Association between liver X receptor-α and neuron-derived orphan nuclear receptor-1 in Kupffer cells of C57BL/6 mice during inflammation

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Abstract. The liver X receptor (LXR) isoform LXR-α has a significant role in lipid metabolism and innate immunity. Overexpression of neuron-derived orphan nuclear receptor-1 (NOR-1) in macrophages reduces the synthesis of inflammatory cytokines and chemokines. However, to date, the mechanisms via which NOR-1 inhibits lipopolysaccharide (LPS)-induced inflammation in Kupffer cells (KCs) via LXR-α have not been elucidated. T0901317 is the most potent LXR-α ligand, leading to its activation. In the present study, KCs were isolated from C57BL/6 mice and randomly divided into five groups: Control, T0901317, LPS, LPS + T0901317 and LPS + T0901317 + NOR-1 small hairpin (sh)RNA groups. In order to investigate the role of NOR-1 in inflammation, shRNA targeting NOR-1 was used to specifically knock down NOR-1 mRNA in KCs. The expression levels of LXR-α and NOR-1 in KCs were determined by reverse transcription quantitative polymerase chain reaction and western blot analyses. The protein levels of tumor necrosis factor (TNF)-α and interleukin (IL)-10 in the supernatant of KCs were evaluated by ELISA. The results revealed that LXR-α expression in the T0901317 group was higher than that in the control group; furthermore, LXR-α expression was higher in KCs treated with LPS + T0901317 compared with that in KCs treated with LPS only. The expression levels of NOR-1 in each group showed a similar trend. shRNA targeting of NOR-1 suppressed the mRNA expression of NOR-1, but had no influence on LXR-α mRNA expression. NOR-1 protein expression was augmented in the LPS + T0901317 group compared with that in the LPS + T09 + shRNA group. In the supernatant of KCs, the TNF-α levels in the LPS + T0901317 group were lower than those in the LPS group, whereas the IL-10 levels were higher in the LPS + T0901317 group compared with those in the LPS group. The results of the present study suggested that ligand T0901317 promotes LXR-α expression, which consequently suppresses LPS-induced inflammation by elevating NOR-1 expression in KCs.

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Introduction

The liver X receptor (LXR) is a nuclear receptor which has an essential role in the regulation of metabolism and inflammation through inducing and blocking target genes (1,2). Two isoforms of LXR have been identified and are named as LXR-α and LXR-β. LXR-β is expressed ubiquitously in all the tissues examined, while LXR-α is more restricted, being most highly expressed in the liver, adipose tissue, intestine, lung, macrophages, spleen and kidney (3). A previous study has shown that stimulating Kupffer cells (KCs) by lipopolysaccharide (LPS) decreases the mRNA and protein expression of LXR-α (4). LXR-α has recently drawn attention due to its anti-inflammatory properties; however, the underlying molecular mechanism has remained elusive. The neuron-derived orphan nuclear receptor-1 (NOR-1) functions as an early-response gene which regulates key cellular processes, including proliferation, differentiation and survival (5,6). It has been shown to function in the regulation of important genes involved in metabolic homeostasis in the liver and adipocytes (7-9). It has been reported that the overexpression of NOR-1 in macrophages reduced the synthesis of inflammatory cytokines and chemokines (10). Kumar *et al* (8) discovered that the two nuclear receptors LXR and NOR-1 have an interdependent regulatory association in adipocytes and that LXR regulates gene transcription of NOR-1 in adipocytes.

KCs, constituting 80-90% of the tissue-resident macrophages present in the body, are increasingly recognized as important modulators which control the liver's response to injury and repair. They have a crucial role in liver homeostasis as well as in initiation, maintenance and outcome of liver inflammation (11). Upon activation, KCs release various substances, including cytokines, nitric oxide and reactive oxygen species. Therefore, KCs are intimately involved in

the liver's response to infection, toxins, ischemia, resection and other stresses (12). KCs have been implicated in the pathogenesis of various liver diseases, including viral hepatitis, steatohepatitis, alcoholic liver disease, intrahepatic cholestasis, activation or rejection of the liver during liver transplantation and liver fibrosis (13).

