

# Effect of peroxisome proliferator-activated receptor $\gamma$ on the cholesterol efflux of peritoneal macrophages in inflammation

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Received September 19, 2013; Accepted March 12, 2014

DOI: 10.3892/mmr.2014.2200

**Abstract.** Atherosclerosis, a chronic inflammatory disorder characterized by lipid and cholesterol accumulation, is the principal contributing factor to the pathology of cardiovascular disease. Macrophages contribute to plaque development by internalizing native and modified lipoproteins that convert them into cholesterol-rich foam cells. With multiple factors, including hypercholesterolemia and inflammation, promoting atherosclerosis, it is of great significance to elucidate how the mechanism of cholesterol efflux from the macrophages changes and the role of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) in these situations. Following isolation and culture of peritoneal macrophages from C57BL/6 mice in the present study, the cells were divided into three groups: The control group, the ciglitazone group and the PPAR $\gamma$  antisense oligonucleotide group. The expression of PPAR $\gamma$  and nuclear factor of  $\kappa$  light polypeptide gene enhancer in B-cells inhibitor  $\alpha$  (I $\kappa$ B $\alpha$ ) in each group was observed through the levels of protein and mRNA, and then the cholesterol efflux of each group was investigated. In addition, the same experiments were repeated following stimulation of each group with lipopolysaccharide (LPS). No significant difference in the expression levels of PPAR $\gamma$  between the control group and ciglitazone group was observed. The expression levels of PPAR $\gamma$  in the PPAR $\gamma$  antisense oligonucleotide group were evidently lower than those in the control group. Subsequent to stimulation with LPS, the expression levels of PPAR $\gamma$  in the three groups were higher than those of each group prior to stimulation. The cholesterol efflux of the PPAR $\gamma$  antisense oligonucleotide group was clearly suppressed following stimulation with LPS in comparison with that of the other groups. PPAR $\gamma$  contributes to anti-inflammation by protecting I $\kappa$ B $\alpha$  from being

phosphorylated and degraded and promoting cholesterol efflux from peritoneal macrophages in inflammation.

## Introduction

Atherosclerosis, a chronic inflammatory disease of blood vessels, is one of the main causes of cardiovascular disease, which is the most common cause of mortality in industrialized societies and is increasingly becoming the leading cause of mortality worldwide (1). Atherosclerosis is induced by multiple factors and regulated by a number of genes (2). Macrophages, which are known to reside within atherosclerotic plaques, contribute to the pathology of atherosclerosis by internalizing native or modified lipoproteins or lipoprotein remnants that have invaded the vessel wall to form cholesterol-rich foam cells (3). As macrophages are one of the precursors of foam cells, their cholesterol counter transport system (cholesterol efflux) is important to maintain the balance of cholesterol in cells and influence the formation of foam cells (4). The conversion of macrophages into foam cells is orchestrated by disruption of the normal cholesterol homeostatic mechanism that controls the uptake, intracellular metabolism and efflux of cholesterol (5).

The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), a member of a superfamily of ligand-dependent transcription factors that regulate immunity and inflammation, is one of the nuclear receptors expressed in macrophages (6,7). Numerous studies have indicated that PPAR $\gamma$  and its ligands promote cholesterol efflux from macrophages through the PPAR $\gamma$ -liver X receptor  $\alpha$ -ATP-binding cassette, sub-family A, member 1 signaling pathway (8), and this process may downregulate the expression of pro-inflammatory genes in macrophages that may be associated with the transrepression of the transcription factor nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells (NF- $\kappa$ B) (9,10). A study concerning the anti-inflammatory effects of PPAR $\gamma$  has shown that its agonists markedly inhibit the secretion of pro-inflammatory mediators, including tumor necrosis factor- $\alpha$  and interleukin-1 and -6 in activated macrophages (11). However, the effect of PPAR $\gamma$  on the cholesterol efflux of macrophages in inflammation remains unclear. Pretreating wild-type mice with PPAR $\gamma$  ligands may reduce the expression of pro-inflammatory cytokines and alleviate injury of local and distant tissues (12), which

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**Key words:** peroxisome proliferator-activated receptor  $\gamma$ , cholesterol efflux, peritoneal macrophage

has a therapeutic effect in numerous inflammatory diseases, including acute myocarditis, autoimmune encephalitis and multiple sclerosis (13).

