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Lipid Synthesis in Macrophages during Inflammation *in vivo*: Effect of Agonists of Peroxisome Proliferator Activated Receptors α and γ and of Retinoid X Receptors

E. N. Posokhova[1], O. M. Khoshchenko[1], M. I. Chasovskikh[2], E. N. Pivovarova[3], and M. I. Dushkin[2]*

[1]*Institute of Internal Medicine, Siberian Branch of the Russian Academy of Medical Sciences, ul. B. Bogatkova 175/1, 630089 Novosibirsk, Russia; fax: (383) 264-2516*
[2]*Institute of Clinical Immunology, Siberian Branch of the Russian Academy of Medical Sciences, ul. Yadrintsevskaya 14, 630099 Novosibirsk, Russia; fax: (383) 334-8373; E-mail: midushkin@soramn.ru*
[3]*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, pr. Lavrent'eva 10, 630090 Novosibirsk, Russia; fax: (383) 333-1278*

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Abstract—The effects of peroxisome proliferator activated receptors α and γ (PPAR-α and PPAR-γ) and retinoid X receptor (RXR) agonists upon synthesis and accumulation of lipids in murine C57Bl macrophages during inflammation induced by injection of zymosan and *Escherichia coli* lipopolysaccharide (LPS) have been studied. It is significant that intraperitoneal injection of zymosan (50 mg/kg) or LPS (0.1 mg/kg) in mice led to a dramatic increase of [^{14}C]oleate incorporation into cholesteryl esters and triglycerides and [^{14}C]acetate incorporation into cholesterol and fatty acids in peritoneal macrophages. Lipid synthesis reached its maximum rate 18-24 h after injection and was decreased 5-7 days later to control level after LPS injection or was still heightened after zymosan injection. In macrophages obtained in acute phase of inflammation (24 h), degradation of ^{125}I-labeled native low density lipoprotein (NLDL) was 4-fold increased and degradation of ^{125}I-labeled acetylated LDL (AcLDL) was 2-3-fold decreased. Addition of NLDL (50 μg/ml) or AcLDL (25 μg/ml) into the incubation medium of activated macrophages induced 9-14- and 1.25-fold increase of cholesteryl ester synthesis, respectively, compared with control. Addition of NLDL and AcLDL into the incubation medium completely inhibited cholesterol synthesis in control macrophages but had only slightly effect on cholesterol synthesis in activated macrophages. Injection of RXR, PPAR-α, or PPAR-γ agonists—9-*cis*-retinoic acid (5 mg/kg), bezafibrate (10 mg/kg), or rosiglitazone (10 mg/kg), respectively—30 min before zymosan or LPS injection led to significant decrease of lipid synthesis. Ten hour preincubation of activated *in vivo* macrophages with the abovementioned agonists (5 μM) decreased cholesteryl ester synthesis induced by NLDL and AcLDL addition into the cell cultivation medium. The data suggest that RXR, PPAR-α, or PPAR-γ agonists inhibited lipid synthesis and induction of cholesteryl ester synthesis in inflammatory macrophages caused by capture of native or modified LDL.

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Key words: macrophages, lipid metabolism, PPAR, RXR, inflammation

Inflammation induced in mammals by various pathogenic components leads to universal changes in lipid and lipoprotein metabolism in different tissues and cells including macrophages [1, 2]. These changes are associated with the development of atherosclerosis, which is now considered to be a kind of chronic vascular inflammation [3]. Macrophages play a central role in atherogenesis. In macrophages under conditions of acute inflammation in "unstable" atherosclerotic plaques, cholesteryl ester synthesis is activated, which led to formation of lipid inclusions in the cytoplasm [3]. Recent clinical and experimental data demonstrate the formation of lipid inclusions in macrophages in case of bacterial [4] and viral [5] infections. It is obvious now that in spite of a variety of mechanisms of macrophage transformation into foam cells, these mechanisms, undoubtedly, include

Abbreviations: AcLDL) acetylated low density lipoprotein; DMSO) dimethylsulfoxide; LPS) *E. coli* lipopolysaccharide; NLDL) native low density lipoprotein; PBS) phosphate-buffered saline (0.9% NaCl, 50 mM phosphate buffer); PPAR) peroxisome proliferator activated receptor; RXR) retinoid X receptor; SR) scavenger receptor.

* To whom correspondence should be addressed.

pro-atherogenic changes of lipid metabolism, which take place on activation of these cells by pathogenic components [6].

It is known that changes in lipid metabolism in macrophages during inflammation is a result of regulatory suppression of transcription factors from the nuclear hormone receptor super family, which are commonly activated by hydrophobic low molecular weight ligands [7]. Peroxisome proliferator activated receptors (PPAR)-α and -γ, in particular, belong in a group of transcription factors regulating lipid metabolism in macrophages. These receptors form heterodimers with retinoid X receptors (RXR), which activate transcription of target genes of enzymes involved in lipid metabolism [7]. However, there are no data about changes of lipid metabolism in tissue macrophages during inflammation *in vivo* in connection with changes in the activity of these receptors.

In the present study, changes in lipid metabolism and metabolism of native and acetylated low density lipoproteins (NLDL and AcLDL) in macrophages during inflammation induced by zymosan (α-D-mannan and β-D-glucan polymer from *Saccharomyces cerevisiae*), *E. coli* lipopolysaccharide (LPS), and non-specific stimulation of macrophages by starch injections were investigated. Effects of PPAR-α, PPAR-γ, and RXR agonists on these characteristics in acute phase of inflammation were also studied.