However, to date, the association between the two nuclear receptors in KCs during LPS-induced inflammation has remained elusive, and the specific mechanisms are subject to present research. It has been reported that T0901317 is the most potent LXR-α ligand (2). The present study hypothesized that upregulation of LXR-α by T0901317 may suppress LPS-induced inflammation by controlling the expression of NOR-1 in KCs. The present study used T0901317 to activate LXR-α as well as NOR-1 knockdown in order to identify a novel role of LXR-α in regulating LPS-induced inflammation by affecting NOR-1 expression in KCs.

Materials and methods

Animals and experimental protocol. Male C57BL/6 mice (6-8 weeks) were obtained from the laboratory animal research center of Chongqing Medical University (Chongqing, China). All animals (n=60) were housed under specific pathogen-free condition and allowed free access to sterile water and food. The animals received humane care in compliance with the institution's guidelines, as outlined in the guide for the care and use of laboratory animals prepared by the National Academy of Sciences (Beijing, China). The present study was approved by the ethics committee of Chongqing Medical University (Chongqing, China).

In vitro experiments. Primary KCs were isolated from mouse livers according to a previously described procedure (14). Briefly, a three-step procedure was applied to isolate KCs in sufficient number and purity from murine liver, including enzymatic tissue treatment, gradient centrifugation, and selective adherence. KCs were cultured in 24-well plates at a density of $1x10^6$ cells in Dulbecco's modified Eagle's medium (DMEM) (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Hyclone) and antibiotics (100 U/ml penicillin G and 100 mg/ml streptomycin sulphate; Beyotime Institute of Biotechnology, Haimen, China) at 37˚C in the presence of 5% CO_2. The viability of KCs (>90%) was determined by trypan blue exclusion (Beyotime Institute of Biotechnology). KCs were observed under a light microscope (TL-800C; Nikon Corporation, Tokyo, Japan). KCs were randomly divided into six groups (six wells per group): Control (CON), T0901317 (T09; Sigma-Aldrich, St. Louis, MO, USA), LPS (LPS; 10 mg; Sigma-Aldrich), LPS + T0901317 (LPS + T09), LPS + T0901317 + NOR-1 small hairpin (sh) RNA (LPS + T09 + shRNA) and NOR-1 shRNA (shRNA) groups.

Cultured KCs were transfected with a NOR-1 shRNA plasmid (sc-38843-SH; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) using a transfection reagent [NOR-1 shRNA Plasmid (m); Santa Cruz Biotechnology, Inc.] according to a previously described shRNA transfection protocol (15), and manufacturer's instructions. In brief, KCs were seeded into a six-well tissue culture plate at a density of $1x10^6$ cells/well and grown overnight in antibiotic-free normal growth medium prior to transfection. Cells were washed twice with 2 ml shRNA transfection medium (Santa Cruz Biotechnology, Inc.). 200 μl of the DNA/shRNA plasmid transfection reagent/shRNA (425 μg/ml) complexes were added to each well and the cells were cultured at 37˚C in a CO_2 incubator for 5 h. Following incubation, 1 ml growth medium containing twice the amount of serum and antibiotics of normal medium was added to each well and the cells were cultured for 24 h. The transfection efficiency of NOR-1 shRNA was measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

24 h after transfection, the T09, LPS + T09 and LPS + T09 + shRNA groups were incubated with DMEM containing T0901317 (1 μM), and the other three groups were incubated in DMEM in the absence of T0901317 for 6 h. Fresh medium was added to the CON, T09 and shRNA groups, while fresh medium containing LPS (10 ng/ml) was added to the LPS, LPS + T09 and LPS + T09 + shRNA groups, followed by an additional 6-h incubation. The total incubation time in each group was 30 h, which did not include the incubation time with LPS/control (6 h).