Atherosclerosis has been acknowledged as a consequence of lipid metabolism disorder and chronic inflammation (14). Thus, with multiple factors, including hypercholesterolemia and inflammation, promoting atherosclerosis either individually or in combination, it is of great significance to clarify how the mechanism of cholesterol efflux from macrophages changes and the role of PPAR $\gamma$  in these situations. This will help to explain the formation of foam cells and provide novel methods of preventing and curing atherosclerosis.

In view of the action of PPAR $\gamma$  on various key transcriptional factors, we proposed the hypothesis that PPAR $\gamma$  is the primary regulator of macrophage cholesterol efflux and suppressor of the inflammatory response. The present study aimed to provide evidence to elucidate the possible mechanism of PPAR $\gamma$  on the cholesterol efflux of peritoneal macrophages in inflammation and the role of PPAR $\gamma$  in maintaining the balance between the cholesterol efflux and anti-inflammatory response.

## Materials and methods

**Reagents and kits.** LPS (*Escherichia coli*, O111:B4) was purchased from Sigma (St. Louis, MO, USA) and reconstituted in phosphate-buffered saline (PBS). PPAR $\gamma$  antibody (rabbit anti-mouse) and PPAR $\gamma$  antibody (goat anti-rabbit) were purchased from Sigma. Phosphor-nuclear factor of  $\kappa$  light polypeptide gene enhancer in B-cells inhibitor  $\alpha$  (I $\kappa$ B $\alpha$ ; Ser32) was purchased from Youyizhonglian Bio-Corporation (Beijing, China). [ $^3$ H] cholesterol and apolipoprotein AI (ApoAI) were purchased from Sigma. Ciglitazone was purchased from Sigma, and the final concentration of ciglitazone dissolved in dimethylsulfoxide (DMSO) was 3  $\mu$ mol/ml. The sequences of the PPAR $\gamma$  antisense and missense oligonucleotides were 5'-CATGAGGCTTATTGTAGAGCTGA-3' and 5'-GCCAGGTACCACTCACTCTGCAGT-3', respectively. The procedure of synthesis, purification and subpackage of the sequence was operated by Shengong Bio-Corporation (Shanghai, China).

**Animals.** Fifteen C57BL/6 mice (8-10 weeks old, males, weighing 20-26 g) were obtained from the Laboratory Animal Centre of Chongqing Medical University (Chongqing, China). These mice were housed in an animal room and fed a standard diet. All experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation of Chongqing Medical University.

**Experimental protocol.** The 15 mice were randomly divided into three groups. Proceeding from isolation and culture of peritoneal macrophages from the C57BL/6 mice, the cells were divided into three groups: The control group, the ciglitazone group and the PPAR $\gamma$  antisense oligonucleotide group. The expression of PPAR $\gamma$  and I $\kappa$ B $\alpha$  in each group was observed through the levels of protein and mRNA, and then the cholesterol efflux of each group was investigated. The intraperitoneal injection of LPS into mice is a widely used method of constructing inflammatory animal models (15). In

Table I. Primer sequences for RT-PCR.

DNA amplified	Primer sequence (5'→3')	Size (bp)
PPAR $\gamma$		476
Sense	CAATCCGAATTTTCAAGGGTGCCA	
Antisense	GAGCACCTTGGCGAACAGCTGAGAG	
$\beta$ -actin		355
Sense	GAGAAGAGCTATGAACTTCCTGACG	
Antisense	TTGCTGGAAGGTGGACAGAGAGGC	

RT-PCR, reverse transcription-polymerase chain reaction; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ .

addition, the same experiment was repeated subsequent to stimulation of each group with LPS.

**Isolation and treatment of peritoneal macrophages.** Pre-cooled PBS (2 ml) was injected into the abdominal cavity of the mice, whilst the abdomen was kneaded softly for 2 min. The PBS was drawn out and collected, and then centrifuged for 10 min at 2,000  $\times$  g. The supernatant liquid was discarded and placed in RPMI-1640, which regulated the concentration of the cells at 3-5 $\times$ 10<sup>6</sup> cells/ml. The cells were cultivated in 24-well plates at 37°C for 2 h until they had adhered, then the cultivation holes were washed with pre-cooled PBS. The adherent cells were peritoneal macrophages. The peritoneal macrophages were cultivated for 24 h and then randomly divided into three groups: The control group (RMPI-1640+25  $\mu$ l DMSO), the ciglitazone group (RMPI-1640+25  $\mu$ l DMSO+ciglitazone; final concentration, 10  $\mu$ mol/l), and the PPAR $\gamma$  antisense oligonucleotide group (RMPI-1640+25  $\mu$ l DMSO+PPAR $\gamma$  antisense oligonucleotide; final concentration, 400 nmol/l). The final concentration of LPS was 80 ng/ml.

