ISSN 0006-2979, Biochemistry (Moscow), 2019, Vol. 84, No. 5, pp. 529-539. © Pleiades Publishing, Ltd., 2019.
Russian Text © A. M. Bogomolova, V. S. Shavva, A. A. Nikitin, E. V. Nekrasova, E. B. Dizhe, E. E. Larionova, I. V. Kudriavtsev, S. V. Orlov, 2019, published in Biokhimiya, 2019, Vol. 84, No. 5, pp. 692-703.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM18-346, April 1, 2019.

Hypoxia as a Factor Involved in the Regulation of the *apoA-1*, *ABCA1*, and Complement *C3* Gene Expression in Human Macrophages

A. M. Bogomolova[1,2], V. S. Shavva[2], A. A. Nikitin[1,2], E. V. Nekrasova[2], E. B. Dizhe[2], E. E. Larionova[2], I. V. Kudriavtsev[2], and S. V. Orlov[1,2,a,b]*

[1]St. Petersburg State University, 199034 St. Petersburg, Russia
[2]Institute of Experimental Medicine, 197376 St. Petersburg, Russia
[a]e-mail: s.orlov@spbu.ru
[b]e-mail: serge@iem.sp.ru

Received December 10, 2018
Revised January 29, 2019
Accepted January 29, 2019

Abstract—Hypoxia plays a critical role in progression of atherosclerosis. Local oxygen deficiency in a plaque creates a specific microenvironment that alters the transcriptome of resident cells, particularly of macrophages. Reverse cholesterol transport from plaque to liver is considered a main mechanism for regression of atherosclerosis. Ubiquitously expressed ATP-binding cassette transporter A1 (ABCA1) and liver- and small intestine-derived apolipoprotein A-1 (ApoA-1) are two main actors in this process. We recently reported endogenous *apoA-1* expression in human macrophages. While ABCA1 and ApoA-1 have antiatherogenic properties, the role of complement factor C3 is controversial. Plasma C3 level positively correlates with the risk of cardiovascular diseases. On the other hand, *C3* gene knockout in a murine atherosclerosis model increases both plaque size and triglycerides level in blood. In the present study, we show for the first time that a hypoxia-mimicking agent, $CoCl_2$, induces the upregulation of the *apoA-1* and *C3* genes and the accumulation of intracellular and membrane protein ApoA-1 in THP-1 macrophages. The MEK1/2-Erk1/2 and MKK4/7-JNK1/2/3 cascades are involved in upregulation of *ABCA1* and *C3* via activation of transcription factor NF-κB, which interacts with the HIF-1α subunit of hypoxia-inducible factor 1 (HIF-1). The three major MAP-kinase cascades (Erk1/2, JNK1/2/3, and p38) and the NF-κB transcription factor are involved in the hypoxia-induced expression of the *apoA-1* gene in THP-1 macrophages.

DOI: 10.1134/S0006297919050079

Keywords: atherosclerosis, hypoxia, macrophages, THP-1, gene expression regulation, *apoA-1*, *ABCA1*, *C3*

Atherosclerosis is a chronic inflammatory disease associated with the formation of plaques in the arterial tunica intima. It is developed mainly in distorted arteries under hemodynamic shear stress [1]. Shear stress can be caused by a laminar or disturbed blood flow, with the latter leading to vessel damage and subsequent endothelium activation and thickening of tunica media and intima. Endotheliocytes trigger expression of adhesive agents, integrins, selectins, interleukins, interferons, and cytokines. Concurrent increase of vessel permeability leads to infiltration of the subendothelial space by low density lipoproteins (LDL). These molecules are bound in the intima by proteoglycans of the extracellular matrix and undergo various modifications like oxidation, proteolysis, etc. Modified LDL are absorbed by macrophages engaged during endothelium activation, which induces accumulation of intracellular cholesterol and leads to transformation of macrophages into foam cells [2].

Attraction of monocytes and thickening of intima by proliferation of smooth muscle cells leads to growth of atherosclerotic plaques and sparseness of capillaries that nourish the artery middle coat. An increased demand for oxygen triggers vascularization of the intima and plaque, but new vessels tend to originate at the perimeter of the plaque, with its center remaining under permanent hypoxia [3].

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ApoA-1, apolipoprotein A-1; HIF-1, hypoxia-inducible factor 1; LXR, liver X receptor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells.
* To whom correspondence should be addressed.

Transcription factor HIF-1 (hypoxia-inducible factor 1) is a key hypoxia mediator. It is a heterodimer of constitutively expressed subunit HIF-1β and inducible HIF-1α [4]. Under normoxia, proteins with a prolyl hydroxylase domain (PHD1, PHD2, PHD3) and asparaginyl hydroxylase FIH mediate hydroxylation-induced proteasome degradation of HIF-1α. Under low oxygen, no catalysis occurs, and HIF-1α is stabilized at the protein level [5]. HIF-1 induces proliferation of smooth muscle cells and recruitment of monocytes, other leukocytes, and T-lymphocytes to the plaque [6, 7], lowers the migration activity of macrophages, and detains them in the intima [3]. Thus, the population of macrophages and foam cells is constantly growing.

The core pathway of atherosclerosis regression is now considered to be the reverse transport of cholesterol in the form of high density lipoprotein (HDL) from plaque macrophages to the liver. The main proteins involved are apolipoprotein A-1 (ApoA-1) and ATP-binding cassette transporter A1 (ABCA1). ApoA-1 is the major protein component of antiatherogenic HDL, which regulates their plasma level. ApoA-1 is derived mainly from liver and small intestine and enhances ABCA1-mediated cholesterol efflux from peripheral tissues. ABCA1 is a ubiquitous cassette transporter that canalizes the membrane and mediates energy-dependent lipid efflux from the cell. Earlier, we reported endogenic ApoA-1 synthesis in human macrophages [8, 9]. The ApoA-1-homogeneous population of monocytes in human peripheral blood differentiates into ApoA-1-poor and ApoA-1-rich macrophages. This protein is localized in intracellular vesicles and at the external surface of the plasma membrane. Membrane ApoA-1 is either associated with ABCA1 or localizes unbound in lipid rafts [8]. Flow cytometry studies show that the membrane ABCA1 level is higher in ApoA-1-rich macrophages, and transfection with ApoA-1-specific small interfering RNA (siRNA) downregulates the transporter. Macrophage-derived ApoA-1 thus stabilizes ABCA1 in the plasma membrane. Also, ApoA-1 was demonstrated to affect proinflammatory status of the macrophage: its siRNA-induced knockdown results in upregulation of IL-1β and NOS2 in the presence of lipopolysaccharides [8].

While ApoA-1 and ABCA1 have atheroprotective properties, the role of complement C3 is highly controversial. Higher C3 levels in plasma are associated with atherosclerosis and cardiac infarctions, while the level of anaphylatoxin C3a, a hydrolysis derivate of C3, correlates with thickening of the intima and carotid middle coat and the development of acute coronary syndrome [10, 11]. Components of the complement system are present in atherosclerotic plaques arriving to the intima from blood and being synthesized by resident cells. Cascade activation in the plaque can follow three pathways. Classically, it is triggered by oxidized LDL-specific autoantibodies [12], and alternatively by cholesterol crystals, which ultimately induces the assembly of NLRP3 inflammasome and production of proinflammatory cytokines [13]. Plaque macrophages express receptors binding anaphylatoxins C3a and C5a, which trigger oxidative burst and synthesis of TNFα and IL-1. Also, C3a and C5a are chemoattractants for eosinophils, mast cells, monocytes, and B- and T-lymphocytes; they increase the permeability of small blood vessels and induce contraction of smooth muscle cells [10].

A series of studies report the systemic antiatherogenic role of C3. Atherosclerotic plaques in LDL-R- and C3-knockout mice contain more foam cells [14]. The same models also with knockout of ApoE exhibit higher levels of cholesterol and plasma triglycerides and have plaques 84% larger in size compared to C3-intact knockouts [15]. Inactivated C3b (iC3b) was also shown to play an important role in antiinflammatory clearance of apoptotic cells. The interaction of macrophages and dendrite cells with iC3b inhibits NF-κB signaling, lowers the TGF-β, and increases IL-10 secretion [16].