F4/80 staining. KCs were identified by immunofluorescence using fluorescein isothiocyanate-conjuugated monoclonal anti-F4/80 antibody [BM8] (Abcam, Cambridge, UK), according to the manufacturer's instructions. Briefly, KCs were seeded in 24-well chamber slides at a density of $2x10^4$ cells/ml. The cells were then fixed with 4% paraformaldehyde for 10 min and incubated in 1% bovine serum albumin (Sigma-Aldrich)/10% normal goat serum (Beyotime Institute of Biotechnology)/0.3M glycine (Beyotime Institute of Biotechnology) in 0.1% phosphate-buffered saline (PBS)-Tween for 1 h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the anti-F4/50 antibody (1μg/ml), overnight at 4˚C. Propidium iodide (Wuhan Boster Biological Technology Ltd., Wuhan, China) was used to stain the cell nuclei (red) at a concentration of 1.43 μM. After washing with PBS, the cells were covered with mounting medium (Wuhan Boster Biological Technology Ltd.) and the slides were viewed by laser scanning confocal microscopy (C2 Plus; Nikon Corporation).

RNA analysis. To investigate the association between LXR-α and NOR-1 during inflammation, the mRNA expression of LXR-α and NOR-1 in KCs was analyzed by RT-qPCR. Briefly, total RNA was extracted from cell samples of the experimental groups using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions and quantified via the 260 nm/280 nm absorption ratio of RNA samples (Model 722; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each total RNA sample was stored at -70˚C. Equivalent amounts of particle RNA extracted from each sample served as the template for cDNA synthesis using the PrimeScript RT reagent kit with a gDNA Eraser (cat no. DRR037A; Takara Bio Inc., Otsu, Japan). The amplification of sample cDNA was monitored using the SsoFast EvaGreen supermix (Bio-Rad Laboratories, Inc.) and the DNA fluorescent dye SYBR Green (Molecular Probes, Eugene, OR, USA). β-actin served as an endogenous normalization control

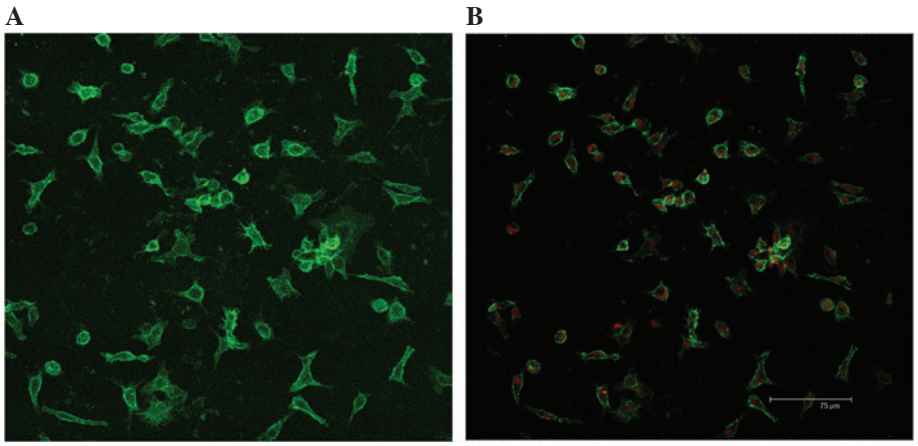

Figure 1. Immunofluorescence images of Kupffer cells stained with F4/80 antibodies. (A) Freshly isolated cells and (B) cells cultured for 4 days exhibited green fluorescence when observed under a laser scanning confocal microscope (magnification, x200). Green, F4/80; red, propidium iodide.

