] Schaffler A, Muller-Ladner U, Scholmerich J, Buchler C. Role of adipose tissue as an inflammatory organ in human diseases. Endocr Rev 2006;27:449–67.
[2] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.
[3] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473–81.
[4] Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 2003;112:1785–8.

[5] Juge-Aubry CE, Somm E, Chicheportiche R, Burger D, Pernin A, Cuenod-Pittet B, et al. Regulatory effects of interleukin (IL)-1, interferon-beta, and IL-4 on the production of IL-1 receptor antagonist by human adipose tissue. J Clin Endocrinol Metab 2004;89:2652–8.
[6] Bunout D, Munoz C, Lopez M, de la Maza MP, Schlesinger L, Hirsch S, et al. Interleukin 1 and tumor necrosis factor in obese alcoholics compared with normal-weight patients. Am J Clin Nutr 1996;63:373–6.
[7] Boden G, She P, Mozzoli M, Cheung P, Gumireddy K, Reddy P, et al. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. Diabetes 2005;54:3458–65.
[8] Ajuwon KM, Spurlock ME. Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3-L1 adipocytes. J Nutr 2005;135:1841–6.
[9] Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol 2001;1:135–45.
[10] Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001;2:675–80.
[11] Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005;17:1–14.
[12] Hwa Cho H, Bae YC, Jung JS. Role of toll-like receptors on human adipose-derived stromal cells. Stem Cells 2006;24:2744–52.
[13] Kopp A, Buechler C, Neumeier M, Weigert J, Aslanidis C, Scholmerich J, et al. Innate immunity and adipocyte function: ligand-specific activation of multiple Toll-like receptors modulates cytokine, adipokine, and chemokine secretion in adipocytes. Obesity (Silver Spring) 2009;17:648–56.
[14] Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000;406:782–7.
[15] Medzhitov R, Janeway Jr CA. Innate immunity: the virtues of a nonclonal system of recognition. Cell 1997;91:295–8.
[16] O'Neill LA. Targeting signal transduction as a strategy to treat inflammatory diseases. Nat Rev Drug Discov 2006;5:549–63.
[17] Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004;4:499–511.
[18] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006;124:783–801.
[19] O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 2007;7:353–64.
[20] Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, et al. A toll-like receptor that prevents infection by uropathogenic bacteria. Science 2004;303:1522–6.
[21] Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 2005;308:1626–9.
[22] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006;116:3015–25.
[23] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006;346:739–45.
[24] Vitseva OI, Tanriverdi K, Tchkonia TT, Kirkland JL, McDonnell ME, Apovian CM, et al. Inducible Toll-like receptor and NF-kappaB regulatory pathway expression in human adipose tissue. Obesity (Silver Spring) 2008;16:932–7.
[25] Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, et al. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007;56:1986–98.
[26] Radin MS, Sinha S, Bhatt BA, Dedousis N, O'Doherty RM. Inhibition or deletion of the lipopolysaccharide receptor Toll-like receptor-4 confers partial protection against lipid-induced insulin resistance in rodent skeletal muscle. Diabetologia 2008;51:336–46.
[27] Senn JJ. Toll-like receptor-2 is essential for the development of palmitate-induced insulin resistance in myotubes. J Biol Chem 2006;281:26865–75.
[28] Murakami K, Bujo H, Unoki H, Saito Y. High fat intake induces a population of adipocytes to co-express TLR2 and TNFalpha in mice with insulin resistance. Biochem Biophys Res Commun 2007;354:727–34.
[29] Creely SJ, McTernan PG, Kusminski CM, Fisher M, Da Silva NF, Khanolkar M, et al. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 2007;292:E740–7.
[30] Schaffler A, Scholmerich J, Salzberger B. Adipose tissue as an immunological organ: Toll-like receptors, C1q/TNFs and CTRPs. Trends Immunol 2007;28:393–9.
[31] Pietsch J, Batra A, Stroh T, Fedke I, Glauben R, Okur B, et al. Toll-like receptor expression and response to specific stimulation in adipocytes and preadipocytes: on the role of fat in inflammation. Ann N Y Acad Sci 2006;1072:407–9.
[32] Batra A, Pietsch J, Fedke I, Glauben R, Okur B, Stroh T, et al. Leptin-dependent toll-like receptor expression and responsiveness in preadipocytes and adipocytes. Am J Pathol 2007;170:1931–41.
[33] Arner P. Not all fat is alike. Lancet 1998;351:1301–2.
[34] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 1957;226:497–509.
[35] Choi YH, Kim HI, Seong JK, Yu DY, Cho H, Lee MO, et al. Hepatitis B virus X protein modulates peroxisome proliferator-activated receptor gamma through protein-protein interaction. FEBS Lett 2004;557:73–80.
[36] Israel A. A role for phosphorylation and degradation in the control of NF-kappa B activity. Trends Genet 1995;11:203–5.