MATERIALS AND METHODS

Chemicals. Analytical grade chemicals were used: [1-^{14}C]oleate, [2-^{14}C]acetate, [^{125}I]Na with specific radioactivity of 56 mCi/mol and 11 mCi/μg, respectively (Amersham, England); zymosan (Serva, Germany), *E. coli* LPS, bezafibrate, 9-*cis*-retinoic acid, and BSA (Sigma, USA); rosiglitazone (Cayman Chemical Company, USA). Scintillation toluene, PPO, POPOP, hexane, isopropanol, RPMI-1640 cell culture medium, L-glutathione, and fetal bovine serum were purchased from domestic companies.

Preparative methods. LDLs (1.019-1.063 g/ml) were isolated from plasma of healthy volunteers by ultracentrifugation [8] and acetylated using acetic anhydride [9]. ^{125}I-Labeled NLDL and ^{125}I-labeled AcLDL were generated using the iodomonochloric method [10]. Low molecular weight reaction products were removed by chromatography on Sephadex G-25 and dialysis against 1 liter of solution containing 0.15 M NaCl and 0.3 mM EDTA (pH 7.4) for 24 h at 4°C. Radioactivity of ^{125}I-labeled NLDL and ^{125}I-labeled AcLDL was 70-100 pulses/min per ng protein. The lipoproteins were sterilized by filtration through a 0.45-μm filter (TPP AG, Switzerland).

Experiments with animals and injection of chemicals. Male C57Bl/6 mice maintained in the animal facility of the Institute of Cytology and Genetics (Siberian Branch of the Russian Academy of Sciences) and fed a standard laboratory diet were used. Aseptic inflammation was induced by intraperitoneal injection of zymosan or LPS in doses 50 and 0.1 mg/kg of body weight, respectively, in 1 ml of PBS (phosphate-buffered saline). One milliliter of 4% starch solution in PBS was injected. Control mice were injected with 1 ml of PBS. Nuclear hormonal receptor agonists bezafibrate (PPAR-α ligand) [11], rosiglitazone (PPAR-γ ligand) [12], and 9-*cis*-retinoic acid (RXR ligand) [13] were injected once 30 min before zymosan or LPS in doses 10, 10, and 5 mg/kg of body weight, respectively. Animals were sacrificed by cervical dislocation 2-168 h after injection of agonists; peritoneal macrophages were isolated and cultivated.

Isolation and cultivation of peritoneal macrophages. Peritoneal cells in concentration $2 \cdot 10^6$/ml were plated onto Petri dishes (diameter of 2.5 cm) or 6-well plates (TPP AG) in RPMI-1640 medium supplemented with 5% fetus bovine serum, 2 mM L-glutathione, 100 U/ml penicillin, and 100 U/ml gentamicin [14]. Cells were allowed to adhere at 37°C in a CO_2-incubator (Sanyo, Japan) for 2 h and washed with Hanks medium to remove non-adherent cells.

Incorporation of [^{14}C]oleate into cholesteryl esters and triglycerides and incorporation of [^{14}C]acetate into cholesterol and fatty acids in macrophages. Macrophage monolayer was incubated with 5 μl of an ethanol mixture containing 5 μCi of 0.2 mM [1-^{14}C]oleate added to RPMI-1640 medium supplemented with 0.2% fatty acid-free BSA at 37°C for 4 h in the presence or absence of 50 μg/ml NLDL or 25 μg/ml AcLDL [13]. Incorporation of [2-^{14}C]acetate into cholesterol was measured after 6 h incubation of cells with 1 mM [2-^{14}C]acetate added to the culture medium mentioned above [15]. In studies with PPAR-α, PPAR-γ, and RXR agonists, cells were preincubated for 10 h with 5 μl of dimethylsulfoxide (DMSO) containing bezafibrate, rosiglitazone, or 9-*cis*-retinoic acid (final concentration 5 μM). Cells were washed three times in Hanks medium and further incubated with [1-^{14}C]oleate, and intracellular synthesis of cholesteryl esters was measured in the presence or absence of NLDL and AcLDL. After the incubation, cells were washed three times with PBS containing 0.2% BSA. Lipids were then extracted with hexane–isopropanol (3 : 2 v/v) [9]. Triglycerides, fatty acids, cholesterol, and cholesteryl esters were separated by thin-layer chromatography using Silufol plates (Slovakia) as previously described [15]. Lipid bands were detected by exposure to iodine vapors, scraped off the TLC plate, and radioactivity was measured in toluene scintillator with a Mark-3 scintillation counter (Tracor Analytical, USA). Results are expressed in nmol of [1-^{14}C]oleate or [2-^{14}C]acetate incorporated into cholesterol per mg cellular protein. Protein concentration was determined by the Lowry method [16].

Degradation of ^{125}I-labeled NLDL and ^{125}I-labeled AcLDL in macrophages. The rate of degradation of ^{125}I-labeled NLDL and ^{125}I-labeled AcLDL in macrophages was determined by the standard method of Goldstein and Brown [9]. After 4 h of cellular monolayer incubation with ^{125}I-labeled lipoproteins, 2-ml aliquots of medium were assayed and subjected to 0.5 ml of 50% TCA solution and then centrifugation for 10 min at 15,000*g*. For degradation measurements, the trichloroacetic acid-soluble solution was assayed for ^{125}I radioactivity after removal of free ^{125}I by chloroform extraction. Incubation with labeled LDL in the absence of cells was used as the control. Degradation was less the 5%. Results are expressed in μg of hydrolyzed protein of lipoproteins per mg of cellular protein.

Statistical analysis was performed using Student's *t*-test.