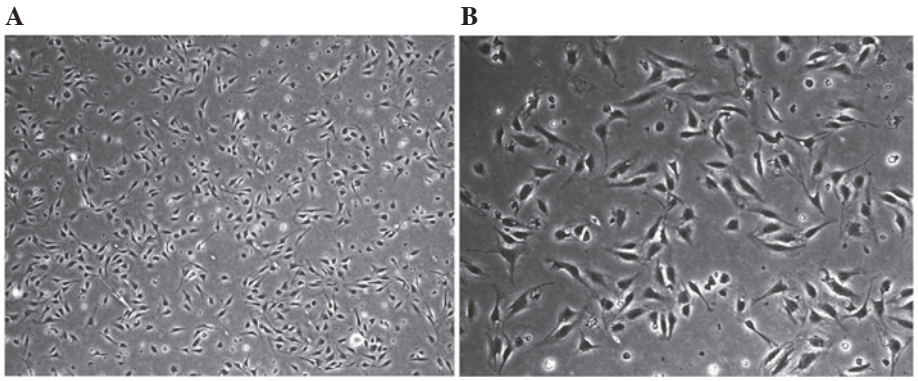

Figure 2. Morphology of Kupffer cells observed at different time-points under a phase contrast microscope. (A) Twenty-four hours after isolation, the cells showed typical morphologic features with irregular shape (magnification, x100). (B) Two days later, the cells became larger and more prominent, exhibiting a transparent cytoplasm and kidney-like nuclei (magnification, x200).

(reference gene). PCR cycling conditions were as follows: Initial denaturation at 94˚C for 2 min, 30 cycles at 94˚C for 20 sec, 60˚C for 30 sec and 72˚C for 30 sec. The thermal cycler was from Bio-Rad Laboratories (S1000™ Thermal Cycler with 96-Well Fast Reaction module). All PCR products were separated by electrophoresis on 2% agarose gels. The DNA bands of LXR-α or NOR-1 were normalized to the corresponding β-actin band using the Bio-Image analysis system (Gel Doc2000; Bio-Rad Laboratories, Inc.), and the ratio of LXR-α or NOR-1 to β-actin was used to assess the relative mRNA expression. Bio-Rad CFX Manager software (version 2.0; Bio-Rad Labotatories, Inc.) was used for data analysis, and the relative mRNA expression levels were calculated using the Vandesompele method (16). All values are expressed as the ratio of the RNA concentration of the target amplicon to the RNA concentration of the housekeeping gene (β-actin), to take into account differences betweensample RNA concentrations.

Sequences of specific LXR-α, NOR-1 and β-actin primers were as follows: LXR-α forward, 5'-GAGACATCTCGG AGGTACAACCC-3' and reverse, 5'-AGCAAGGCAAACTC GGCATC-3'; NOR-1 forward, 5'-TGTCTCAGTGTCGGGATG GTT-3' and reverse, 5'-TCCTGTTGTAGTGGGCTCTTTG-3'; and β-actin forward, 5'-TGCCCATCTACGAGGGCTAT-3' and reverse, 5'-TGATGTCACGCACGATTTCC-3'.

Western blot analysis. The protein expression of LXR-α and NOR-1 in KCs was detected by western blot analysis. Protein extracts were obtained by homogenizing samples in a cell lysis buffer containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.42 mM NaCl, 15 mM $MgCl_2$, 25% glycerol, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride and 0.5 mM dithiothreitol. The protein concentration was determined using a Bradford Assay kit (Bio-Rad Laboratories, Inc.). Equal amounts of protein samples were each separated on 12% Tris-HCl gels (Bio-Rad Laboratories, Inc.) by electrophoresis and transferred onto a polyvinylidene fluoride membrane (Merck Millipore, Darmstadt, Germany). The membrane was then blocked for 1 h with 5% nonfat dry milk and incubated with goat anti-mouse LXR-α (1/1,000 dilution; SC1202; Santa Cruz Biotechnology, Inc.) and rabbit anti-mouse NOR-1 (1/1,000 dilution; SC133840; Santa Cruz Biotechnology, Inc.) polyclonal antibodies at 4˚C overnight. The membrane was washed and incubated for 1 h at room temperature with a 1/1,200-dilution of rabbit anti-goat (cat. no. BA1006) or goat anti-rabbit (cat. no. BA1003) immunoglobulin G (Wuhan Boster Biological Technology Ltd.) for 1 h. Finally, the membrane was developed using an Enhanced Chemiluminescence Detection kit (Pierce Biotechnology, Inc., Thermo Fisher Scientific, Waltham, MA, USA) and exposed