In this work we studied the expression mechanisms of the important for atherogenesis genes *apoA-1*, *ABCA1*, and *C3* in THP-1 macrophages under hypoxic conditions.

MATERIALS AND METHODS

Cell cultures. Human acute monocytic leukemia cell line THP-1 was obtained from the Collection of Vertebrate Cell Cultures maintained by the Institute of Cytology of the Russian Academy of Sciences. The cells were cultivated under 5% CO_2 at 37°C in RPMI medium (Biolot, Russia) containing 10% fetal calf serum (FCS, HyClone, USA). Differentiation of THP-1 monocytes to macrophages was induced by adding phorbol-12-myristate-13-acetate (PMA) to the concentration of 50 ng/ml (81 nM) for over 24 h. The cells were afterwards rinsed free of PMA and passaged in fresh RPMI medium with 10% FCS for over 48 h. Differentiating THP-1 macrophages were cultivated for three days in total.

Antibodies. Monoclonal murine antibodies against human β-actin (ab3280) were purchased from Abcam (GB). Intracellular ApoA-1 protein was detected with goat polyclonal antibodies against human ApoA-1 described earlier [17] and horseradish peroxidase-conjugated rabbit secondary antibodies against goat IgG (A5420; Sigma, USA). Surface ApoA-1 was detected with murine monoclonal antibodies against human ApoA-1 (0650-0050; Bio-Rad, USA) and Alexa Fluor 647-labeled rabbit secondary antibodies F(ab′)2 against murine IgG (Abcam).

Signaling pathway inhibitors. Inhibitors of MAPK and NF-κB were purchased from Biomol (USA), and LXR agonist from Sigma. One hour prior to administration of $CoCl_2$, the culture medium was supplemented

with one of the following compounds: p38 inhibitor SB203580 (EI-286, 12.5 μM), JNK1/2/3 inhibitor SP600125 (EI-305, 10 μM), MEK1/2 inhibitor U0126 (EI-282, 10 μM), NF-κB inhibitor QNZ (EI-352, 10 nM), LXR agonist TO901317 (2.5 μM). The solvent dimethyl sulfoxide (DMSO) was used as a control in concentrations corresponding to the probes with inhibitors.

RNA extraction. For RNA extraction and subsequent RT-PCR, cells were seeded in 24-well plates with density $1 \cdot 10^4$ cells/cm^2 and incubated under 5% CO_2 at 37°C. After 16- or 24-h incubation with $CoCl_2$, the cells were lysed with commercial RNA Extract Kit (Evrogen, Russia), and total RNA was extracted following the manufacturer's protocol. Degradation level of total RNA was verified by electrophoresis in 1% agarose gel using 28S and 18S rRNA bands as relative markers. The concentration and purity of RNA was determined with an Avaspec-2048 spectrophotometer (Avantes, The Netherlands). Optical density ratios of RNA samples at 260 and 280 nm were considered as corresponding to pure RNA if they exceeded 2.0 and 1.7 at 260 and 230 nm, respectively.

Reverse transcription and real-time PCR. In this study, expression of the *β-actin* and *GAPDH* genes was considered as reference, and *apoA-1*, *ABCA1*, *C3*, *IL-8* were the experimental genes. In reverse transcription assays we used reversed primers for experimental genes and oligo-dT18; specific primers were used because of the low mRNA concentrations of the experimental genes and to avoid reverse transcription of long 3′-untranslated regions. The reaction mixture (25 μl) contained 2 μg RNA, specific primers (0.5 μl each) (2 pmol/μl), 0.5 μl oligo-dT18 (50 pmol/μl), reverse transcriptase M-MLV (50 U), nucleotides dATP, dGTP, dCTP, and dTTP (0.5 mM each), 5 μl 5× reverse transcription buffer (250 mM Tris-HCl, pH 8.3, at 25°C, 500 mM KCl, 15 mM $MgCl_2$, 50 mM dithiothreitol). Immediately prior to the reaction, the mixture was preheated to 42°C for subsequent thermocycling (1.5 h – 42°C, 15 min – 70°C, hold at 4°C). cDNA obtained was stored at –20°C and used for RT-PCR assays.

A commercial kit from Syntol (Russia) was used for RT-PCR assays. Primers and hybridization probes for *β-actin* [18], *GAPDH* [19], *apoA-1* [19], *ABCA1* [20], and *C3* [18] have been described earlier. The reaction mixture (25 μl) contained 0.5 μl primers (10 μM), 0.2 μl Taq polymerase (1.25 U), 2.5 μl 10× PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$), 1 μl cDNA (corresponds to reverse transcription reaction products obtained from 0.08 μg total RNA), and deionized water to 25 μl. Control wells contained the reaction mixture for PCR without cDNA. Assays were performed with a CFX-96 thermocycler (Bio-Rad) under the following conditions: 95°C – 5 min, 50 cycles: 95°C – 25 s, 59.5°C – 45 s (for TaqMan); 95°C – 5 min, 50 cycles: 95°C – 30 s, 60°C – 20 s, 72°C – 30 s (for SYBR Green).

The number of cycles (Ct) necessary to reach fluorescence threshold of 10 standard deviations from the background fluorescent signal was estimated with the built-in original software from Bio-Rad. Relative cDNA contents (in percent of control) were estimated as $(2^{\text{Ct control} - \text{Ct sample}}) \times 100$. The estimates for each gene were normalized based on the geometric mean of the two reference gene expression levels (*GAPDH*, *β-actin*) as described earlier [21] and represented as relative gene expression levels with the control expression set to 100%.

Western blotting. Cells were triple-rinsed in phosphate buffered saline (PBS), pH 7.6, and lysed in RIPA-50 buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1 mM EDTA, 0.1% SDS, 0.01% NaN_3, 1 mM phenylmethylsulfonylfluoride (PMSF), pH 7.4). Protein concentrations were measured by the Lowry assay. SDS electrophoresis was done in 12% or 20% polyacrylamide gels. Proteins were transferred to nitrocellulose membrane in 2 h. A 5% dried skim milk solution in PBS with 0.02% Tween 20 was used as the blocking buffer. The samples were incubated in the blocking buffer for 1 h, with primary antibodies for 12 h at 4°C, then with horseradish peroxidase-conjugated secondary antibodies for 1 h. Peroxidase was visualized by the ECL (enhanced chemiluminescence) method. Chemiluminescent signal was detected with the ChemiDoc MP Imaging System (Bio-Rad).

Flow cytometry. For detection of surface membrane ApoA-1, THP-1 macrophages were fixed in 4% formaldehyde/PBS solution for 20 min at 4°C and triple-rinsed in PBS. The samples were further blocked for 40 min at 22°C in the blocking buffer (1% BSA, 0.02% Tween-20/PBS). The cells were exposed to murine antibodies against human ApoA-1 in titer 1 : 250 in 1% BSA, 0.02% Tween-20/PBS at 25°C for 2 h. After double-rinsing in the blocking buffer, Alexa Fluor 647-labeled secondary antibodies were added in titer 1 : 1000 in 1% BSA, 0.02% Tween-20/PBS and incubated for 1 h at 25°C in the dark. The isotype controls used secondary antibodies only. After rinsing in PBS, the cells were transferred into tubes for an Epics Altra (Beckman Coulter, USA) flow cytometer and analyzed using the Beckman Coulter Navios system.

To detect toxic effects of $CoCl_2$, THP-1 cells were washed off the plates with Versene solution and stained live with DNA-binding dyes propidium iodide and YO-PRO-1. Propidium iodide does not permeate intact cell membranes and stains dead cells, emitting in the red spectrum. YO-PRO-1 permeates cell membranes of apoptotic but not live cells and emits in the green spectrum. The analyses were done with the Beckman Coulter Navios system.

Statistical analysis. Results are presented as the mean ± mean standard error. Statistical confidence of differences between samples was estimated with Student's unpaired *t*-test. Multiple comparisons were performed

with Dunnett's test. Differences were considered significant if $p < 0.05$. Statistics were calculated in Microsoft Excel.