**Immunocytochemical staining analysis of peritoneal macrophages.** The peritoneal macrophages were cultured on a chamber slide, which was washed with PBS and air-dried. The slide was fixed with methanol for 30 min at -20°C and then stained with PPAR $\gamma$  antibody (rabbit anti-mouse; Abcam, Cambridge, MA, USA) and PPAR $\gamma$  antibody (goat anti-rabbit; Abcam) for 24 h at room temperature. The cells that were stained purple were considered positive.

**Assessment of gene expression of PPAR $\gamma$  by quantitative reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA samples of the peritoneal macrophages were extracted using an RNA extraction kit Takara Bio Inc. (Shiga, Japan) according to the manufacturer's instructions. Total RNA was quantified with the ratio of absorption values of RNA samples at 260 and 280 nm. Each total RNA sample was reversely transcribed to complementary DNA using an RT-PCR kit and stored at -70°C. All PCR products were electrophoresed on 2% agarose gels. The RT-PCR was performed using the sense and antisense primers for PPAR $\gamma$  or  $\beta$ -actin (Table I). The relative expression of mRNAs were assessed by taking the ratio of the intensity of the DNA bands of PPAR $\gamma$

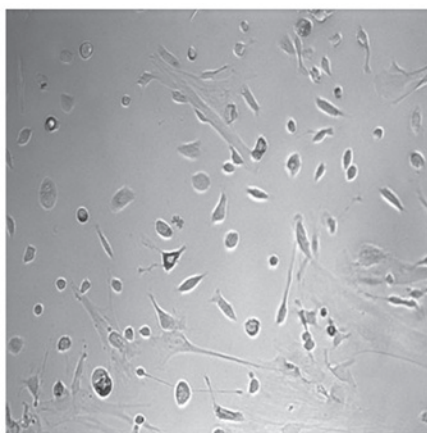


Figure 1. Normal peritoneal macrophages. Magnification, x200.

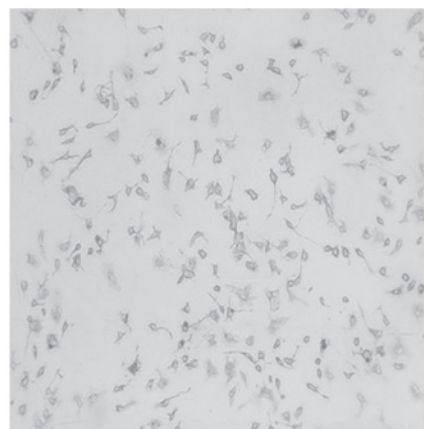


Figure 2. Immunocytochemical staining of PPAR $\gamma$  in peritoneal macrophages prior to stimulation by LPS. Magnification, x200. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; LPS, lipopolysaccharide.

to the  $\beta$ -actin band using the Bio-Image analysis system (Gel Doc 2000; Bio-Rad, Hercules, CA, USA) and expressed as arbitrary units.

**Western blotting analysis of PPAR $\gamma$  and I $\kappa$ B $\alpha$ .** Total protein of the peritoneal macrophages was extracted by homogenizing the macrophages in a cell lysis buffer (Beyotime Institute of Biotechnology, Shanghai, China), then by two cycles of centrifugation at 12,000  $\times$  g for 15 min. Protein concentration was determined using a Bradford assay kit (Beyotime Institute of Biotechnology). The total protein was separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes, which were then incubated with rabbit anti-mouse PPAR $\gamma$  polyclonal antibody (diluted 1:1000, IMG-441; Sigma) and horseradish peroxidase-conjugated goat anti-rabbit IgG (diluted 1:2000; Zhongshan Jinqiao, Beijing, China). The immune complexes were developed with an Enhanced Chemiluminescence Detection kit (Pierce Biotechnology, Inc., Rockford, IL, USA) and the membranes were then immediately exposed to autoradiographic film (Kodak, Rochester, NY, USA). The relative amount of PPAR $\gamma$  protein was quantified from the optical density of the corresponding band by Bio-Image analysis system (Gel Doc 2000; Bio-Rad).