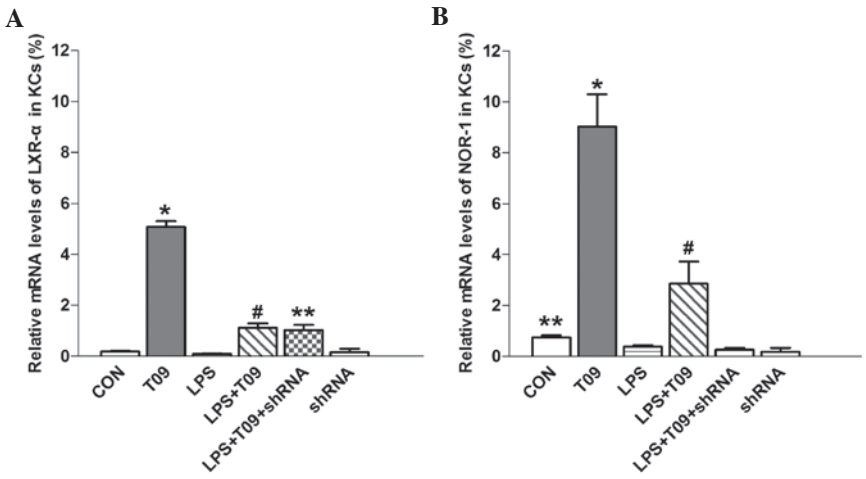

Figure 3. Relative mRNA levels of (A) LXR-α and (B) NOR-1 in KCs as determined by reverse transcription quantitative polymerase chain reaction analysis. Values are expressed as the mean ± standard deviation of four individual experiments. *P<0.05 vs. control group; $^{\#}$P<0.05 vs. LPS group; **P>0.05 vs. LPS + T09 group in A and vs. shRNA group in B. LXR, liver X receptor; NOR, neuron-derived orphan nuclear receptor; KC, Kupffer cell; LPS, lipopoylsaccharide; CON, control; shRNA, small hairpin RNA targeting NOR-1; T09, T0901317 (LXR-α-activating ligand).

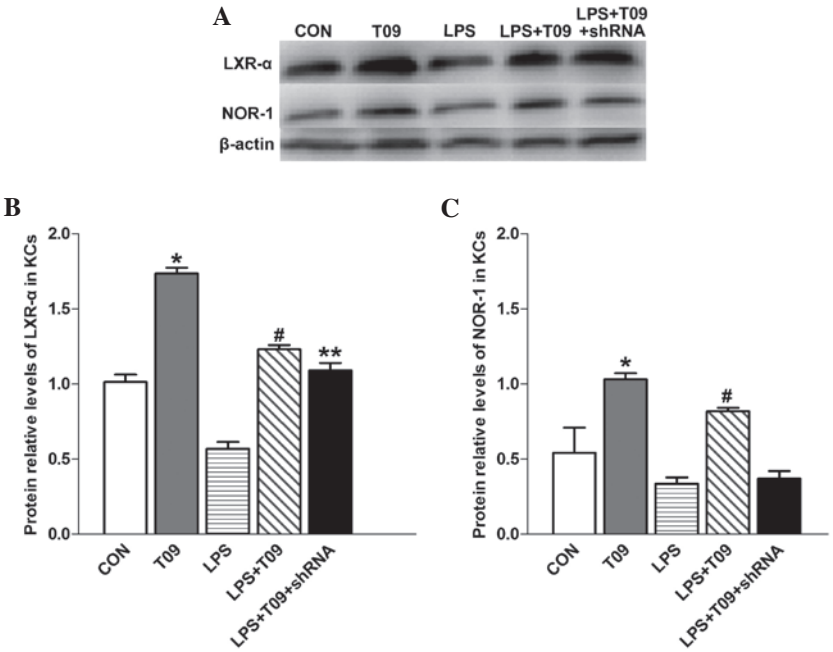

Figure 4. Protein levels of LXR-α and NOR-1 in KCs. LXR-α and NOR-1 protein levels in KCs were determined by western blot analysis. (A) Western blot representative of four individual experiments. Quantified levels of (B) LXR-α and (C) NOR-1 normalized to β-actin. Values are expressed as the mean ± standard deviation of four individual experiments. *P<0.05 vs. control group, $^{\#}$P<0.05 vs. LPS group, and **P>0.05 vs. LPS + T09 group in B or LPS + T09 + shRNA group in C. LXR, liver X receptor; NOR, neuron-derived orphan nuclear receptor; KC, Kupffer cell; LPS, lipopoylsaccharide; CON, control; shRNA, small hairpin RNA targeting NOR-1; T09, T0901317 (LXR-α-activating ligand).

to an autoradiographic film (Eastman Kodak, Rochester, NY, USA). The relative amounts of LXR-α and NOR-1 protein were quantified via the relative density of the protein bands using the Gel Doc 2000 image analysis system (Bio-Rad Laboratories, Inc.).