RESULTS

$CoCl_2$ is a time- and dose-dependent regulator of *apoA-1* and *C3* genes and upregulates ApoA-1 protein in THP-1 macrophages. To study expression regulation of *apoA-1* and *C3* in THP-1 macrophages, we used the hypoxia-mimetic agent $CoCl_2$. It stabilizes the HIF-1α protein by inhibiting PHD-containing enzymes that mediate proteasomal HIF-1α degradation in normoxia [5, 22]. Plausible cytotoxic effects of $CoCl_2$ were revealed with flow cytometry. After incubation with $CoCl_2$, the cells were stained with the DNA-binding dyes propidium iodide and YO-PRO-1 and analyzed by flow cytometry. $CoCl_2$ at concentrations of 50-300 μM induces apoptosis in less than 10% of cells compared to the control. Higher concentrations are toxic (Fig. 1a). Figure 1b shows cell distribution by fluorescence levels of propidium iodide and YO-PRO-1 after incubation with 500 μM $CoCl_2$. As a result, only 54% of the cells remain viable, and the rest go through different stages of apoptosis.

Hypoxia is known to positively regulate expression of *IL-8* and *ABCA1* in macrophages [6, 23, 24]. In this work, *IL-8* induction in response to hypoxia was used as a positive control. Levels of *IL-8* and *ABCA1* mRNA reach their peak values under 100 μM $CoCl_2$ during 16-h incubation (Fig. 2). These conditions are therefore optimal for hypoxia response induction in THP-1 macrophages. $CoCl_2$ upregulates *apoA-1* mRNA over the entire concentration range at incubation times of 16 and 24 h (Fig. 3a). The quantities of intracellular (Fig. 3b) and surface protein ApoA-1 (Fig. 4) also increase. Expression of *C3* mRNA as well increases under 100 μM $CoCl_2$ during 16-h-, but not 24-h incubation. On 24-h incubation, the gene expression lowers at concentrations of 200 μM and higher (Fig. 3c).

Role of signaling cascades MEK1/2-Erk1/2, MKK4/7-JNK1/2/3, and MKK3/6-p38 and transcription factors NF-κB and LXRs in $CoCl_2$-dependent activation of *ABCA1*, *C3*, and *apoA-1* genes in THP-1 macrophages. Transporter ABCA1, a key regulator of cholesterol efflux from macrophages, is known to be positively regulated by HIF-1α at the mRNA level [24]. However, the regulation mechanism remains unstudied. Transcription factor NF-κB plays a pivotal role in the adaptation of macrophage to hypoxia [25]. In a hypoxic environment, macrophages induce signaling that involves stress-activated MAP kinases Erk1/2 [26], p38 [27], and JNK [28]. Low oxygen-mediated activation of these kinases was also reported for other cell types [29-32].

To define the role of MAP kinases and transcription factor NF-κB in $CoCl_2$-dependent expression of *ABCA1*, *C3*, and *apoA-1* in THP-1 macrophages, inhibitors of p38 (SB203580), JNK1/2/3 (SP600125), and MEK1/2 kinases (U0126) and of transcription factor NF-κB (QNZ) were added to the culture medium 1 h prior to

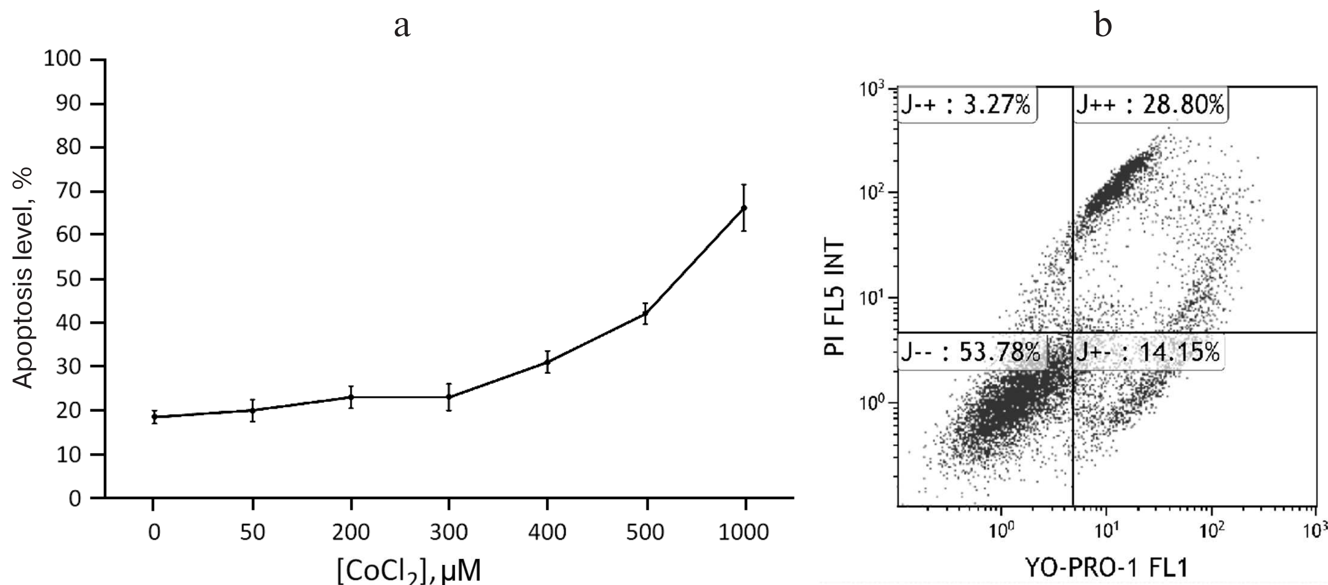

Fig. 1. Cytotoxic effects of $CoCl_2$. a) Flow cytometry. Each dot represents a sum of quadrants J+– and J++. The graph is plotted based on three independent experiments. Error bars correspond to the mean standard error. b) Scatter dot plot showing the distribution of THP-1 macrophages over fluorescence levels of YO-PRO-1 and propidium iodide under 500 μM $CoCl_2$. Axes YO-PRO-1 FL1 and PI FL5 INT represent YO-PRO-1 and propidium iodide fluorescence levels, respectively. Quadrants correspond to percentage of cells in different states: live cells (J– –); cells in early (J+–) and late (J++) apoptosis; dead cells (cell debris; J–+).

administration of $CoCl_2$. The LXR agonist (TO901317) that strongly upregulates *ABCA1* [24] and *apoA-1* mRNA [9] in macrophages was used as a positive control.

Inhibitors of MEK1/2 and JNK1/2/3, but not of p38, arrest the $CoCl_2$-dependent expression activation of *ABCA1* (Fig. 5a) and *C3* (Fig. 5b). Apart from abolishing the effect of $CoCl_2$, inhibitor of NF-κB downregulates both genes in $CoCl_2$-intact cells. The LXR agonist, as expected, strongly enhances *ABCA1* expression by itself and in combination with $CoCl_2$. The opposite effect of the agonist is observed for *C3* gene; its expression lowers compared to the control in either presence or absence of $CoCl_2$. The Co^{2+}-mediated induction of *apoA-1* expression is dependent on all three major MAP kinase cascades (Fig. 5c). Inhibition of JNK kinases dampens the effect of hypoxia on the *apoA-1* expression, while inhibition of p38 and MEK1/2 kinases was associated with reversal of the impact of Co^{2+} (Fig. 5c). The inducing effect of Co^{2+} was also reversed by the inhibitor of NF-κB but not by the LXR agonist. Although the 5′-regulatory region of *apoA-1* does not contain NF-κB-binding sites, NF-κB-dependent regulation of *apoA-1* in macrophages was reported earlier [9]. NF-κB probably regulates *apoA-1* via interaction with ligand-activated nuclear receptor PPARα (a negative regulator of *apoA-1* in macrophages) leading to inactivation of both transcription factors.