**Determination of peritoneal macrophage cholesterol efflux.** The concentration of macrophages was regulated at  $3.0 \times 10^9$  cells/l and the macrophages were transferred into RPMI-1640 containing fetal bovine serum, penicillin and streptomycin, and [ $^3$ H] cholesterol. After 24 h, the cells were cultivated in new medium containing 50  $\mu$ g/ml ApoAI for 12 h. The [ $^3$ H]cholesterol in the culture solution and cells was detected by liquid scintillation counting. The effluxion of cholesterol was calculated using the following formula: [ $^3$ H] (culture solution)/[ $^3$ H] (culture solution and cells)  $\times$  100.

**Statistical analysis.** Data are reported as the mean  $\pm$  standard deviation and were analyzed using one-way analysis of variance with Tukey's multiple comparison test, and the statistical program SPSS, version 11.0 (SPSS, Inc., Chicago, IL, USA).  $P \leq 0.05$  was considered to indicate a statistically significant difference.

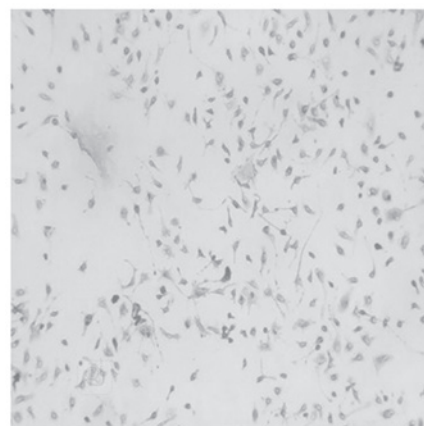


Figure 3. Immunocytochemical staining of PPAR $\gamma$  in peritoneal macrophages following stimulation by LPS. Magnification, x200. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; LPS, lipopolysaccharide.



Figure 4. RT-PCR analysis of PPAR $\gamma$  mRNA expression in each group prior to and following stimulation with LPS. Group 1, stimulated ciglitazone group; group 2, unstimulated ciglitazone group; group 3, stimulated AODN group; group 4, unstimulated AODN group; group 5, stimulated AODN group; group 6, unstimulated AODN group. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; RT-PCR, reverse transcription-polymerase chain reaction; LPS, lipopolysaccharide; AODN, antisense oligonucleotide group.

## Results

**Immunocytochemical staining analysis of peritoneal macrophages.** Peritoneal macrophages from C57BL/6 mice were isolated and cultured (Fig. 1). Subsequently, PPAR $\gamma$  in peritoneal macrophages prior to and following stimulation by LPS

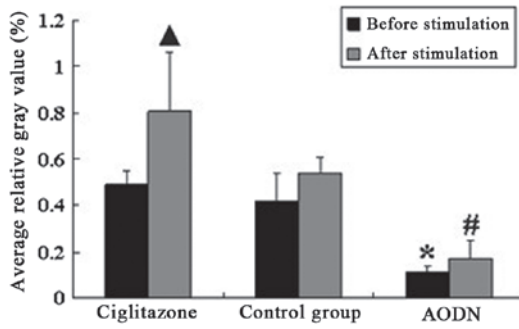


Figure 5. RT-PCR analysis of PPAR $\gamma$  mRNA expression in each group prior to and following stimulation by LPS. (\* $P < 0.01$  vs. unstimulated control group,  $\Delta P < 0.05$  vs. stimulated control group,  $\#P < 0.01$  vs. stimulated control group). Data are presented as the mean  $\pm$  standard deviation. AODN, antisense oligonucleotide group; RT-PCR, reverse transcription-polymerase chain reaction; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; LPS, lipopolysaccharide.

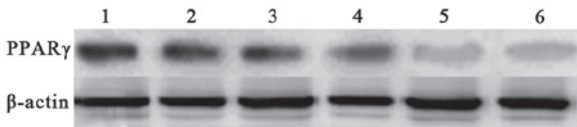


Figure 6. Expression of PPAR $\gamma$  protein in each group prior to and following stimulation with LPS. Group 1, stimulated ciglitazone group; group 2, unstimulated ciglitazone group; group 3, stimulated control group; group 4, unstimulated control group; group 5, stimulated AODN group; group 6, unstimulated AODN group. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; LPS, lipopolysaccharide; AODN, antisense oligonucleotide group.

