# MICROBIOLOGY AND IMMUNOLOGY

# The Content of PPAR, LXR, and RXR and the PPAR DNA-Binding Activity in Macrophages over the Course of Inflammation in Mice

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 3, pp. 317-321, March, 2009
Original article submitted July 4, 2008

The content of peroxisome proliferation activating proteins PPAR-α and PPAR-γ, liver X receptors (LXR), and retinoid X receptors (RXR) and activity of PPAR-α, PPAR-γ, and PPAR-δ binding to DNA response elements in C57Bl/6 mouse macrophages were studied during different phases of aseptic inflammation, induced by intraperitoneal injection of 50 mg/kg zymosan A. The DNA-binding activities of PPAR-α and PPAR-γ and the levels of PPAR-α, PPAR-γ, LXR, and RXR in peritoneal macrophages dropped on days 1 and 3 after zymosan injection. On days 7 and 14 the DNA-binding activity of PPAR-γ and content of PPAR-γ and LXR-β protein increased in comparison with the control, while the DNA-binding activity and content of PPAR-α in the cells remained low. Recovery of RXR protein content in macrophages was observed only on day 14 after zymosan injection.

**Key Words:** *peroxisome proliferation activating receptors; liver X receptors; retinoid X receptors; macrophages; time course of inflammation*

Peroxisome proliferation activation receptors (PPAR) and liver X receptors (LXR), members of the nuclear hormonal receptor superfamily, play the key role in the regulation of integration changes in lipid metabolism and immune response during inflammation in macrophages [12]. These transcription factors form heterodimers with retinoid X receptors (RXR) in the cytoplasm, which are then translocated to the nucleus and bind to the DNA response elements, modulating the transcription of target genes [1]. The PPAR and LXR regulate the transcription programs involved in the capture and elimination of lipids, lipogenesis, and lipoprotein metabolism in macrophages, and hence, these factors play an important role in the development of such diseases as atherosclerosis, metabolic syndrome, and type 2 diabetes mellitus [11]. Their activities, intracellular content, and mRNA expression reduce under conditions of acute inflammatory response caused by injection of bacterial LPS or proinflammatory cytokines [8]. By contrast, activation of PPAR, LXR, or RXR by natural or synthetic agonists leads to suppression of inflammatory response and recovery of lipid metabolism parameters in various cells, including macrophages [14]. The greater part of studies in these sphere

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were carried out on macrophage cultures during the acute period of inflammation, while changes in the content and activities of these receptors in macrophages were never studied over the course of an inflammatory process.

Zymosan A, containing 50-57% 1→3-β-glycanes, induces the production of proinflammatory mediators in macrophages, binding to glycane receptors and activating NF-κB [16]. Previously we studied the changes in lipid synthesis, degradation of low density lipoproteins [3], and effects of PPAR, LXR or RXR agonists on the formation of lipid incorporations [2] in macrophages over the course of an inflammatory process on the model of aseptic inflammation induced by zymosan A injection.

Now we studied the content of PPAR, LXR or RXR and activity of binding to the DNA response elements for three PPAR isoforms in macrophages obtained during different periods of inflammation induced by zymosan A.

## MATERIALS AND METHODS

Experiments were carried out on male C57Bl/6 mice, kept on standard rations at vivarium (Institute of Cytology and Genetics). Aseptic inflammation was induced by intraperitoneal injection of zymosan A (Sigma) in a dose of 1 mg/20 g in 1 ml 0.05 M phosphate buffer, containing 0.9% NaCl. Control mice were intraperitoneally injected with 1 ml PBS. The animals were sacrificed by cervical dislocation on days 1, 3, 7, and 14 after zymosan A injection. Peritoneal macrophages were collected by peritoneal lavage and cultured [3]. Each group consisted of 18 animals (3 subgroups of 6 mice for immunoblotting of nuclear receptor content, for evaluating DNA binding activity, and for measuring serum TNF-α).

Immunoblotting of nuclear receptors was carried out in lyzed macrophages [9]. The cells were lyzed by buffer containing 10 mM HEPES (pH 7.9), 0.5% Nonidet P40, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, and 1% protease inhibitor cocktail (Sigma) during 15 min at 4°C. The supernatant was collected and protein concentration was measured by Bredfrd's method [5]. The samples were frozen and stored at -20°C. Proteins were separated in 10% PAAG and transferred onto a nitrocellulose membrane. For immunodetection, blocking was carried out in PBS with 5% degreased milk during 1 h at ambient temperature and the sample was incubated with the first rabbit polyclonal antibodies to mouse PPAR-α (Sigma), PPAR-γ (Santa Cruz Biotechnology), LXR-β (Santa Cruz Biotechnology), LXR-α (Abcam), and β-actin (Santa Cruz Biotechnology) during 2 h at 37°C. All antibodies except LXR-α were diluted 1:500; LXR-α were diluted 1:1000. After incubation the samples were washed in PBS with 5% degreased milk (15 min×3 times). Immune complexes were detected using second antibodies (diluted 1:400) labeled by phosphatase (Santa Cruz Biotechnology). The results were processed using TotalLab software. The macrophage nuclear extract was obtained as described previously [10]. The cells were resuspended and homogenized in 10 mM Tris-buffer (pH 7.4) containing 2 mM $MgCl_2$, 140 mM NaCl, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride, and centrifugated (1000*g*, 30 min, 40°C). The precipitate was resuspended in HEPES buffer (pH 7.9) with 2.5% glycerol, 140 mM NaCl, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride, and centrifugated repeatedly under the same conditions. Protein in the nuclear fraction was measured after Bredford [5]. Specific DNA-binding activity was measured in the macrophage nuclear extract using the PPAR-α, PPAR-δ, and PPAR-γ Complete Transcription Factor Assay Kit (Cayman Chemical, Cat. No. 10008878) by the method based on enzyme immunoassay detection of PPAR isoforms linked with response elements of oligonucleotides immobilized on the plastic. Serum level of TNF-α in animals was measured using a ProCon solid-phase EIA kit (R&D Systems Inc.) as described previously [2]. The significance of differences ($p<0.05$) in the compared means was evaluated using Student's *t* test with Statgraphics statistical software.