In summary, induction of the cell response to hypoxia activates the *apoA-1*, *ABCA1*, and *C3* genes and also triggers accumulation of intracellular and surface-bound ApoA-1 protein in THP-1 macrophages. $CoCl_2$-dependent regulation of *ABCA1* and *C3* gene expression engages signaling cascades NF-κB, MKK4/7-JNK1/2/3, and MEK1/2-Erk1/2, but not MKK3/6-p38. The $CoCl_2$-dependent induction of *apoA-1* expression is mediated by all three studied MAP kinase cascades and transcription factor NF-κB. Activation of nuclear receptor LXRα upregulates *ABCA1* and *apoA-1* in either the presence or absence of $CoCl_2$ but has the opposite impact on the expression of *C3*.

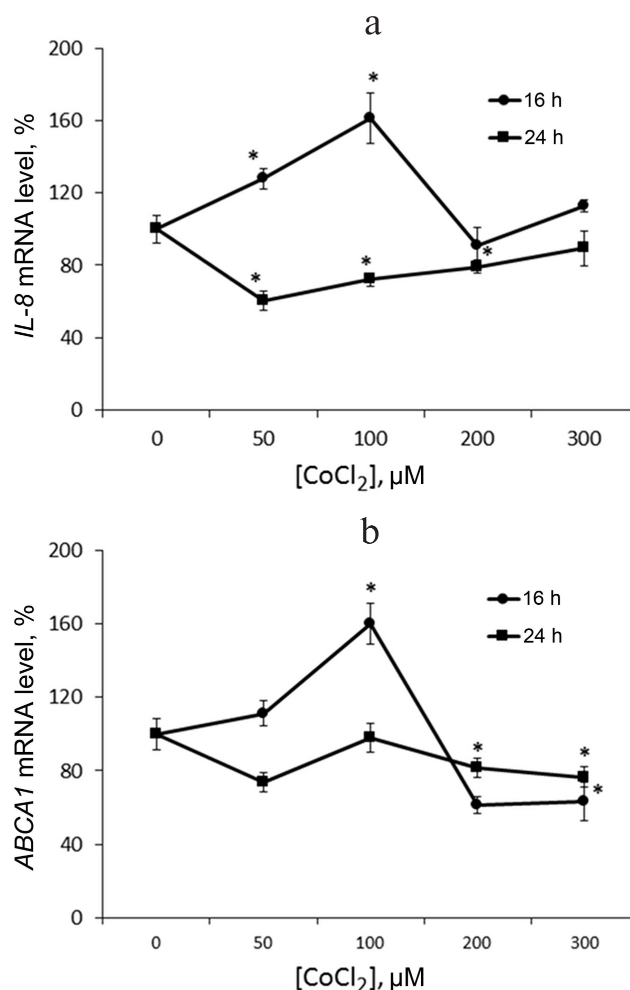

Fig. 2. $CoCl_2$ is a dose- and time-dependent expression regulator of *IL-8* (a) and *ABCA1* (b) genes in THP-1 macrophages. Real-time PCR. Cells were incubated at the indicated $CoCl_2$ concentrations for 16 and 24 h. Error bars correspond to the mean standard error. The data are derived from three independent experiments. Statistically significant differences between mRNA samples in the control and $CoCl_2$ treatments are marked with asterisks (Student's *t* test, * $p < 0.05$).

DISCUSSION

Local hypoxia of atherosclerotic plaques has been described in the literature [33]. Hypoxic microenvironment is among the inducers of monocyte differentiation into macrophages, particularly via activation of proinflammatory genes [34]. Hypoxia-induced and inflammatory signalings are largely similar, which is particularly obvious from the hierarchical interaction of transcription factors NF-κB and HIF-1α, the major modulators of cell adaptation to hypoxia. NF-κB not only facilitates the cytokine-dependent induction of gene *HIF1A* under hypoxia, but also mediates its constitutive expression [35]. In turn, HIF-1α upregulates the *p65* subunit of NF-κB and IKKα [36]. Interestingly, NF-κB activation under hypoxia may be partly mediated by a mechanism of HIF-1α stabilization. IKKβ kinase that induces dissociation of the NF-κB/IκBα complex contains conserved motif LxxLAP involved in proline hydroxylation. The same motif of HIF-1α is hydroxylated by PHD-containing enzymes in normoxia. Inhibiting of PHD1 and PHD2 leads to accumulation of IKKβ in the cell and subsequent activation of NF-κB [37].

In this work, we have shown that inhibition of NF-κB not only reverses the $CoCl_2$-dependent upregulation of the *ABCA1* and *C3* genes, but also downregulates them without the induction of response to hypoxia. In the murine macrophage model, it was shown that expression of *ABCA1* increases after treatment with TNFα in the NF-κB-dependent manner [38]. It was also shown that

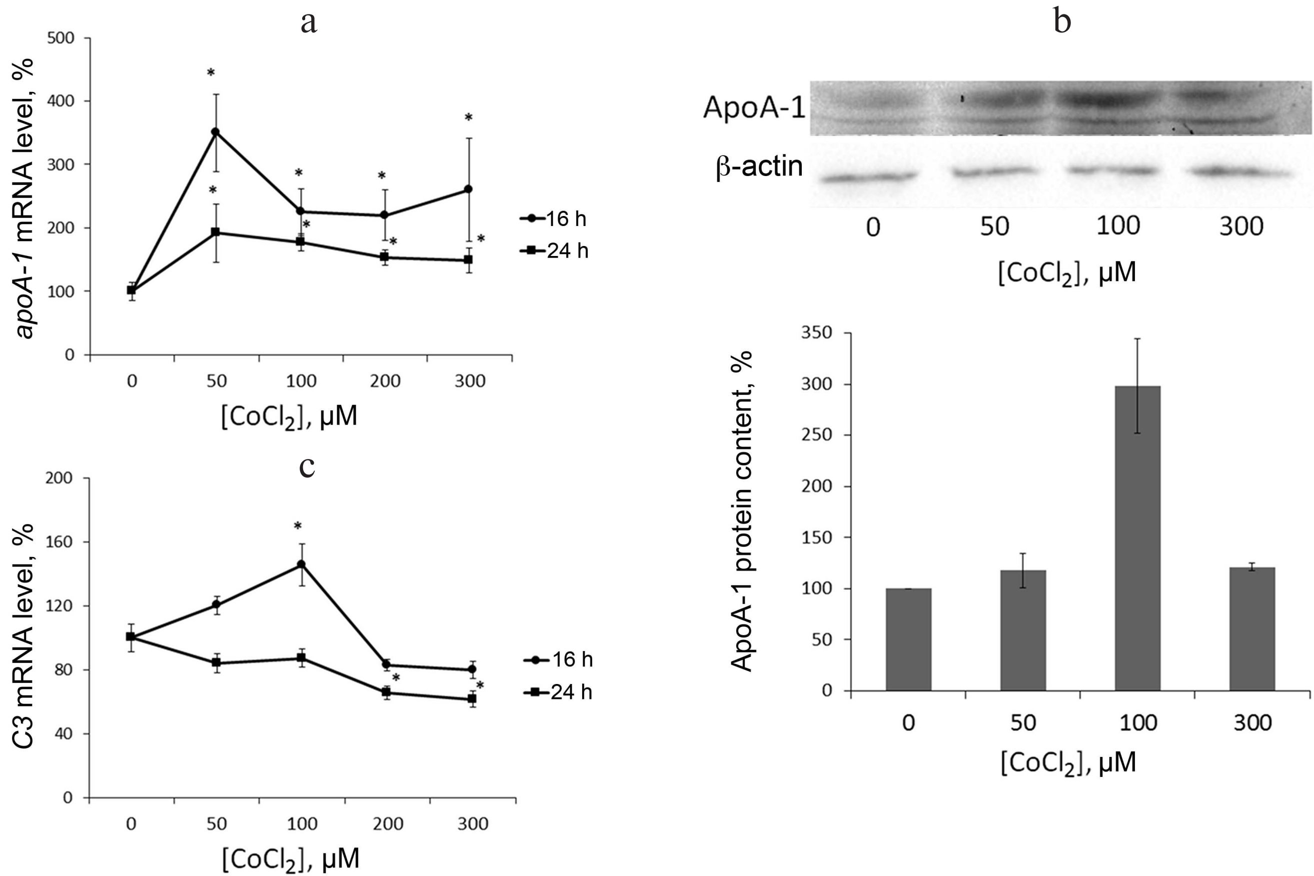

Fig. 3. $CoCl_2$ is a dose- and time-dependent expression regulator of *apoA-1* (a) and *C3* (c) genes and a factor of intracellular ApoA-1 protein accumulation in THP-1 macrophages (b). a, c) Real-time PCR. Cells were incubated under the indicated $CoCl_2$ concentrations for 16 and 24 h. Error bars correspond to the mean standard error. The data are derived from three independent experiments. The Y-axis shows the mRNA level in percent, with 100% being the level in intact cells. Statistically significant differences between mRNA levels in the samples are marked with asterisks (Student's *t* test, * $p < 0.05$). b) Western blotting. Cells were incubated under the indicated $CoCl_2$ concentrations for 16 h. The plot represents data from three immunoblots quantified with densitometry. The Y-axis shows relative ApoA-1 levels, with 100% corresponding to intact cells. Values are normalized to β-actin levels.