RESULTS

Cholesteryl ester, triglyceride, cholesterol, and fatty acid synthesis in zymosan- or LPS-activated peritoneal macrophages. Intraperitoneal injection of zymosan (50 mg/kg body weight) or LPS (0.1 mg/kg body weight) in mice led to a dramatic increase of [^{14}C]oleate incorporation into cholesteryl esters, triglycerides, and phospholipids (Fig. 1) and [^{14}C]acetate incorporation into cholesterol and fatty acids in peritoneal macrophages (Fig. 2). Both zymosan and LPS led to a similar increase of triglyceride and fatty acid synthesis 6-8 h after injection (Figs. 1b and 2b) in contrast to cholesterol and cholesteryl ester synthesis (Figs. 1a and 2a). Incorporation of labeled precursors into lipids reached its maximum 18-24 h after aseptic inflammation had been induced. We observed 16-, 9-, 19-, and 23-fold increase of ^{14}C-labeled cholesterol, cholesteryl ester, triglyceride, and fatty acid production 18 h after zymosan administration compared with PBS control (Figs. 1 and 2). The rate of lipid synthesis gradually decreased at 72, 120, and 168 h though they were still 2-to-4-fold heightened 7 days later compared with solvent control. LPS administration resulted in a less significant increase of lipid synthesis at early stages of inflammation compared with zymosan injection. Also, the lipid synthesis level after LPS injection returns to normal level more rapidly (Figs. 1 and 2). In 120 h after LPS injection, the values of radioactive precursor incorporation into lipids were similar to control values, and no significant difference was observed at 168 h.

Degradation of ^{125}I-labeled NLDL and ^{125}I-labeled AcLDL in macrophages obtained 24 h after zymosan or LPS injection. The ability of macrophages isolated during acute stage of inflammatory response (24 h after zymosan or LPS injection) for degradation of native and modified LDL was altered. The investigation of ^{125}I-labeled NLDL degradation (50-200 μg of protein/ml medium) in cultured macrophages demonstrated that NLDL degradation was 4-fold heightened in cells obtained from mice treated with zymosan compared with control cells (Fig. 3a). On the contrary, ^{125}I-labeled AcLDL degradation (25-150 μg/ml) in murine macrophages after zymosan injection was reduced 2-3-fold (Fig. 3b).

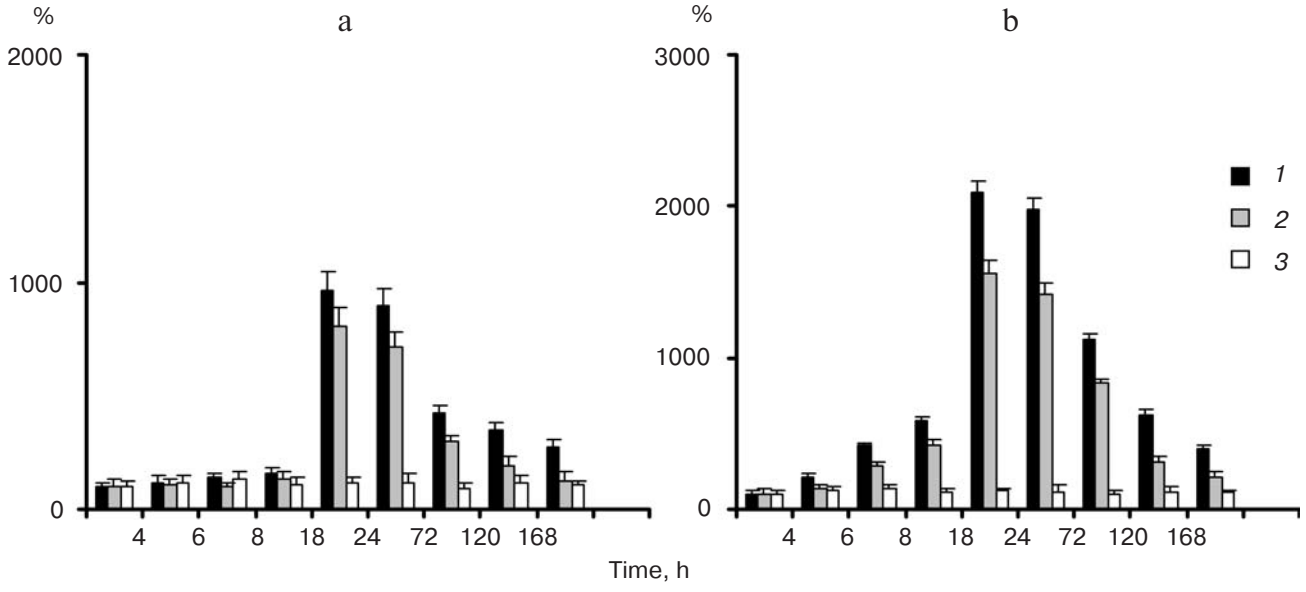

Fig. 1. Time course of [^{14}C]oleate incorporation into cholesteryl esters (a) and triglycerides (b) in peritoneal macrophages after intraperitoneal injection of zymosan (*1*) and LPS (*2*) at a dose of 50 and 0.1 mg/kg body weight, respectively, in mice. Control animals were injected with PBS (*3*). Ordinate axis, [^{14}C]oleate incorporation into cholesteryl esters and triglycerides measured in percent of control values. The 100% values were 8.58 and 32.79 nmol of esterified oleate per mg cellular protein (a and b, respectively).