## RESULTS

The activity of inflammatory process caused by injection of zymosan A was evaluated by measuring serum level of TNF-α. On days 1 and 3 after zymosan injection serum TNF-α level increased 9.4 and 6.8 times, respectively, in comparison with the control (512.0±68.5 and 372.0±44.8 ng/ml on days 1 and 3, respectively, *vs.* 54.6±5.9 ng/ml in the control; $p<0.001$). On days 7 and 14 the level of TNF-α gradually decreased, but its level remained 2.4 and 1.6 times higher than in the control (131.0±18.8 and 87.4±10.8 ng/ml on days 7 and 14, respectively, *vs.* 54.6±5.9 ng/ml in the control; $p<0.05$).

Study of binding of 3 nuclear factor PPAR isoforms to specific DNA response elements showed a reduction of PPAR-α and PPAR-γ DNA-binding activity by 47 and 42% on days 1 and 3 after zymosan A injection, respectively, in comparison with the control level (injection of PBS; Table 1). The

PPAR-γ DNA-binding activity increased by 27% on day 7 and by 39% on day 14 in comparison with the control, while PPAR-α DNA-binding remained 36 and 31% reduced on days 7 and 14, respectively. No appreciable changes in macrophage PPAR-δ DNA-binding activity were detected over 14 days of aseptic inflammation.

The concentrations of nuclear factor protein in macrophage lysates, measured by immunoblotting with specific antibodies, also changed significantly (Fig. 1). The content of PPAR-α protein remained low throughout the entire period of inflammation studied (20-fold on days 1 and 3). On days 7 and 14 this value constituted only 29 and 49% of con-