ELISA analysis. ELISA was used to measure the protein levels of tumor necrosis factor (TNF)-α (TNF-α Mouse ELISA kit; cat. no. ab100747; Abcam) and interleukin (IL)-10 (IL-10 Mouse ELISA kit; cat. no. ab46103; Abcam) in the supernatant of cultured cells according to the manufacturer's instructions.

Statistical analysis. Values are expressed as the mean ± standard deviation, and comparisons between values were performed by analysis of variance using the statistical package SPSS version 18.0 (International Business Machines, Armonk, NY, USA). The comparison mean values was performed using the Student's t-test. P<0.05 was considered to indicate a statistically significant difference between values.

Results

Identification of KCs. The viability of KCs determined by trypan blue staining after isolation was ≥98%. The purity of

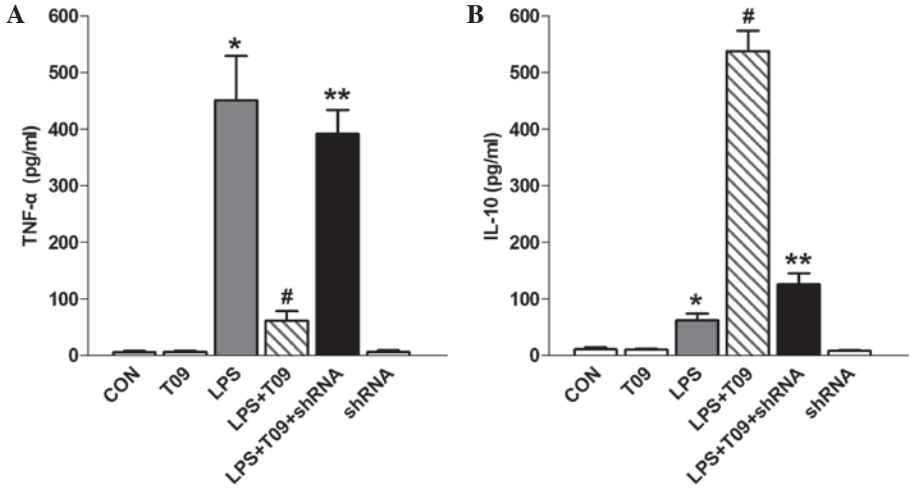

Figure 5. Levels of (A) TNF-α and (B) IL-10 in the supernatant of KCs as determined by ELISA. Values are expressed as the mean ± standard deviation of four individual experiments. *P<0.05 vs. control group, #P<0.05 vs. LPS group, and **P<0.05 vs. LPS + T09 group. KC, Kupffer cell; LPS, lipopoylsaccharide; CON, control; shRNA, small hairpin RNA targeting neuron-derived orphan nuclear receptor-1; T09, T0901317 (liver X receptor-α-activating ligand); TNF, tumor necrosis factor; IL, interleukin.

KC fractions was determined by morphological observation in combination with F4/80 staining (Fig. 1). The freshly isolated cells had a round shape and were 8-10 μm in diameter as indicated by light microscopy observation. 2 h later, most of the cells adhered to the wall of plastic flask and exhibited a fried-egg shape. With increasing culture time, the cells became larger and more prominent, showing typical morphological features of macrophages with irregular shape, transparent cytoplasm and kidney-like nuclei (Fig. 2).