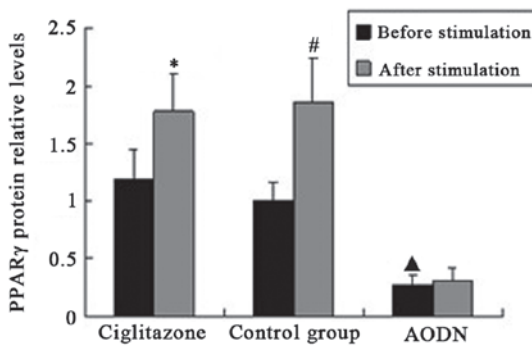


Figure 7. Expression of PPAR $\gamma$  protein in each group prior to and following stimulation with LPS. (\* $P < 0.05$  vs. the unstimulated ciglitazone group,  $\#P < 0.05$  vs. unstimulated control group,  $\Delta P < 0.01$  vs. unstimulated control group). Data are presented as the mean  $\pm$  standard deviation. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; AODN, antisense oligonucleotide group; LPS, lipopolysaccharide.

was examined using immunocytochemistry (Figs. 2 and 3). It was found that the number of PPAR $\gamma$ -positive cells following stimulation by LPS was greater than that prior to stimulation by LPS. The PPAR $\gamma$ -positive cells were stained purple.

*Expression of PPAR $\gamma$  mRNA in peritoneal macrophages pre- and post-LPS stimulation.* The results of the RT-PCR indicated that there was no significant difference in the average relative gray value between the control group and ciglitazone group. The average relative gray value of the

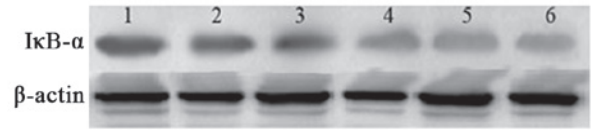


Figure 8. Expression of I $\kappa$ B- $\alpha$  protein in each group prior to and following stimulation with LPS. Group 1, unstimulated control group; group 2, unstimulated ciglitazone group; group 3, unstimulated AODN group; group 4, stimulated control group; group 5, stimulated ciglitazone group; group 6, stimulated AODN group. I $\kappa$ B- $\alpha$ , NF- $\kappa$ B inhibitor; LPS, lipopolysaccharide; AODN, antisense oligonucleotide group.

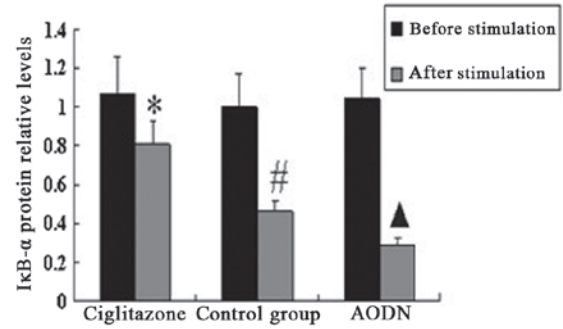


Figure 9. Expression of I $\kappa$ B- $\alpha$  protein in each group prior to and following stimulation with LPS. (\* $P < 0.05$  vs. unstimulated ciglitazone group,  $\#P < 0.05$  vs. unstimulated control group,  $\Delta P < 0.01$  vs. unstimulated AODN group). Data are presented as the mean  $\pm$  standard deviation. I $\kappa$ B- $\alpha$ , nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells inhibitor  $\alpha$ ; AODN, antisense oligonucleotide group; LPS, lipopolysaccharide.

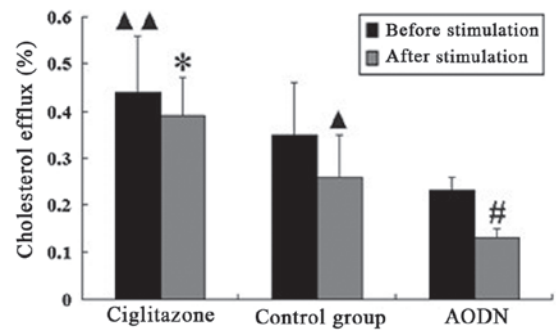


Figure 10. Cholesterol efflux of peritoneal macrophages in each group prior to and following stimulation with LPS. (\* $P < 0.05$  vs. unstimulated ciglitazone group,  $\Delta P < 0.05$  vs. unstimulated control group,  $\#P < 0.05$  vs. unstimulated AODN group,  $\Delta\Delta P < 0.05$  vs. unstimulated control group). Data are presented as the mean  $\pm$  standard deviation. AODN, antisense oligonucleotide group; LPS, lipopolysaccharide.