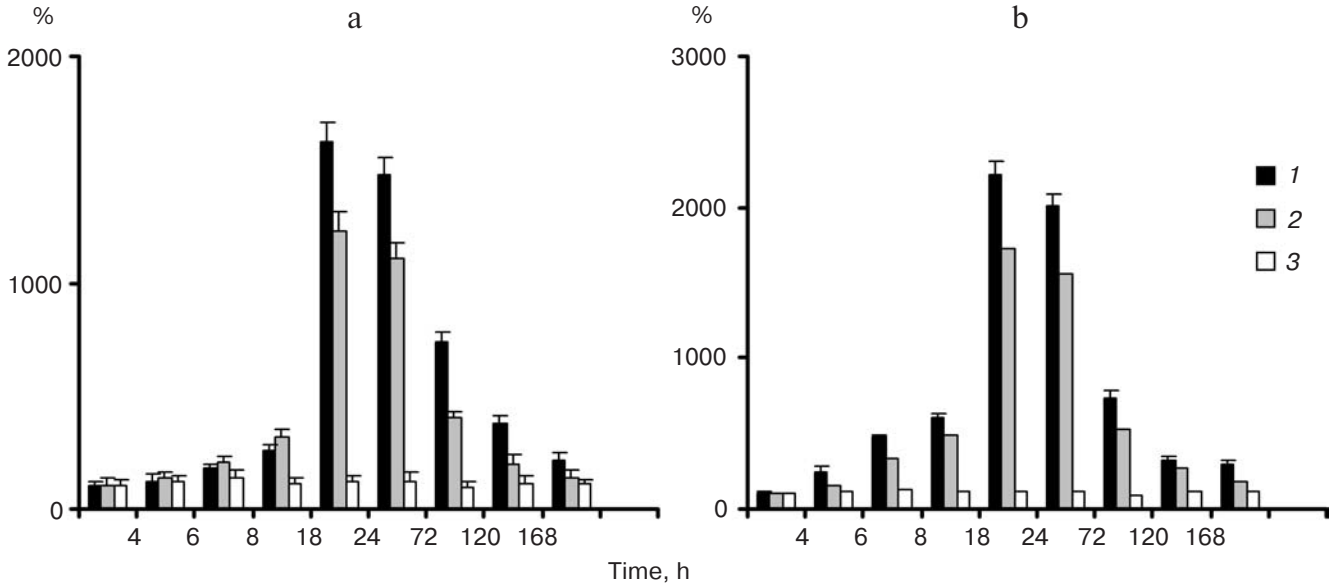

Fig. 2. Time course of [^{14}C]acetate incorporation into cholesterol (a) and fatty acids (b) in peritoneal macrophages after intraperitoneal injection of zymosan (*1*) and LPS (*2*) at a dose of 50 and 0.1 mg/kg, respectively, in mice. Control animals were injected with PBS (*3*). Ordinate axis, [^{14}C]acetate incorporation into cholesterol and fatty acids measured in percent of control values. The 100% values were 6.6 and 8.2 nmol of acetate per mg cellular protein (a and b, respectively).

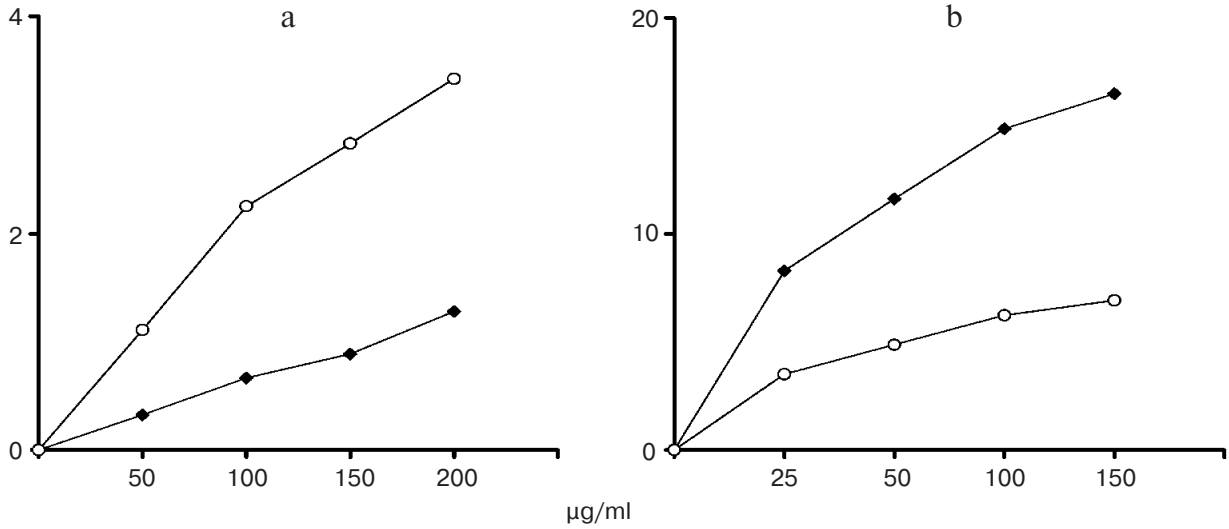

Fig. 3. Dependence of degradation rate of ^{125}I-labeled NLDL (a) and ^{125}I-labeled AcLDL (b) in resident macrophages obtained 24 h after PBS injection (closed symbols) and macrophages obtained 24 h after zymosan injection (open symbols) on concentration of ^{125}I-labeled NLDL and ^{125}I-labeled AcLDL, respectively. Ordinate, degradation of labeled LDL in μg of LDL protein per mg cellular protein in 4 h. Abscissa, concentration of labeled LDL in culture medium.