NOR-1 mRNA expression is partially controlled by LXR-α under inflammatory and non-inflammatory conditions. T0901317, a synthetic ligand of LXR-α, significantly increased LXR-α mRNA expression in T09-KCs as compared with that in the control group (P<0.05), and LXR-α expression in the LPS + T09 group was higher than that in the LPS group (P<0.05) (Fig. 3A). These results encouraged the further exploration of the effect of NOR-1 shRNA on LXR-α mRNA expression. However, the knockdown of NOR-1 using shRNA had no effects on LXR-α mRNA expression, and there was no significant difference in LXR-α expression between the LPS + T09 and LPS + T09 + shRNA groups (P>0.05) (Fig. 3A). The mRNA expression of NOR-1 in the T09 group was significantly higher than that in the control group (P<0.05), and the expression was also higher in the LPS + T09 group than that in the LPS or LPS + T09 + shRNA group (P<0.05) (Fig. 3B). Furthermore, NOR-1 mRNA expression was reduced by shRNA compared with that in the control cells (P<0.05), indicating that NOR-1 shRNA effectively suppressed the mRNA expression of NOR-1 (Fig 3B).

These results suggested that increased LXR-α expression by its ligand can elevate NOR-1 mRNA expression under normal and inflammatory conditions, and that there is a close association between LXR-α and NOR-1. By contrast, shRNA targeting NOR-1 suppressed NOR-1 mRNA expression but had no impact on LXR-α mRNA levels (Fig. 3). These findings indicated that the mRNA expression of NOR-1 is partially controlled by LXR-α.

NOR-1 protein expression is partially controlled by LXR-α under inflammatory and non-inflammatory conditions. The protein expression of LXR-α was significantly higher in T0901317 group than that in the control group (P<0.05), and LXR-α was enhanced in the LPS + T09 group compared with that in the LPS group (P<0.05) (Fig. 4A). However, there was no significant difference in LXR-α levels between the LPS + T09 group and the LPS + T09 + shRNA group (P>0.05). NOR-1 protein expression was significantly higher in the T09 group than that in the control group (P<0.05), and it was augmented in the LPS + T09 group compared with that in the LPS + T09 + shRNA group (P<0.05) (Fig. 4B). These observations indicated that increased LXR-α expression enhances the protein levels of NOR-1 under normal and inflammatory conditions. The fact that NOR-1 shRNA had no effect on LXR-α expression in KCs further confirms the interdependent association between LXR-α and NOR-1.

LXR-α inhibits LPS-induced inflammation in KCs through elevating NOR-1 expression. To further investigate the involvement of LXR-α and NOR-1 in LPS-induced secretion of pro-inflammatory and anti-inflammatory cytokines in KCs, TNF-α and IL-10 were quantified in each KC group by ELISA.

TNF-α levels in the LPS group were significantly higher than those in the control group (P<0.05), whereas they were obviously reduced in the LPS + T09 group compared with those in the LPS group (P<0.05) (Fig 5A). The effect of NOR-1 shRNA on the production of TNF-α in KCs was then explored. The production of TNF-α in KCs was significantly enhanced in response to NOR-1 shRNA and T0901317 pre-treatment followed by LPS stimulation in comparison with that in KCs treated with LPS and T0901317 (P<0.05). The levels of the anti-inflammatory cytokine IL-10 in LPS-treated KCs was higher than that in control cells (P<0.05), and of note, it was significantly higher in the LPS + T09 group than that in the LPS group (P<0.05) (Fig. 5B). Furthermore, the IL-10 levels were decreased in the LPS + T09 + shRNA group, which may be attributed to the suppression of NOR-1 (P<0.05). T0901317

blocked LPS-mediated TNF-α production and induced IL-10 secretion. Of note, T0901317 or NOR-1 shRNA alone had no effect on the expression levels of TNF-α or IL-10 (Fig. 5).

The ELISA results showed that LPS induced a marked level of inflammation in KCs, which was partially suppressed through ligand-induced upregulation of LXR-α expression. Upregulated LXR-α inhibited LPS-induced inflammation through elevating NOR-1 expression in KCs. These results suggested that LPS-induced inflammation may be partially suppressed by LXR-α, which may have an anti-inflammatory role by increasing NOR-1 expression and promoting ultimate secretion of the anti-inflammatory cytokine IL-10 in KCs.