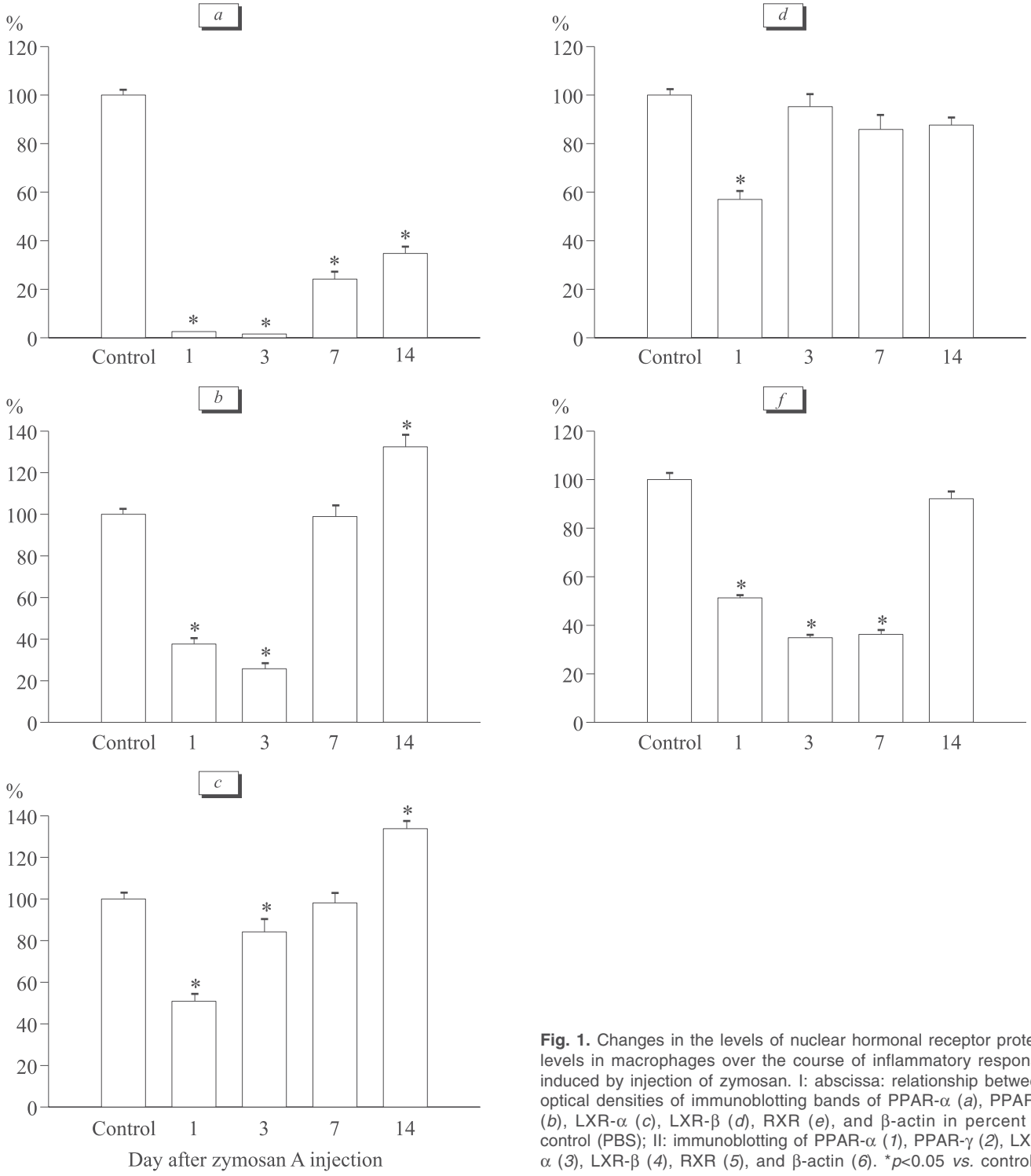


**Fig. 1.** Changes in the levels of nuclear hormonal receptor protein levels in macrophages over the course of inflammatory response induced by injection of zymosan. I: abscissa: relationship between optical densities of immunoblotting bands of PPAR-α (*a*), PPAR-γ (*b*), LXR-α (*c*), LXR-β (*d*), RXR (*e*), and β-actin in percent of control (PBS); II: immunoblotting of PPAR-α (*1*), PPAR-γ (*2*), LXR-α (*3*), LXR-β (*4*), RXR (*5*), and β-actin (*6*). **p*<0.05 *vs.* control.

**TABLE 1.** Changes in PPAR-a, PPAR-g, and PPAR-d Specific DNA-Binding Activity in Macrophages over the Course of Inflammatory Process Caused by Injection of Zymosan A (*M*±*m*, *n*=6)

| Day after zymosan injection | DNA-binding activity, % of control | | |
|---|---|---|---|
| | PPAR-α | PPAR-γ | PPAR-δ |
| Control | 100.0±5.6 | 100.0±7.2 | 100.0±7.1 |
| 1 | 54.6±6.1** | 64.8±6.8** | 103.8±10.2 |
| 3 | 52.3±4.9** | 51.3±5.4** | 120.4±12.1 |
| 7 | 64.0±7.8** | 127.2±11.1* | 110.2±8.9 |
| 14 | 68.7±7.6* | 138.9±12.3* | 113.1±10.2 |

**Note.** The mean optical density at λ=450 nm is taken for 100% DNA-binding activity in the control for PPAR-α, PPAR-γ, and PPAR-δ. *$p<0.05$ and **$p<0.01$ *vs.* control (injection of PBS).

trol, respectively. The time course of PPAR-γ levels in macrophages during inflammatory reaction development was different. On day 1 after zymosan injection the concentration of PPAR-γ protein reduced 4-fold, on day 3 5-fold, on day 7 it reached the control level, while on day 14 increased by 35% *vs.* the control. Similarly to PPAR-γ, the content of LXR-α protein reduced on days 1 and 3 after zymosan injection. On day 7 the level of this protein increased, reaching the control level, while on day 14 it surpassed the control levels by 44%. The level of LXR-β in the cells dropped on day 1 and virtually did not differ from the control on days 3, 7, and 14. The cell level of RXR protein (forming heterodimers with PPAR and LXR) decreased significantly on days 1, 3, and 7 and normalized by day 14.

At some stages of the inflammatory process, depending on its specific features, macrophages exhibit functional heterogeneity, caused by different mechanisms of their activation [4]. Our study showed a different picture of changes in the PPAR, LXR, and RXR protein levels and of three PPAR isoform activities during the acute (days 1 and 3) and chronic (days 7 and 14) stages of inflammation. It was shown previously on surviving macrophage cultures that classical activation of macrophages by γ-IFN, LPS, or TNF-α was realized through NF-κB induction and associated with the mechanism of macrophage activation during the acute phase of inflammation induced by zymosan A [16]. It was shown that classical activation of macrophages was paralleled by suppressed expression of PPAR-α, PPAR-γ, LXR, and RXR [15]. By contrast, alternative activation of macrophages by IL-4, IL-13 [6,7], or prostaglandin $D_2$ [12] can lead to selective expression of PPAR-γ. Some authors claim that alternative activation of macrophages is characteristic of the reparative stage of inflammation [4]. This suggests the use of a differentiated approach to the use of PPAR-α or PPAR-γ agonists in patients with different forms of inflammatory and autoimmune diseases.

The study was supported by the Russian Foundation for Basic Research (grants Nos. 06-04-48273 and 07-04-00864).

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