NF-κB knockdown in epithelium of small intestine precludes higher transcription of *C3* gene in response to IL-1β [39]. Obviously, TNFα and IL-1β signalings can be considered hypoxia-specific, as in both cases NF-κB is the main modulator.

Another important element of hypoxia-induced signal cascades is MAP kinases. We demonstrated that kinases MEK1/2 and JNK1/2/3, but not p38, are involved in the regulation of the *ABCA1* and *C3* genes in THP-1 macrophages in response to hypoxia. Ample evidence suggests interactions between the MEK1/2-Erk1/2, HIF-1, and NF-κB pathways. HIF-1 activation is known to recruit the p300/CBP complex. MAPK inhibition was shown to disturb interaction with HIF-1α/p300. Overexpression of MEK1 results in transactivation of both proteins [40]. Apart from interaction with co-activators, the transcription activity of HIF-1α depends on certain posttranslational modifications. Phosphorylation of Ser641/643 residues in HIF-1α by Erk1/2 kinase arrests its exportin CRM1-geared efflux from the nucleus. This allows the buildup of the nuclear fraction of active HIF-1α [41]. Also, Erk1/2, but not p38, is involved in transactivation of NF-κB subunit p65. This interaction upregulates the *MIP-2* gene in murine macrophages under hypoxia [42]. Activation of JNK1/2/3 under low O_2 has been reported for macrophages [28] and other cell types [30]. Induction of *apoA-1* in hypoxic macrophages depends on the activation of proinflammatory MAP kinase cascade p38, apart from the Erk1/2 and JNK1/2/3 cascades. Hypoxia is a known enhancement factor for phosphorylated p38 (in active form) in hippocampus cortex neurons [43]. Hypoxia enhances activation of p38 in macrophages in the presence of palmitic acid, which causes endoplasmic reticulum stress [44]. The arrest of p38 and Erk1/2 cascades leads to an increase of normal *apoA-1* expression and reversal of the stimulating effect of Co^{2+}. Previously [45, 46], we demonstrated enhancement of the *apoA-1*

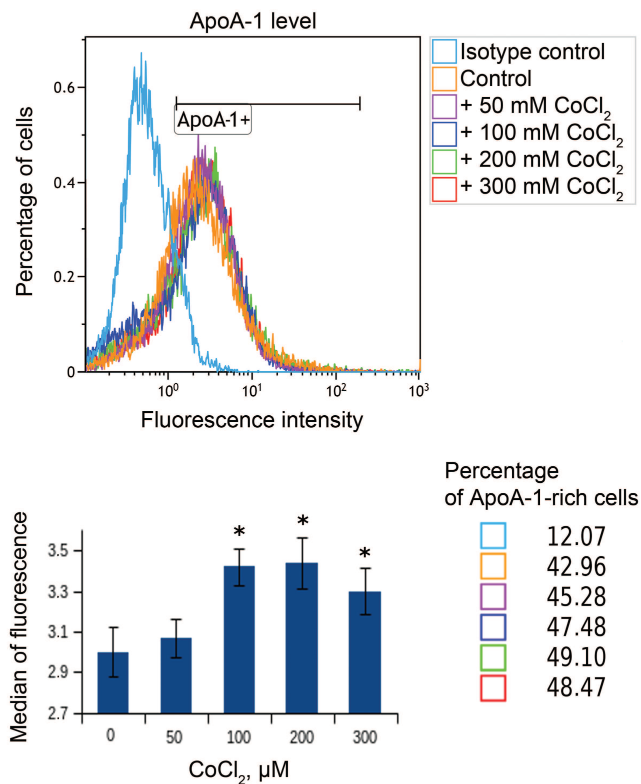

Fig. 4. $CoCl_2$ induces accumulation of membrane surface ApoA-1 protein in THP-1 macrophages. Flow cytometry. Plots reflect changes in surface ApoA-1 quantities under the indicated $CoCl_2$ treatments. ApoA1+ marks ApoA-1-rich macrophages. The plot shows medians of surface ApoA-1 content distributions in cells at different $CoCl_2$ concentrations. Statistically significant deviations from the control (zero concentration of $CoCl_2$) are marked with asterisks (Student's *t* test, * $p < 0.01$). Cell percentage corresponds to that of ApoA-1-rich macrophages in different $CoCl_2$ treatments (color-coded).

gene under arrest of the Erk1/2 cascade in human hepatoma line HepG2. This effect was found to be associated with phosphorylation of transcription factor complex FOXO1/LXRβ interacting with the B and C sites of the hepatic enhancer of *apoA-1* gene [45]. Moreover, damage of B or C sites of the hepatic enhancer of *apoA-1* reverses the effect of hydrogen peroxide (oxidative stress) on activity of the 5′-regulatory region of the *apoA-1* gene in HepG2 cells [46]. Considering (i) high similarity between proinflammatory cytokine-activated signaling pathways in hypoxia and in oxidative stress [25, 31-37, 44] and (ii) the interaction of transcription factors FOXO1 and LXRβ with B and C sites of the hepatic enhancer of the *apoA-1* gene not only in HepG2 cells but in human macrophages as well [9], we suggest that the reversal of the effect of Co^{2+} on *apoA-1* expression, when MAP kinase cascades p38 and Erk1/2 are blocked, is mediated by changes in phosphorylation level of transcription factors LXRβ and FOXO1. Further research is necessary to elucidate this question.

In our design, the synthetic LXR agonist TO901317 that strongly upregulates the *ABCA1* gene was used as the positive control. As expected, it increased the *ABCA1* mRNA level in THP-1 macrophages independently and in