Discussion

LXR-α is a transcription factor belonging to the nuclear receptor family, which has a central role in metabolic homeostasis, being master regulators of key target genes in the glucose and lipid pathways (17). Previous studies have shown that LXR-α has a direct anti-inflammatory effect. Musso *et al* (18) reported that agonists of LXR-α improve cholesterol-induced hepatic inflammation and fibrosis by reducing the activation of KCs and hepatic satellite cells. Spann *et al* (19) provided evidence that regulated accumulation of desmosterol effects numerous homeostatic responses, including activation of LXR target genes, inhibition of sterol regulatory element-binding protein target genes, selective re-programming of fatty acid metabolism and suppression of inflammatory-response genes, observed in macrophage foam cells.

NOR-1 (also known as NR4A3) together with Nurr77 (NR4A1) and Nurr1 (NR4A2) form a superfamily named NR4As. The NR4A proteins are among the most evolutionarily conserved nuclear hormone receptor superfamilies (20). Members of the NR4A sub-family, including NOR-1, lack a classical ligand-binding domain, function as constitutively active transcription factors and respond to stimuli such as immediate early response genes (21). Although initially proved to control certain key processes, including differentiation, proliferation and apoptosis, and associated with central nervous system disorders (22), recent observations identified roles of NR4As in controlling lipid metabolism and inflammation (23). Zhao *et al* (24) discussed how NR4As control hepatic and skeletal muscle glucose metabolism, plasma and hepatic lipid metabolism, as well as differentiation and function of white and brown adipocytes.

It has been shown that NR4A receptors in murine macrophages can be activated by LPS, and NOR-1 is potently and transiently induced at an early time-point (21); however, the exact underlying mechanism has remained elusive. Based on the anti-inflammatory action of NOR-1 and the regulation of NOR-1 by activated LXR-α, the present study hypothesized that LXR-α may inhibit inflammation by regulating NOR-1 expression. The present study reported several findings supporting a novel role of LXR-α and NOR-1 in controlling LPS-induced inflammation. KCs were treated with LPS, LXR-α agonist and NOR-1 shRNA to examine their effects. First, it was found that LPS and/or LXR-α agonist led to profound changes in LXR-α expression, which were paralleled by similar alterations in NOR-1 expression. Furthermore, it was revealed that LXR-α agonist resulted in upregulation of NOR-1 at the mRNA and protein level; however, NOR-1 shRNA had no effect on LXR-α expression, indicating that LXR-α regulated NOR-1 expression. Finally, the levels of TNF-α and IL-10, which are classic pro-inflammatory and anti-inflammatory factors, respectively, were all elevated in KCs treated with LPS. Of note, TNF-α was decreased but IL-10 was elevated in KCs treated with LPS + T09. However, T0901317 alone affected neither TNF-α nor IL-10 levels. NOR-1 knockdown in KCs treated with LPS + T09 increased the levels of TNF-α to a similar extent to that in the LPS group, while the levels of IL-10 were markedly decreased. These results indicated that enhancement of LXR-α expression regulates inflammation in KCs by reducing the secretion of pro-inflammatory factor TNF-α and elevating the secretion of anti-inflammatory factor IL-10, which is closely associated with the expression of NOR-1.

The results of the present study suggested that during inflammation, NOR-1 has an anti-inflammatory role by increasing the expression of the anti-inflammatory cytokine IL-10 in KCs. This phenomenon underscores the fact that the cross-talk and expression modulation between the nuclear receptor families has, at large, remained elusive. Further study is required to confirm that LXR-α regulates LPS-mediated inflammation through controlling NOR-1 expression. An enhanced understanding of the complex association between LXR-α and NOR-1 during KC growth is important for revealing the role of adaptive immunity in inflammation, which may further benefit the application of adaptive immunity in clinical treatment of inflammation.

In conclusion, the present study discovered that promoting LXR-α expression by its ligand T0901317 elevated NOR-1 expression in KCs, which in turn upregulated cytokine IL-10 to resist LPS-induced inflammation. The association between LXR-α and NOR-1 in regulating metabolism, inflammation and immunity provides novel insight into the feasibility of using LXR-α to regulate inflammation; therefore, the present study suggested LXR-α as a novel target to treat inflammatory diseases.

Acknowledgements

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