[37] Lee JY, Hwang DH. The modulation of inflammatory gene expression by lipids: mediation through Toll-like receptors. Mol Cells 2006;21:174–85.
[38] Honda K, Yanai H, Mizutani T, Negishi H, Shimada N, Suzuki N, et al. Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. Proc Natl Acad Sci U S A 2004;101:15416–21.
[39] Honda K, Yanai H, Negishi H, Asagiri M, Sato M, Mizutani T, et al. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 2005;434:772–7.
[40] Kawai T, Sato S, Ishii KJ, Coban C, Hemmi H, Yamamoto M, et al. Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat Immunol 2004;5:1061–8.
[41] Moynagh PN. TLR signalling and activation of IRFs: revisiting old friends from the NF-kappaB pathway. Trends Immunol 2005;26(9):469–76.
[42] Negishi H, Fujita Y, Yanai H, Sakaguchi S, Ouyang X, Shinohara M, et al. Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. Proc Natl Acad Sci U S A 2006;103:15136–41.
[43] Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, et al. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 2003;301:640–3.
[44] Wasserman F. Handbook of Physiology. Washington, DC, USA: American Physiology Society; 1965.
[45] Suganami T, Ogawa Y. J Adipose tissue macrophages: their role in adipose tissue remodeling. Leukoc Biol 2010;88:33–9.
[46] Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. Mol Cell 1998;2:253–8.
[47] Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, et al. Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. J Immunol 2002;168:4531–7.
[48] Gelman AE, Zhang J, Choi Y, Turka LA. Toll-like receptor ligands directly promote activated CD4+ T cell survival. J Immunol 2004;172:6065–73.
[49] Ramis JM, Franssen-van Hal NL, Kramer E, Llado I, Bouillaud F, Palou A, et al. Carboxypeptidase E and thrombospondin-1 are differently expressed in subcutaneous and visceral fat of obese subjects. Cell Mol Life Sci 2002;59:1960–71.
[50] Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G. Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. J Lipid Res 2004;45:148–54.
[51] Larsson B, Bengtsson C, Bjorntorp P, Lapidus L, Sjostrom L, Svardsudd K, et al. Is abdominal body fat distribution a major explanation for the sex difference in the incidence of myocardial infarction? The study of men born in 1913 and the study of women, Goteborg, Sweden. Am J Epidemiol 1992;135:266–73.
[52] Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009;15:914–20.
[53] Abe M, Matsuda M, Kobayashi H, Miyata Y, Nakayama Y, Komuro R, et al. Effects of statins on adipose tissue inflammation: their inhibitory effect on MyD88-independent IRF3/IFN-beta pathway in macrophages. Arterioscler Thromb Vasc Biol 2008;28:871–7.
[54] Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, et al. 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 2003;278:37041–51.
[55] Krebs DL, Hilton DJ. SOCS proteins: negative regulators of cytokine signaling. Stem Cells 2001;19:378–87.
[56] Håkansson-Ovesjö ML, Collin M, Meister B. Down-regulated STAT3 messenger ribonucleic acid and STAT3 protein in the hypothalamic arcuate nucleus of the obese leptin-deficient (ob/ob) mouse. Endocrinology 2000;141:3946–55.
[57] Lang JE, Williams ES, Mizgerd JP, Shore SA. Effect of obesity on pulmonary inflammation induced by acute ozone exposure: role of interleukin-6. Am J Physiol Lung Cell Mol Physiol 2008;294:L1013–20.
[58] Baumann H, Morella KK, White DW, Dembski M, Bailon PS, Kim H, et al. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc Natl Acad Sci U S A 1996;93:8374–8.
[59] Zhang F, Basinski MB, Beals JM, Briggs SL, Churgay LM, Clawson DK, et al. Crystal structure of the obese protein leptin-E100. Nature 1997;387:206–9.
[60] Busso N, So A, Chobaz-Peclat V, Morard C, Martinez-Soria E, Talabot-Ayer D, et al. Leptin signaling deficiency impairs humoral and cellular immune responses and attenuates experimental arthritis. J Immunol 2002;168:875–82.
[61] Mancuso P, Gottschalk A, Phare SM, Peters-Golden M, Lukacs NW, Huffnagle GB. Leptin-deficient mice exhibit impaired host defense in Gram-negative pneumonia. J Immunol 2002;168:4018–24.
[62] Mandel MA, Mahmoud AA. Impairment of cell-mediated immunity in mutation diabetic mice (db/db). J Immunol 1978;120:1375–7.
[63] Matarese G, Di Giacomo A, Sanna V, Lord GM, Howard JK, Di Tuoro A, et al. Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. J Immunol 2001;166:5909–16.
[64] Siegmund B, Lehr HA, Fantuzzi G. Leptin: a pivotal mediator of intestinal inflammation in mice. Gastroenterology 2002;122:2011–25.