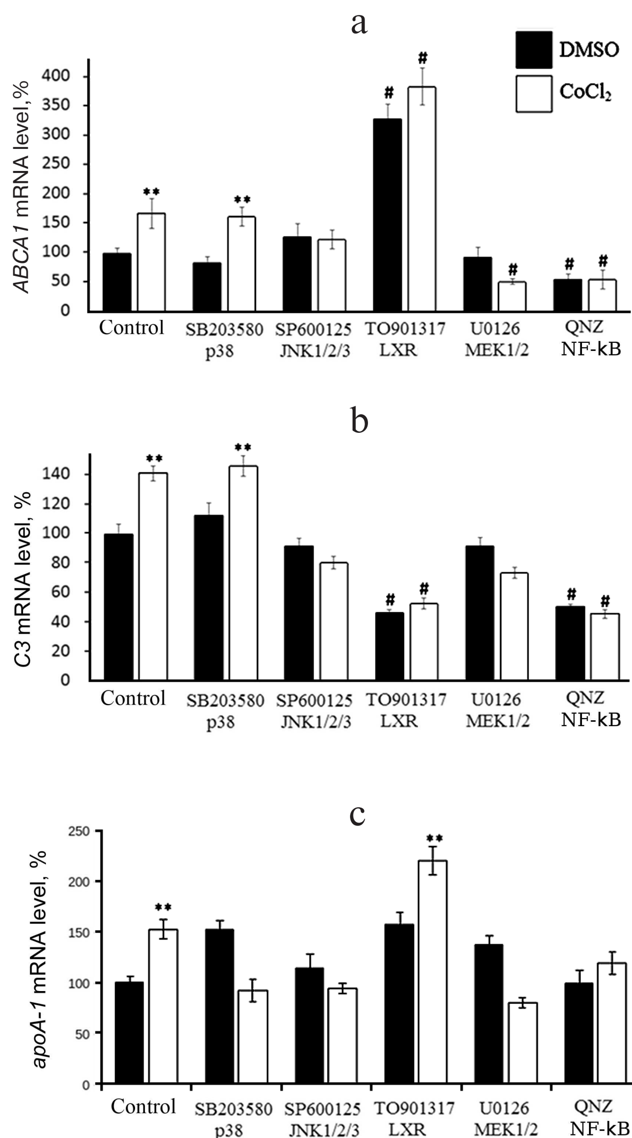

Fig. 5. Role of MAP kinase cascades MEK1/2-Erk1/2, MKK4/7-JNK1/2/3, and MKK3/6-p38 and transcription factors NF-κB and LXRs in $CoCl_2$-dependent activation of *ABCA1* (a), *C3* (b), and *apoA-1* genes (c) in THP-1 macrophages. Real-time PCR. Cells were incubated with one of the following compounds – SB203580 (12.5 μM), SP600125 (10 μM), U0126 (10 μM), QNZ (10 nM), TO901317 (2.5 μM) – for 1 h prior to administration of $CoCl_2$ (100 μM). The data are derived from three independent assays. The Y-axis shows mRNA levels in percent, with 100% corresponding to DMSO-treated cells. Error bars correspond to the mean standard error. Statistically significant differences between mRNA levels in compared groups are marked with asterisks (** $p < 0.05$, Dunnett's test). Statistically significant differences from mRNA level in DMSO-treated cells are marked with # symbol ($^{\#}$ $p < 0.05$, Student's *t*-test).

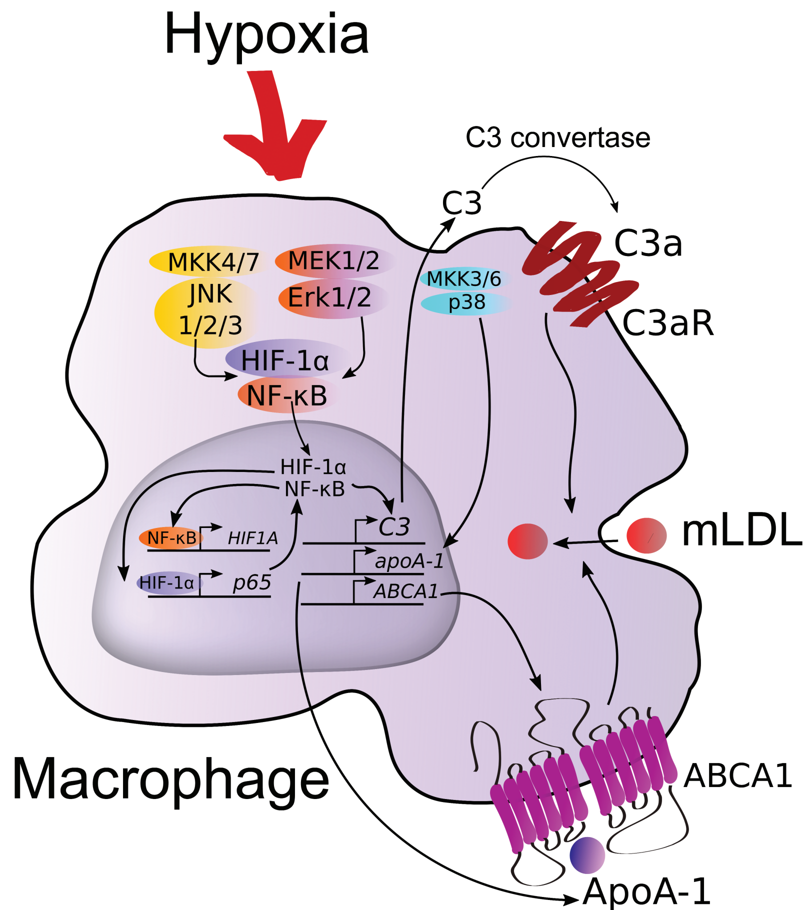

Fig. 6. Hypothetical regulation of the *ABCA1*, *C3*, and *apoA-1* genes in human macrophages under hypoxia. (For details see "Discussion" section.)

combination with $CoCl_2$, thus suggesting independent action of LXR and NF-κB/HIF-1 reported earlier [38]. Of interest is the reverse effect of TO901317 on expression of the *C3* gene. We previously reported the TO901317-dependent upregulation of the *C3* gene in THP-1 macrophages after 5-day differentiation in culture [18]. In the present study, THP-1 macrophages were cultivated for 3 days. This effect can be compared with the TNFα-induced mechanism of *apoA-1* expression regulation that we described earlier. The TNFα treatment results in more than a 5-fold increase of *apoA-1* expression in THP-1 monocytes, while it grows only 1.5-fold in THP-1 macrophages. Time-dependent *apoA-1* expression in THP-1 monocytes was also shown. A 5-fold increase in expression is observed after 24-h incubation with TNFα, while after 48-h incubation the *apoA-1* expression drops to the control level [9].

We can now consider the mechanism of positive regulation of expression of the *apoA-1*, *ABCA1*, and *C3* genes in macrophages in response to hypoxia (Fig. 6). Elevated levels of *C3* mRNA and plausibly its protein product result in the production of anaphylatoxin C3a. Binding to its receptor on the macrophage surface membrane, C3a induces expression of *C3* and activates uptake of modified LDL (mLDL) by the cells [18]. Although endogenous ApoA-1 stabilizes ABCA1 and has antiinflammatory properties, its level does not correlate with the intensity of reverse cholesterol transport [8]. On the other hand, transmission of the expression vector of the human *apoA-1* gene into macrophages of mice with *apoA-1* knockout results in noticeable reduction of plaques [47]. Also, knockouts of ABCA1 and ABCG1 were shown to disturb chemotaxis in macrophages [48]. Considering the impaired migration activity of hypoxic macrophages, this effect is likely negated by endogenous ApoA-1 via stabilization of ABCA1. Therefore, if induction of *C3* expression in hypoxia can be considered atherogenic, the role of highly expressed *apoA-1* and *ABCA1* remains controversial and demands further study.

Funding

This work was supported by the Russian Science Foundation, grant no. 17-15-01326; expression regula-

tion of *ABCA1* and *C3* genes under hypoxic conditions (Figs. 1, 2, 3b, 5a, 5b) and by the Russian Foundation for Basic Research, grant no. 17-04-01947; expression of *apoA-1* gene (Figs. 3a, 3c, 4, 5c).

Conflict of Interest

The authors declare no conflict of interest.

Ethical Approval

This article does not contain any description of studies involving humans and animals as objects.