PPAR $\gamma$  antisense oligonucleotide group was evidently lower than that of control group. Following stimulation with LPS, the expression levels of PPAR $\gamma$  mRNA in the ciglitazone group were higher than those in the control group, while the PPAR $\gamma$  mRNA expression levels of the PPAR $\gamma$  antisense oligonucleotide group were lower than those of the control group (Figs. 4 and 5).

*Expression of PPAR $\gamma$  and I $\kappa$ B $\alpha$  protein in peritoneal macrophages pre- and post-LPS stimulation.* The results of the western blotting suggested that there was no significant

Table II. Cholesterol efflux of the peritoneal macrophages in each group prior to and following stimulation with LPS (mean  $\pm$  standard deviation, %).

Measurement	Ciglitazone	Control	AODN
Cholesterol efflux			
Prior to stimulation	0.44 $\pm$ 0.12 <sup>d</sup>	0.35 $\pm$ 0.11	0.23 $\pm$ 0.03
Following stimulation	0.39 $\pm$ 0.08 <sup>a</sup>	0.26 $\pm$ 0.09 <sup>b</sup>	0.13 $\pm$ 0.02 <sup>c</sup>
Suppression ratio	11.37	25.72	43.48

<sup>a</sup>P<0.05 vs. unstimulated ciglitazone group; <sup>b</sup>P<0.05 vs. unstimulated control group; <sup>c</sup>P<0.05 vs. unstimulated AODN group; <sup>d</sup>P<0.05 vs. unstimulated control group. LPS, lipopolysaccharide; AODN, antisense oligonucleotide group.

difference in the expression of PPAR $\gamma$  protein between the control group and ciglitazone group. The PPAR $\gamma$  protein expression levels of the PPAR $\gamma$  antisense oligonucleotide group were considerably lower than those of the control group. Subsequent to stimulation with LPS, the expression levels of PPAR $\gamma$  protein in the three groups were higher than those of each group prior to stimulation, and the I $\kappa$ B $\alpha$  protein expression levels of the three groups were lower than those of each group prior to stimulation (Figs. 6-9).

*Cholesterol efflux of peritoneal macrophages in each group pre- and post-LPS stimulation.* The cholesterol efflux of the ciglitazone group was suppressed following stimulation with LPS, and the suppression ratio was lower than that of the control group. However, the cholesterol efflux of the PPAR $\gamma$  antisense oligonucleotide group was greatly suppressed following stimulation with LPS, and the suppression ratio was higher than that of the control group (Table II, Fig. 10).

## Discussion

It has been universally acknowledged that atherosclerosis is a disease associated with lipid metabolic disturbance and chronic inflammation (16). Foam cells form from macrophages, in which the cholesterol accumulation is the significant pathological characteristic of atherosclerotic lesions (17). The balance of cholesterol in macrophages depends on coordinated regulation of cholesterol, including intake, storage, *de novo* synthesis and efflux (18). As a thiazolidinedione, ciglitazone is a high-affinity ligand for PPAR $\gamma$  (19,20), and is able to activate PPAR $\gamma$  to suppress the activation of inflammatory cells and delivery of inflammatory mediators (21).

In the present study, measurement of the cholesterol efflux of peritoneal macrophages in each group indicated that pretreating macrophages with ciglitazone increases the cholesterol efflux. However, the cholesterol efflux was weaker in the PPAR $\gamma$  antisense oligonucleotide group compared with that in the control group. This demonstrates that PPAR $\gamma$ , when activated by its ligand ciglitazone, greatly reinforces the cholesterol efflux of peritoneal macrophages. In inflammation, the cholesterol efflux of the three groups was suppressed, but the suppression ratio varied. The cholesterol efflux of the PPAR $\gamma$  antisense oligonucleotide group was evidently suppressed following stimulation with LPS, and the suppression ratio was

higher than that of the other two groups. The technique of knockdown using antisense nucleic acids was selected due to its benefits, which include strong specificity to target site, few side-effects and a precise depression effect (22). The results indicate that PPAR $\gamma$  is associated with the suppression of the cholesterol efflux resulting from LPS stimulation. This study demonstrated that stimulation of peritoneal macrophages with LPS suppresses the cholesterol efflux, even when the expression of PPAR $\gamma$  is upregulated, so it was presumed that the activation of PPAR $\gamma$  was affected due to its anti-inflammatory characteristic. When pro-atherosclerotic factors, including inflammation and hypercholesterolemia coexist, the anti-inflammatory effect of PPAR $\gamma$  is of great significance.