Effect of NLDL and AcLDL on [^{14}C]oleate incorporation into cholesteryl esters and triglycerides and on [^{14}C]acetate incorporation into cholesterol in macrophages obtained 24 h after zymosan or LPS injection. As evident from Table 1, activated macrophages obtained in acute stage of zymosan- or LPS-induced inflammation (after 24 h) significantly differed from control cells in their ability to stimulate cholesteryl ester synthesis after incubation with NLDL and AcLDL. Addition of NLDL (50 μg/ml) in cultured medium had no effect on the rate of labeled oleate incorporation in control cells. However, NLDL addition led to 1.9- and 2.3-fold increase of cholesterol esterification rate in zymosan- and LPS-stimulated macrophages, respectively, compared with the same cells

incubated without LDL and 14- and 9.2-fold increase compared with control cells. Cholesteryl ester changes had a different tendency after AcLDL addition into the medium. The addition of AcLDL (25 μg/ml) resulted in increase of cholesteryl ester synthesis in resident macrophages 22.6-fold. At the same time, cholesteryl ester increased 2.7- and 3.1-fold only in zymosan- and LPS-stimulated macrophages, respectively. Both NLDL and AcLDL had no reliable effect on labeled oleate incorporation into triglycerides in control cells, while the incubation of zymosan- and LPS-stimulated macrophages with NLDL or AcLDL decreased [^{14}C]oleate incorporation into triglycerides by 30-33 and 45-48%, respectively. Data in Table 1 demonstrate significant differences between control and stimulated macrophages in the rate of suppression of [^{14}C]acetate incorporation into choles-

Table 1. Effect of native and acetylated LDL on rate of [^{14}C]oleate and [^{14}C]acetate incorporation into lipids in macrophages 24 h after zymosan and LPS injection ($M \pm m$, $n = 10$)

Injection of inflammation inducers in mice	Cultivation conditions of macrophages	[^{14}C]Oleate incorporation, nmol/4 h per mg protein		[^{14}C]Acetate incorporation, nmol/6 h per mg protein
		cholesteryl esters	triglycerides	cholesterol
PBS	lipid-free medium	0.84 ± 0.04	2.72 ± 0.14	0.67 ± 0.07
Zymosan	»	8.84 ± 0.62*	56.82 ± 3.9*	8.11 ± 0.82*
LPS	»	4.7 ± 0.38*	32.09 ± 2.8*	5.82 ± 0.61*
PBS	NLDL	1.17 ± 0.12	2.61 ± 0.21	0.1 ± 0.02
Zymosan	»	16.55 ± 1.48*	38.13 ± 4.62*	7.96 ± 0.72*
LPS	»	10.88 ± 1.23*	22.85 ± 2.76*	6.02 ± 0.73*
PBS	AcLDL	18.99 ± 2.14	2.17 ± 0.23	0.05 ± 0.02
Zymosan	»	23.68 ± 2.85	29.59 ± 0.39*	7.62 ± 0.74*
LPS	»	14.72 ± 1.68	17.63 ± 0.25*	6.29 ± 0.93*

* $p < 0.001$ compared to control.

Table 2. Effect of native and acetylated LDL on [^{14}C]oleate and [^{14}C]acetate incorporation into lipids in macrophages 5 days after zymosan and starch solution injections ($M \pm m$, $n = 10$)

Injection of inflammation inducers in mice	Cultivation conditions of macrophages	[^{14}C]Oleate incorporation, nmol/4 h per mg protein		[^{14}C]Acetate incorporation, nmol/6 h per mg protein
		cholesteryl esters	triglycerides	cholesterol
PBS	lipid-free medium	0.89 ± 0.063	2.36 ± 0.16	0.76 ± 0.064
Starch	»	2.33 ± 0.26*	17.25 ± 1.32**	3.03 ± 0.22**
Zymosan	»	3.11 ± 0.34*	14.78 ± 1.56**	1.98 ± 0.21*
Starch + zymosan	»	2.98 ± 0.24*	24.55 ± 1.87**	3.71 ± 0.42**
PBS	NLDL	1.13 ± 0.12	2.28 ± 0.17	0.09 ± 0.03
Starch	»	5.44 ± 0.46**	18.48 ± 2.1**	1.94 ± 0.1*
Zymosan	»	3.05 ± 0.33*	9.62 ± 1.2**	0.56 ± 0.08
Starch + zymosan	»	7.67 ± 0.84**	20.86 ± 1.8**	3.82 ± 0.42**
PBS	AcLDL	19.67 ± 2.1	2.17 ± 0.17	0.03 ± 0.01
Starch	»	22.99 ± 2.4	14.66 ± 1.61**	1.84 ± 0.24*
Zymosan	»	24.32 ± 2.1	13.08 ± 1.22**	0.12 ± 0.01*
Starch + zymosan	»	31.27 ± 3.53*	19.82 ± 2.32**	3.59 ± 0.36**

* $p < 0.05$.
** $p < 0.001$.

terol in the presence of NLDL and AcLDL. Preincubation of control cells with NLDL or AcLDL for 10 h resulted in a full suppression of cholesterol synthesis. Inflammation-activated macrophages exhibited an increased base level of cholesterol synthesis without any significant changes after incubation with NLDL or AcLDL.