REFERENCES

1. Heo, K.-S., Fujiwara, K., and Abe, J. (2014) Shear stress and atherosclerosis, *Mol. Cells*, **37**, 435-440, doi: 10.14348/molcells.2014.0078.
2. Bonomini, F., Tengattini, S., Fabiano, A., Bianchi, R., and Rezzani, R. (2008) Atherosclerosis and oxidative stress, *Histol. Histopathol.*, **23**, 381-390, doi: 10.14670/HH-23.381.
3. Fong, G.-H. (2015) Potential contributions of intimal and plaque hypoxia to atherosclerosis, *Curr. Atheroscler. Rep.*, **17**, 32-41, doi: 10.1007/s11883-015-0510-0.
4. Parathath, S., Mick, S. L., Feig, J. E., Joaquin, V., Grauer, L., Habiel, D. M., Gassmann, M., Gardner, L. B., and Fisher, E. A. (2011) Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism, *Circ. Res.*, **109**, 1141-1152, doi: 10.1161/CIRCRESAHA.111.246363.
5. Epstein, A. C., Gleadle, J. M., McNeill, L. A., Hewitson, K. S., O'Rourke, J., Mole, D. R., Mukherji, M., Metzen, E., Wilson, M. I., Dhanda, A., Tian, Y. M., Masson, N., Hamilton, D. L., Jaakkola, P., Barstead, R., Hodgkin, J., Maxwell, P. H., Pugh, C. W., Schofield, C. J., and Ratcliffe, P. J. (2001) *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation, *Cell*, **107**, 43-54, doi: 10.1016/S0092-8674(01)00507-4.
6. Hirani, N., Antonicelli, F., Strieter, R. M., Wiesener, M. S., Ratcliffe, P. J., Haslett, C., and Donnelly, S. C. (2001) The regulation of interleukin-8 by hypoxia in human macrophages – a potential role in the pathogenesis of the acute respiratory distress syndrome (ARDS), *Mol. Med. Camb. Mass*, **7**, 685-697.
7. Murdoch, C., Muthana, M., and Lewis, C. E. (2005) Hypoxia regulates macrophage functions in inflammation, *J. Immunol.*, **175**, 6257-6263, doi: 10.4049/jimmunol.175.10.6257.
8. Mogilenko, D. A., Orlov, S. V., Trulioff, A. S., Ivanov, A. V., Nagumanov, V. K., Kudriavtsev, I. V., Shavva, V. S., Tanyanskiy, D. A., and Perevozchikov, A. P. (2012) Endogenous apolipoprotein A-I stabilizes ATP-binding cassette transporter A1 and modulates Toll-like receptor 4 signaling in human macrophages, *FASEB J.*, **26**, 2019-2030, doi: 10.1096/fj.11-193946.
9. Shavva, V. S., Mogilenko, D. A., Nekrasova, E. V., Trulioff, A. S., Kudriavtsev, I. V., Larionova, E. E., Babina, A. V., Dizhe, E. B., Missyul, B. V., and Orlov, S. V. (2018) Tumor necrosis factor alpha stimulates endogenous apolipoprotein A-I expression and secretion by human monocytes and macrophages: role of MAP-kinases, NF-kB, and nuclear receptors PPARα and LXRs, *Mol. Cell. Biochem.*, **448**, 211-223, doi: 10.1007/s11010-018-3327-7.
10. Speidl, W. S., Kastl, S. P., Huber, K., and Wojta, J. (2011) Complement in atherosclerosis: friend or foe? *J. Thromb. Haemost.*, **9**, 428-440, doi: 10.1111/j.1538-7836.2010.04172.x.
11. Hertle, E., van Greevenbroek, M. M., Arts, I. C., van der Kallen, C. J., Geijselaers, S. L., Feskens, E. J., Jansen, E. H., Schalkwijk, C. G., and Stehouwer, C. D. (2014) Distinct associations of complement C3a and its precursor C3 with atherosclerosis and cardiovascular disease. The CODAM study, *Thromb. Haemost.*, **111**, 1102-1111, doi: 10.1160/TH13-10-0831.
12. Binder, C. J., Chang, M.-K., Shaw, P. X., Miller, Y. I., Hartvigsen, K., Dewan, A., and Witztum, J. L. (2002) Innate and acquired immunity in atherogenesis, *Nat. Med.*, **8**, 1218-1226, doi: 10.1038/nm1102-1218.
13. Samstad, E. O., Niyonzima, N., Nymo, S., Aune, M. H., Ryan, L., Bakke, S. S., Lappegard, K. T., Brekke, O.-L., Lambris, J. D., Damas, J. K., Latz, E., Mollnes, T. E., and Espevik, T. (2014) Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release, *J. Immunol.*, **192**, 2837-2845, doi: 10.4049/jimmunol.1302484.
14. Buono, C., Come, C. E., Witztum, J. L., Maguire, G. F., Connelly, P. W., Carroll, M., and Lichtman, A. H. (2002) Influence of C3 deficiency on atherosclerosis, *Circulation*, **105**, 3025-3031.
15. Persson, L., Boren, J., Robertson, A.-K. L., Wallenius, V., Hansson, G. K., and Pekna, M. (2004) Lack of complement factor C3, but not factor B, increases hyperlipidemia and atherosclerosis in apolipoprotein $E^{-/-}$ low-density lipoprotein receptor$^{-/-}$ mice, *Arterioscler. Thromb. Vasc. Biol.*, **24**, 1062-1067, doi: 10.1161/01.ATV. 0000127302.24266.40.
16. Amarilyo, G., Verbovetski, I., Atallah, M., Grau, A., Wiser, G., Gil, O., Ben-Neriah, Y., and Mevorach, D. (2010) iC3b-opsonized apoptotic cells mediate a distinct anti-inflammatory response and transcriptional NF-κB-dependent blockade, *Eur. J. Immunol.*, **40**, 699-709, doi: 10.1002/eji.200838951.
17. McVicar, J. P., Kunitake, S. T., Hamilton, R. L., and Kane, J. P. (1984) Characteristics of human lipoproteins isolated by selected-affinity immunosorption of apolipoprotein A-I, *Proc. Natl. Acad. Sci. USA*, **81**, 1356-1360.
18. Mogilenko, D. A., Kudriavtsev, I. V., Trulioff, A. S., Shavva, V. S., Dizhe, E. B., Missyul, B. V., Zhakhov, A. V., Ischenko, A. M., Perevozchikov, A. P., and Orlov, S. V. (2012) Modified low density lipoprotein stimulates complement C3 expression and secretion via liver X receptor and Toll-like receptor 4 activation in human macrophages, *J. Biol. Chem.*, **287**, 5954-5968, doi: 10.1074/jbc.M111.289322.
19. Orlov, S. V., Mogilenko, D. A., Shavva, V. S., Dizhe, E. B., Ignatovich, I. A., and Perevozchikov, A. P. (2010) Effect of TNFα on activities of different promoters of human

apolipoprotein A-I gene, *Biochem. Biophys. Res. Commun.*, **398**, 224-230, doi: 10.1016/j.bbrc.2010.06.064.