The activation of NF- $\kappa$ B is an important signal transmission pathway that produces various pro-inflammatory factors (23). NF- $\kappa$ B consists mainly of the heterodimer p50/p65, which is generally bound to I $\kappa$ B, maintaining a state of inactivation in the cytoplasm (24,25). LPS binds to the corresponding receptor in the cytomembrane and leads to phosphorylation and degradation of I $\kappa$ B, and then NF- $\kappa$ B is released into the nucleus to promote the transcription of target genes (26). Certain studies have indicated that PPAR $\gamma$  may suppress several inflammation-correlated signaling pathways, including Janus kinase-signal transducer and activator of transcription, NF- $\kappa$ B, nuclear factor of activated T cell and activator protein 1, to express the anti-inflammatory effect (27-30). In order to explore the association between PPAR $\gamma$  and NF- $\kappa$ B in peritoneal macrophages in inflammation, the three groups in the present study were stimulated with LPS. The results indicated that the expression of I $\kappa$ B $\alpha$  was downregulated in each group by LPS, and the downregulation of I $\kappa$ B $\alpha$  in the PPAR $\gamma$  antisense oligonucleotide group was more significant than that in the other two groups. We consider PPAR $\gamma$  to be closely connected with NF- $\kappa$ B in peritoneal macrophages in inflammation, and PPAR $\gamma$  may produce anti-inflammatory effects by protecting I $\kappa$ B $\alpha$  from being phosphorylated and degraded in order to influence the activation and nuclear translocation of NF- $\kappa$ B.

In conclusion, the present study demonstrates that PPAR $\gamma$  performs a role in anti-inflammation by means of protecting I $\kappa$ B $\alpha$  from being phosphorylated and degraded and promoting cholesterol efflux from peritoneal macrophages in inflammation. As the understanding of the complex association between PPAR $\gamma$  and the cholesterol efflux from peritoneal macrophages in inflammation increases, there will undoubtedly be

an increasing number of opportunities to apply knowledge to the management and ultimately the prevention of atherosclerosis.

### Acknowledgements

This study was granted financial support from the National Natural Science Foundation of China (grant 30772098) and Chongqing Science Technology Commission (No.s cstc2010bb5386 and cstc2012jjA10090).