Effect of NLDL and AcLDL on [^{14}C]oleate incorporation into cholesteryl esters and triglycerides and on [^{14}C]acetate incorporation into cholesterol in macrophages isolated five days after zymosan or starch injection. Intraperitoneal injection of 4% starch solution is a standard model made to obtain activated macrophages for investigation of their functions *in vitro* [17]. Macrophages isolated 5 days after injection of 4% starch solution showed 2.6-, 7.3-, and 4-fold increase in cholesteryl ester, triglyceride, and cholesterol synthesis compared with control cells (PBS injection) (Table 2). Zymosan administration 4 days after starch injection did not produce an additive effect on cholesterol, triglyceride, and cholesteryl ester synthesis in cells isolated 24 h after zymosan injection (Table 2). But at the same time, cholesterol, triglyceride, and cholesteryl ester synthesis values were 2.2-, 2.3-, and 3-fold lower compare with the same values obtained 24 h after single zymosan injection (Table 1). Data shown in Table 2 represent the differences in ability of NLDL and AcLDL to influence cholesteryl ester and cholesterol synthesis in macrophages isolated 5 days after zymosan or starch administration. NLDL (50 μg/ml) had no significant effect on the rate of oleate incorporation into cholesteryl esters in zymosan-stimulated cells. Under these conditions, acetate incorporation into cholesterol decreased 3.5-fold. Starch-activated macrophages cultivated under the same conditions exhibited 2.3-fold increase of cholesteryl ester synthesis and 1.56-fold decrease of cholesterol synthesis. Addition of AcLDL (25 μg/ml) in medium of "starched" macrophage incubation resulted in a less evident increase in cholesteryl ester and cholesterol synthesis compared with cells obtained 5 days after zymosan injection. It was found that cholesteryl ester, triglyceride, and cholesterol synthesis rate changes was similar in starch-activated and in starch together with zymosan-activated cells in the presence of NLDL or AcLDL.

Effect of PPAR-α, PPAR-γ, and RXR agonist injection on lipid synthesis in macrophages isolated after zymosan or LPS injection. The effect of several nuclear hormone receptor agonists, namely bezafibrate (PPAR-α ligand), rosiglitazone (PPAR-γ ligand), and 9-*cis*-retinoic acid (RXR ligand), was investigated. Animals were treated with 5 mg/kg retinoic acid or 10 mg/kg bezafibrate and rosiglitazone of each compound administered in a single intraperitoneal injection. The effect of the agonists on the lipid synthesis rate was determined in LPS- or zymosan-activated macrophages. Figure 4 demonstrates that all investigated agonists significantly repressed the induction of lipid synthesis in macrophages in the acute stage of inflammation (24 h). Bezafibrate was relatively more potent in suppression of [^{14}C]oleate incorporation into triglycerides (4-fold decrease compared to LPS or zymosan injection) (Fig. 4b). At the same time, rosiglitazone was more effective in decreasing [^{14}C]oleate incorporation into cholesteryl esters (2-3-fold) (Fig. 4a). Retinoic acid primarily suppressed [^{14}C]acetate incorporation into cholesterol (Fig. 4c).

Effect of PPAR-α, PPAR-γ, and RXR agonists on the ability of NLDL and AcLDL to stimulate the rate of [^{14}C]oleate incorporation into cholesteryl esters in macrophages isolated after zymosan or LPS administration *in vitro*. The results of our investigation shown in Fig. 5 revealed that bezafibrate, rosiglitazone, and 9-*cis*-retinoic acid (5 μM of each) inhibited cholesteryl ester synthesis in the presence of NLDL or AcLDL both in control (Fig. 5a) and activated cells obtained 24 h after zymosan (Fig. 5b) or LPS (Fig. 5c) administration.

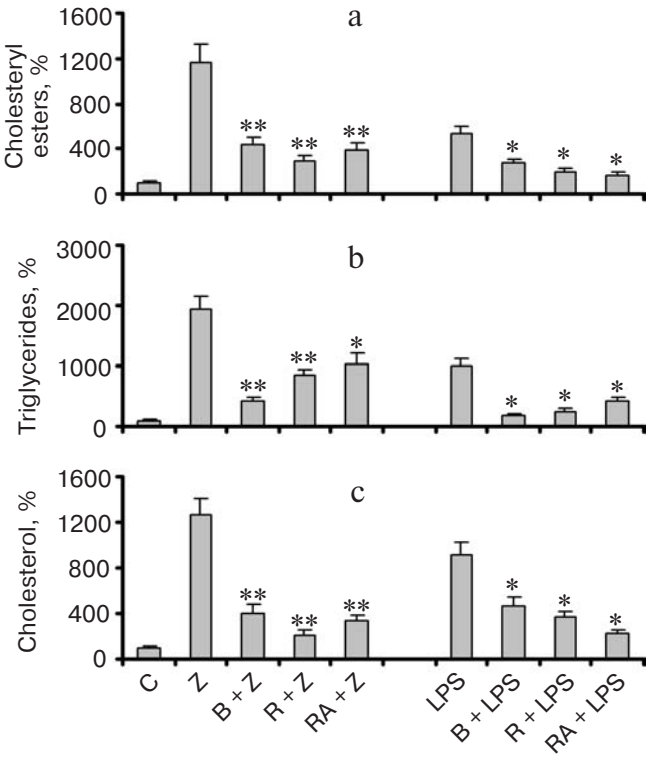

Fig. 4. Effect of intraperitoneal injection of nuclear hormone receptor ligands bezafibrate (B), rosiglitazone (R), and retinoic acid (RA) on [^{14}C]oleate incorporation into cholesteryl esters (a) and triglycerides (b) and [^{14}C]acetate incorporation into cholesterol (c) in peritoneal macrophages after intraperitoneal injection of zymosan (Z) and LPS in doses of 50 and 0.1 mg/kg, respectively. Control (C), PBS injection. Ordinate, [^{14}C]oleate incorporation into cholesteryl esters and triglycerides and [^{14}C]acetate incorporation into cholesterol measured in percent of control values. The 100% values were 8.58 and 32.79 nmol of esterified oleate per mg cellular protein in 4 h (a, b) and 6.6 nmol of acetate incorporated into cholesterol per mg cellular protein in 6 h (c). * $p < 0.05$; ** $p < 0.001$.