20. Mogilenko, D. A., Shavva, V. S., Dizhe, E. B., Orlov, S. V., and Perevozchikov, A. P. (2010) PPARγ activates ABCA1 gene transcription but reduces the level of ABCA1 protein in HepG2 cells, *Biochem. Biophys. Res. Commun.*, **402**, 477-482, doi: 10.1016/j.bbrc.2010.10.053.
21. Mogilenko, D. A., Kudriavtsev, I. V., Shavva, V. S., Dizhe, E. B., Vilenskaya, E. G., Efremov, A. M., Perevozchikov, A. P., and Orlov, S. V. (2013) Peroxisome proliferator-activated receptor alpha positively regulates complement C3 expression but inhibits TNFα-mediated activation of C3 gene in mammalian hepatic derived cells, *J. Biol. Chem.*, **288**, 1726-1738, doi: 10.1074/jbc.M112.437525.
22. Vengellur, A., and LaPres, J. J. (2004) The role of hypoxia inducible factor 1α in cobalt chloride induced cell death in mouse embryonic fibroblasts, *Toxicol. Sci.*, **82**, 638-646, doi: 10.1093/toxsci/kfh278.
23. Rydberg, E. K., Salomonsson, L., Hulten, L. M., Noren, K., Bondjers, G., Wiklund, O., Bjornheden, T., and Ohlsson, B. G. (2003) Hypoxia increases 25-hydroxycholesterol-induced interleukin-8 protein secretion in human macrophages, *Atherosclerosis*, **170**, 245-252, doi: 10.1016/S0021-9150(03)00302-2.
24. Schmitz, G., and Langmann, T. (2005) Transcriptional regulatory networks in lipid metabolism control ABCA1 expression, *Biochim. Biophys. Acta*, **1735**, 1-19, doi: 10.1016/j.bbalip.2005.04.004.
25. Marsch, E., Sluimer, J. C., and Daemen, M. J. A. P. (2013) Hypoxia in atherosclerosis and inflammation, *Curr. Opin. Lipidol.*, **24**, 393-400, doi: 10.1097/MOL.0b013e32836484a4.
26. Li, R. C., Haribabu, B., Mathis, S. P., Kim, J., and Gozal, D. (2011) Leukotriene B4 receptor-1 mediates intermittent hypoxia-induced atherogenesis, *Am. J. Respir. Crit. Care Med.*, **184**, 124-131, doi: 10.1164/rccm.201012-2039OC.
27. Ortiz-Masia, D., Diez, I., Calatayud, S., Hernandez, C., Cosin-Roger, J., Hinojosa, J., Esplugues, J. V., and Barrachina, M. D. (2012) Induction of CD36 and thrombospondin-1 in macrophages by hypoxia-inducible factor 1 and its relevance in the inflammatory process, *PloS One*, **7**, e48535, doi: 10.1371/journal.pone.0048535.
28. Fuhrmann, D. C., Tausendschon, M., Wittig, I., Steger, M., Ding, M. G., Schmid, T., Dehne, N., and Brune, B. (2015) Inactivation of tristetraprolin in chronic hypoxia provokes the expression of cathepsin B, *Mol. Cell. Biol.*, **35**, 619-630, doi: 10.1128/MCB.01034-14.
29. Li, Q., Yu, B., and Yang, P. (2015) Hypoxia-induced HMGB1 in would tissues promotes the osteoblast cell proliferation via activating ERK/JNK signaling, *Int. J. Clin. Exp. Med.*, **8**, 15087-15097.
30. Chiu, C.-Z., Wang, B.-W., and Shyu, K.-G. (2014) Angiotensin II and the JNK pathway mediate urotensin II expression in response to hypoxia in rat cardiomyocytes, *J. Endocrinol.*, **220**, 233-246, doi: 10.1530/JOE-13-0261.
31. Zhang, J., Liu, Q., Fang, Z., Hu, X., Huang, F., Tang, L., and Zhou, S. (2016) Hypoxia induces the proliferation of endothelial progenitor cells via upregulation of Apelin/APLNR/MAPK signaling, *Mol. Med. Rep.*, **13**, 1801-1806, doi: 10.3892/mmr.2015.4691.
32. Wu, Y., Yang, Y., Yang, P., Gu, Y., Zhao, Z., Tan, L., Zhao, L., Tang, T., and Li, Y. (2013) The osteogenic differentiation of PDLSCs is mediated through MEK/ERK and p38 MAPK signaling under hypoxia, *Arch. Oral Biol.*, **58**, 1357-1368, doi: 10.1016/j.archoralbio.2013.03.011.
33. Mayr, M., Sidibe, A., and Zampetaki, A. (2008) The paradox of hypoxic and oxidative stress in atherosclerosis, *J. Am. Coll. Cardiol.*, **51**, 1266-1267, doi: 10.1016/j.jacc.2008.01.005.
34. Strehl, C., Fangradt, M., Fearon, U., Gaber, T., Buttgereit, F., and Veale, D. J. (2014) Hypoxia: how does the monocyte-macrophage system respond to changes in oxygen availability? *J. Leukoc. Biol.*, **95**, 233-241, doi: 10.1189/jlb.1212627.
35. Taylor, C. T., and Cummins, E. P. (2009) The role of NF-κB in hypoxia-induced gene expression, *Ann. N. Y. Acad. Sci.*, **1177**, 178-184, doi: 10.1111/j.1749-6632.2009.05024.x.
36. Walmsley, S. R., Print, C., Farahi, N., Peyssonnaux, C., Johnson, R. S., Cramer, T., Sobolewski, A., Condliffe, A. M., Cowburn, A. S., Johnson, N., and Chilvers, E. R. (2005) Hypoxia-induced neutrophil survival is mediated by HIF-1α-dependent NF-κB activity, *J. Exp. Med.*, **201**, 105-115, doi: 10.1084/jem.20040624.
37. Cummins, E. P., Berra, E., Comerford, K. M., Ginouves, A., Fitzgerald, K. T., Seeballuck, F., Godson, C., Nielsen, J. E., Moynagh, P., Pouyssegur, J., and Taylor, C. T. (2006) Prolyl hydroxylase-1 negatively regulates IκB kinase-β, giving insight into hypoxia-induced NFκB activity, *Proc. Natl. Acad. Sci. USA*, **103**, 18154-18159, doi: 10.1073/pnas.0602235103.
38. Gerbod-Giannone, M.-C., Li, Y., Holleboom, A., Han, S., Hsu, L.-C., Tabas, I., and Tall, A. R. (2006) TNFα induces ABCA1 through NF-κB in macrophages and in phagocytes ingesting apoptotic cells, *Proc. Natl. Acad. Sci. USA*, **103**, 3112-3117, doi: 10.1073/pnas.0510345103.
39. Moon, M. R., Parikh, A. A., Pritts, T. A., Fischer, J. E., Cottongim, S., Szabo, C., Salzman, A. L., and Hasselgren, P. O. (1999) Complement component C3 production in IL-1β-stimulated human intestinal epithelial cells is blocked by NF-κB inhibitors and by transfection with ser 32/36 mutant IκBα, *J. Surg. Res.*, **82**, 48-55, doi: 10.1006/jsre.1998.5503.
40. Sang, N., Stiehl, D. P., Bohensky, J., Leshchinsky, I., Srinivas, V., and Caro, J. (2003) MAPK signaling up-regulates the activity of hypoxia-inducible factors by its effects on p300, *J. Biol. Chem.*, **278**, 14013-14019, doi: 10.1074/jbc.M209702200.
41. Mylonis, I., Chachami, G., Samiotaki, M., Panayotou, G., Paraskeva, E., Kalousi, A., Georgatsou, E., Bonanou, S., and Simos, G. (2006) Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1α, *J. Biol. Chem.*, **281**, 33095-33106, doi: 10.1074/jbc.M209702200.
42. Zampetaki, A., Mitsialis, S. A., Pfeilschifter, J., and Kourembanas, S. (2004) Hypoxia induces macrophage inflammatory protein-2 (MIP-2) gene expression in murine macrophages via NF-κB: the prominent role of p42/p44 and PI3 kinase pathways, *FASEB J.*, **18**, 1090-1092, doi: 10.1096/fj.03-0991fje.
43. Haddad, J. J., and Hanbali, L. B. (2014) Hypoxia upregulates MAPK(p38)/MAPK(ERK) phosphorylation *in vitro*: neuroimmunological differential time-dependent expression of MAPKs, *Protein Pept. Lett.*, **21**, 444-451, doi: 10.2174/09298665210514021811252l.

44. Snodgrass, R. G., Boss, M., Zezina, E., Weigert, A., Dehne, N., Fleming, I., Brune, B., and Namgaladze, D. (2016) Hypoxia potentiates palmitate-induced pro-inflammatory activation of primary human macrophages, *J. Biol. Chem.*, **291**, 413-424, doi: 10.1074/jbc.M115.686709.
45. Shavva, V. S., Bogomolova, A. M., Nikitin, A. A., Dizhe, E. B., Tanyanskiy, D. A., Efremov, A. M., Oleinikova, G. N., Perevozchikov, A. P., and Orlov, S. V. (2017) Insulin-mediated downregulation of apolipoprotein A-I gene in human hepatoma cell line HepG2: the role of interaction between FOXO1 and LXRβ transcription factors, *J. Cell. Biochem.*, **118**, 382-396, doi: 10.1002/jcb.25651.
46. Shavva, V. S., Bogomolova, A. M., Nikitin, A. A., Dizhe, E. B., Oleinikova, G. N., Lapikov, I. A., Tanyanskiy, D. A., Perevozchikov, A. P., and Orlov, S. V. (2017) FOXO1 and LXRα downregulate the apolipoprotein A-I gene expression during hydrogen peroxide-induced oxidative stress in HepG2 cells, *Cell Stress Chaperon.*, **22**, 123-134, doi: 10.1007/s12192-016-0749-6.
47. Ishiguro, H., Yoshida, H., Major, A. S., Zhu, T., Babaev, V. R., Linton, M. F., and Fazio, S. (2001) Retrovirus-mediated expression of apolipoprotein A-I in the macrophage protects against atherosclerosis *in vivo*, *J. Biol. Chem.*, **276**, 36742-36748, doi: 10.1074/jbc.M106027200.
48. Pagler, T. A., Wang, M., Mondal, M., Murphy, A. J., Westerterp, M., Moore, K. J., Maxfield, F. R., and Tall, A. R. (2011) Deletion of ABCA1 and ABCG1 impairs macrophage migration because of increased Rac1 signaling, *Circ. Res.*, **108**, 194-200, doi: 10.1161/CIRCRESAHA.110.228619.