### References

- Klingenberg R and Hansson GK: Treating inflammation in atherosclerotic cardiovascular disease: emerging therapies. *Eur Heart J* 30: 2838-2844, 2009.
- Moore KJ and Tabas I: Macrophages in the pathogenesis of atherosclerosis. *Cell* 145: 341-355, 2011.
- Hansson GK and Hermansson A: The immune system in atherosclerosis. *Nat Immunol* 12: 204-212, 2011.
- Reiss AB and Cronstein BN: Regulation of foam cells by adenosine. *Arterioscler Thromb Vasc Biol* 32: 879-886, 2012.
- McLaren JE, Michael DR, Ashlin TG and Ramji DP: Cytokines, macrophage lipid metabolism and foam cells: implications for cardiovascular disease therapy. *Prog Lipid Res* 50: 331-347, 2011.
- Glass CK and Saijo K: Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells. *Nat Rev Immunol* 10: 365-376, 2010.
- Villacorta L, Schopfer FJ, Zhang J, *et al*: PPAR $\gamma$  and its ligands: therapeutic implications in cardiovascular disease. *Clin Sci (Lond)* 116: 205-218, 2009.
- Bouhlef MA, Staels B and Chinetti-Gbaguidi G: Peroxisome proliferator-activated receptors - from active regulators of macrophage biology to pharmacological targets in the treatment of cardiovascular disease. *J Intern Med* 263: 28-42, 2008.
- Rigamonti E, Chinetti-Gbaguidi G and Staels B: Regulation of macrophage functions by PPAR- $\alpha$ , PPAR- $\gamma$ , and LXRs in mice and men. *Arterioscler Thromb Vasc Biol* 28: 1050-1059, 2008.
- Tabas I: Macrophage death and defective inflammation resolution in atherosclerosis. *Nat Rev Immunol* 10: 36-46, 2010.
- Martinez FO, Helming L and Gordon S: Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol* 27: 451-483, 2009.
- Takano H and Komuro I: Peroxisome proliferator-activated receptor  $\gamma$  and cardiovascular diseases. *Circ J* 73: 214-220, 2009.
- Lamers C, Schubert-Zsilavecz M and Merk D: Therapeutic modulators of peroxisome proliferator-activated receptors (PPAR): a patent review (2008-present). *Expert Opin Ther Pat* 22: 803-841, 2012.
- Kajinami K and Kawai Y: Beyond C-reactive protein; new evidence for another inflammatory biomarker predicting cardiovascular disease risk. *Atherosclerosis* 214: 39-40, 2011.
- Lawrence CB, Brough D and Knight EM: Obese mice exhibit an altered behavioural and inflammatory response to lipopolysaccharide. *Dis Model Mech* 5: 649-659, 2012.
- Lloyd-Jones D, Adams RJ, Brown TM, *et al*: American Heart Association Statistics Committee and Stroke Statistics Subcommittee: Executive summary: heart disease and stroke statistics - 2010 update: a report from the American Heart Association. *Circulation* 121: 948-954, 2010.
- Zhao Y, Pennings M, Vrins CL, *et al*: Hypocholesterolemia, foam cell accumulation, but no atherosclerosis in mice lacking ABC-transporter A1 and scavenger receptor BI. *Atherosclerosis* 218: 314-322, 2011.
- Uto-Kondo H, Ayaori M, Ogura M, *et al*: Coffee consumption enhances high-density lipoprotein-mediated cholesterol efflux in macrophages. *Circ Res* 106: 779-787, 2010.
- Ogawa Y, Yoneda M, Tomeno W, *et al*: Peroxisome proliferator-activated receptor  $\gamma$  exacerbates Concanavalin A-induced liver injury via suppressing the translocation of NF- $\kappa$ B into the nucleus. *PPAR Res* 2012: 940384, 2012.
- Norazmi MN, Mohamed R, Nurul AA and Jaacob NS: The modulation of PPAR $\gamma$ 1 and PPAR $\gamma$ 2 mRNA expression by ciglitazone in CD3/CD28-activated naive and memory CD4<sup>+</sup> T cells. *Clin Dev Immunol* 2012: 849195, 2012.
- Tobiasova Z, Zhang L, Yi T, *et al*: Peroxisome proliferator-activated receptor- $\gamma$  agonists prevent *in vivo* remodeling of human artery induced by alloreactive T cells. *Circulation* 124: 196-205, 2011.
- Visser ME, Witztum JL, Stroes ES and Kastelein JJ: Antisense oligonucleotides for the treatment of dyslipidaemia. *Eur Heart J* 33: 1451-1458, 2012.
- Robinson SM and Mann DA: Role of nuclear factor kappaB in liver health and disease. *Clin Sci (Lond)* 118: 691-705, 2010.
- Tornatore L, Thotakura AK, Bennett J, *et al*: The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation. *Trends Cell Biol* 22: 557-566, 2012.
- Kwak JH, Jung JK and Lee H: Nuclear factor-kappa B inhibitors; a patent review (2006-2010). *Expert Opin Ther Pat* 21: 1897-1910, 2011.
- Kim IT, Ryu S, Shin JS, *et al*: Euscaphic acid isolated from roots of *Rosa rugosa* inhibits LPS-induced inflammatory responses via TLR4-mediated NF- $\kappa$ B inactivation in RAW 264.7 macrophages. *J Cell Biochem* 113: 1936-1946, 2012.
- Bright JJ, Kanakasabai S, Chearwae W and Chakraborty S: PPAR regulation of inflammatory signaling in CNS diseases. *PPAR Res* 2008: 658520, 2008.
- Hirsch J, Johnson CL, Nelius T, *et al*: PEDF inhibits IL8 production in prostate cancer cells through PEDF receptor/phospholipase A2 and regulation of NF $\kappa$ B and PPAR $\gamma$ . *Cytokine* 55: 202-210, 2011.
- Simone RE, Russo M, Catalano A, *et al*: Lycopene inhibits NF- $\kappa$ B-mediated IL-8 expression and changes redox and PPAR $\gamma$  signalling in cigarette smoke-stimulated macrophages. *PLoS One* 6: e19652, 2011.
- Fan B, Ikuyama S, Gu JQ, *et al*: Oleic acid-induced ADRP expression requires both AP-1 and PPAR response elements, and is reduced by Pycnogenol through mRNA degradation in NMuLi liver cells. *Am J Physiol Endocrinol Metab* 297: E112-123, 2009.