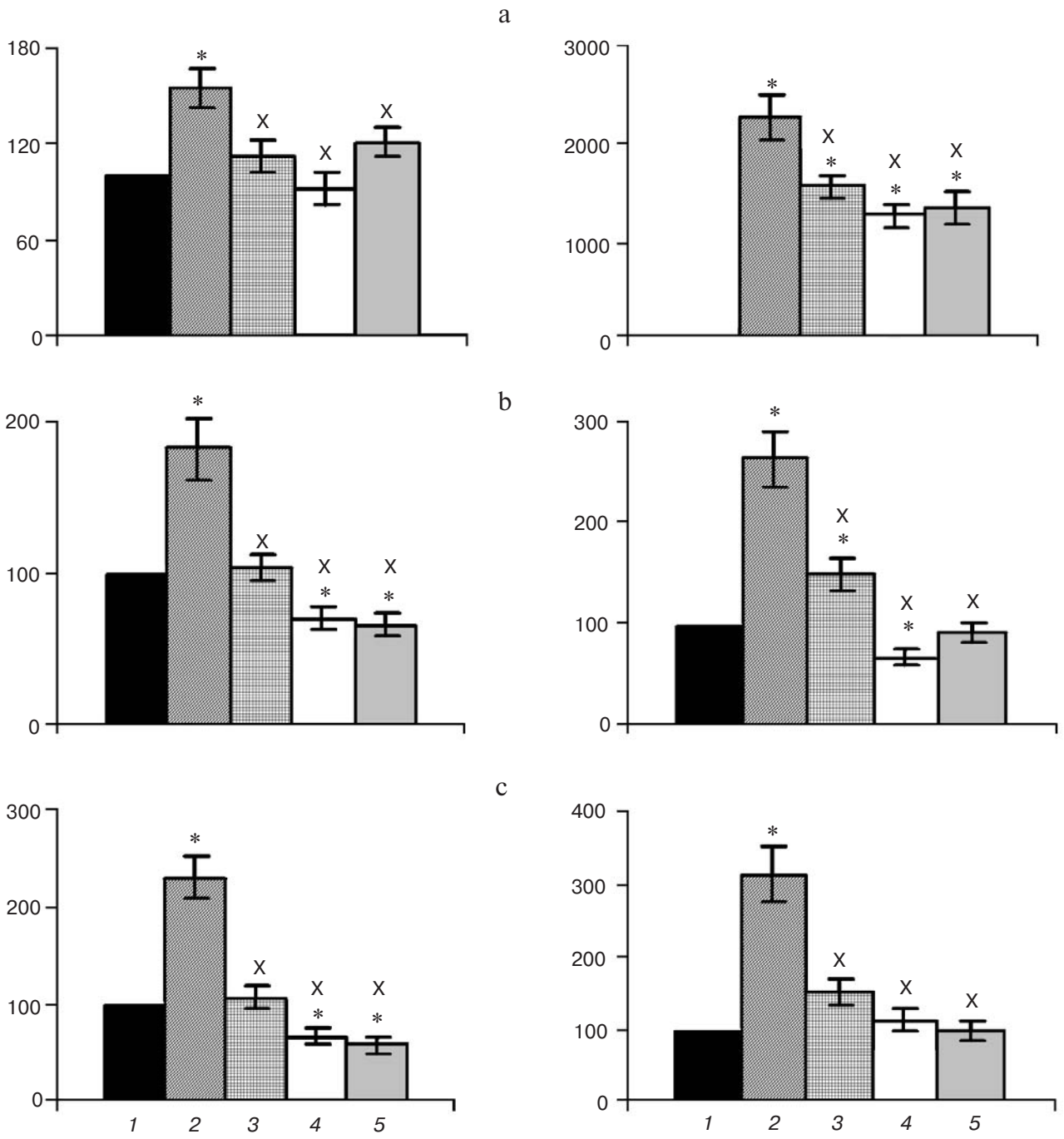

Fig. 5. Effect of preincubation of macrophages obtained after injection of PBS (a), zymosan (b), and LPS (c) with nuclear hormone receptor ligands bezafibrate, rosiglitazone, and 9-*cis*-retinoic acid on [^{14}C]oleate incorporation into cholesteryl esters in the presence of NLDL (50 μg/ml) (left panels) or AcLDL (25 μg/ml) (right panels). Ordinate, [^{14}C]oleate incorporation into cholesteryl esters measured in percent of control values. The 100% values were 0.92, 8.92, and 4.7 nmol of esterified oleate per mg cellular protein in 4 h (a-c, respectively). *1*) Lipid-free medium; *2*) LDL; *3*) LDL + bezafibrate; *4*) LDL + rosiglitazone; *5*) LDL + 9-*cis*-retinoic acid. * $p < 0.05$ compared to control. ^{x} $p < 0.05$ compared to LDL.

Preincubation of control cells (PBS injection) with bezafibrate, rosiglitazone, or retinoic acid for 10 h resulted in cholesteryl ester synthesis decrease in the presence of AcLDL by 34, 76, and 62%, respectively (Fig. 5a). PPAR-α, PPAR-γ, and RXR agonists suppressed cholesteryl ester synthesis in activated macrophages cultured with native LDL to a variable extent. Bezafibrate reduced cholesteryl ester synthesis to the base level (macrophages without LDL), while the effect of rosiglitazone and retinoic acid was even stronger (decrease in cholesteryl

ester synthesis below the base level) (Fig. 5, b and c). Rosiglitazone and retinoic acid also decreased to the base level the labeled oleate incorporation into cholesteryl esters in zymosan- or LPS-stimulated macrophages in the presence of AcLDL. Bezafibrate was less potent and reduced this index only by 40-50%.

DISCUSSION

Mechanisms of activation of cholesteryl ester synthesis in macrophages during inflammation *in vivo* are not investigated completely. On one hand, it can be due to excess of cholesterol intake through receptor-dependent intake of native and modified LDL [3]. On the other hand, it is also known that bacterial LPS stimulate lipid synthesis [18] and decrease expression of class B receptors and ATP binding cassette transporters [19], which limit cholesterol efflux from the cells.

Yeast wall component zymosan, *E. coli* LPS, or starch injection stimulates activation of fatty acid, triglyceride, cholesterol, and cholesteryl ester synthesis in macrophages, which reached its maximum 18-24 h after injection. This suggests that accumulation of fatty acids and cholesterol in macrophages during inflammation may result in increase of triglyceride and cholesteryl ester synthesis independently from receptor-dependent LDL intake. At the same time, the effect of all stimuli investigated, fatty acid consumption for triglyceride synthesis was 1.5-2.0-fold as much as for cholesteryl ester synthesis (Fig. 1). There is no literature data on lipid synthesis changes in macrophages at different stages of inflammation *in vivo*. The only report found describes increase of cholesterol synthesis in J774 cells by 25% after their activation with *E. coli* LPS [20]. However, results of a series of experiments summarized in a review [1] showed that acute inflammation induced by LPS or pro-inflammatory cytokine injection stimulates fatty acid, triglyceride, cholesterol, and phospholipid synthesis in murine liver, muscles, and kidneys with maximum at 24 h. Summarizing the literature and our own data, we suppose the increase of lipid synthesis in macrophages to be a universal response of different cells to inflammation induced by different stimuli. At the same time, our studies revealed that injection of zymosan, a very potent stimulator of inflammation, on the fourth day after starch administration did not lead to stimulation of significant lipid synthesis in macrophages. Even so the level of lipid accumulation remained lower than this level after zymosan injection only (Table 2). These results probably reflect the occurrence of macrophage tolerance on recurring injection of stimulator [21], which needs further investigation.

Our studies revealed macrophages obtained in acute stage of inflammation to have an increased ability for NLDL degradation and decreased ability for AcLDL degradation. These results generally agree with the data obtained by other authors. Early studies in J774 [20] and RAW 264.7 [22] macrophages showed that their activation by LPS leads to an increase in receptor-dependent cellular intake and degradation of NLDL. However, as authors [23] showed, the mechanism of NLDL intake activation in phorbol-myristate-activated macrophages is related to the rate of endocytosis and not to the increase of cell surface LDL receptor expression. LDL modified by acetylation of apolipoprotein B (apoB) considered to be classical ligands of scavenger receptor (SR) class A, which play a prominent role in unregulated uptake and degradation of AcLDL and cholesteryl ester synthesis induction in resident macrophages [9]. Earlier it was shown *in vitro* that tumor necrosis factor secreted by macrophages during their cultivation in the presence of LPS reduces expression of SR class A and SR-dependent degradation of AcLDL [24]. That is why relatively low influence of AcLDL and marked effect of NLDL on cholesteryl ester synthesis in activated macrophages (Table 1) that we observed can be explained both by reduced role of SR class-A-dependent pathways in cholesteryl ester synthesis induction in macrophages during inflammation and activation of NLDL uptake pathway. Addition of NLDL and AcLDL to activated macrophages resulted in decrease of triglyceride synthesis and increase of cholesteryl ester synthesis from oleate at the same rate. Our studies also revealed that neither NLDL nor AcLDL suppressed cholesterol synthesis in activated macrophages. These data suggest that stimulation of fatty acid and cholesterol synthesis together with high level of LDL intake in macrophages during inflammation may lead to a significant consumption of accumulated fatty acids on cholesteryl ester synthesis.

Our data showed that PPAR-α, PPAR-γ, and RXR inhibit triglyceride and cholesteryl ester synthesis during inflammation. This corresponds to the idea that these transcription factors play a crucial role in regulation of lipid metabolism in macrophages. Neutral lipid synthesis stimulation under the exposure of pro-inflammatory stimulators is considered to be due to accumulation of intracellular fatty acids as a result of suppression of mitochondrion β-oxidation and intracellular transport of fatty acids during inflammation [1]. It was shown that PPAR in macrophages regulate positively expression of fatty acid transporter protein and carnitine-palmitoyl transferase-1β participating in these pathways [25]. It is also known that RXR can combine into heterodimers with all types of PPAR, thus regulating PPAR target genes. All studied agonists were also able to inhibit NLDL- and AcLDL-induced cholesteryl ester synthesis in activated macrophages in micromolar concentrations *in vitro*. PPAR-γ and RXR agonists were the most effective *in vitro* decreasing the cholesteryl ester synthesis rate to base level in the presence of AcLDL and even lower in the presence of NLDL. Our results agree with literature data demonstrating the possibility of PPAR-γ [26] and PPAR-α [27]

agonists decreasing cholesteryl ester synthesis and stimulating metabolic pathways of cholesterol efflux from cells in the presence of HDL.

Thus, our results confirm the hypothesis of the prominent role of infection-inflammatory factors in proatherogenic changes in macrophage lipid metabolism. Since the NLDL degradation increases in activated macrophages, it may be assumed that cell cholesteryl ester synthesis during inflammation will increase under conditions of hypercholesterolemia development and by high level of NLDL. In turn, lipid metabolism changes in activated macrophages depend considerably on PPAR-α, PPAR-γ, and RXR activity. That is why their activation inhibits lipid synthesis and the ability of LDL to stimulate cholesteryl ester synthesis.

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