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Tumor necrosis factor α stimulates endogenous apolipoprotein A-I expression and secretion by human monocytes and macrophages: role of MAP-kinases, NF-κB, and nuclear receptors PPARα and LXRs

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Abstract

Apolipoprotein A-I (ApoA-I) is the main structural and functional protein component of high-density lipoprotein. ApoA-I has been shown to regulate lipid metabolism and inflammation in macrophages. Recently, we found the moderate expression of endogenous apoA-I in human monocytes and macrophages and showed that pro-inflammatory cytokine tumor necrosis factor α (TNFα) increases apoA-I mRNA and stimulates ApoA-I protein secretion by human monocytes and macrophages. Here, we present data about molecular mechanisms responsible for the TNFα-mediated activation of apoA-I gene in human monocytes and macrophages. This activation depends on JNK and MEK1/2 signaling pathways in human monocytes, whereas inhibition of NFκB, JNK, or p38 blocks an increase of apoA-I gene expression in the macrophages treated with TNFα. Nuclear receptor PPARα is a ligand-dependent regulator of apoA-I gene, whereas LXRs stimulate apoA-I mRNA transcription and ApoA-I protein synthesis and secretion by macrophages. Treatment of human macrophages with PPARα or LXR synthetic ligands as well as knock-down of LXRα, and LXRβ by siRNAs interfered with the TNFα-mediated activation of apoA-I gene in human monocytes and macrophages. At the same time, TNFα differently regulated the levels of PPARα, LXRα, and LXRβ binding to the apoA-I gene promoter in THP-1 cells. Obtained results suggest a novel tissue-specific mechanism of the TNFα-mediated regulation of apoA-I gene in monocytes and macrophages and show that endogenous ApoA-I might be positively regulated in macrophage during inflammation.

Keywords Apolipoprotein A-I · Macrophages · TNFα · LXR · PPAR · JNK · p38 · MEK1/2

Introduction

Apolipoprotein A-I (ApoA-I) is the main structural and functional component of human high-density lipoprotein (HDL) [1]. The high level of HDL-associated ApoA-I in plasma protects blood vessels against atherosclerotic lesion formation [2–4]. Anti-atherogenic action of ApoA-I appears to

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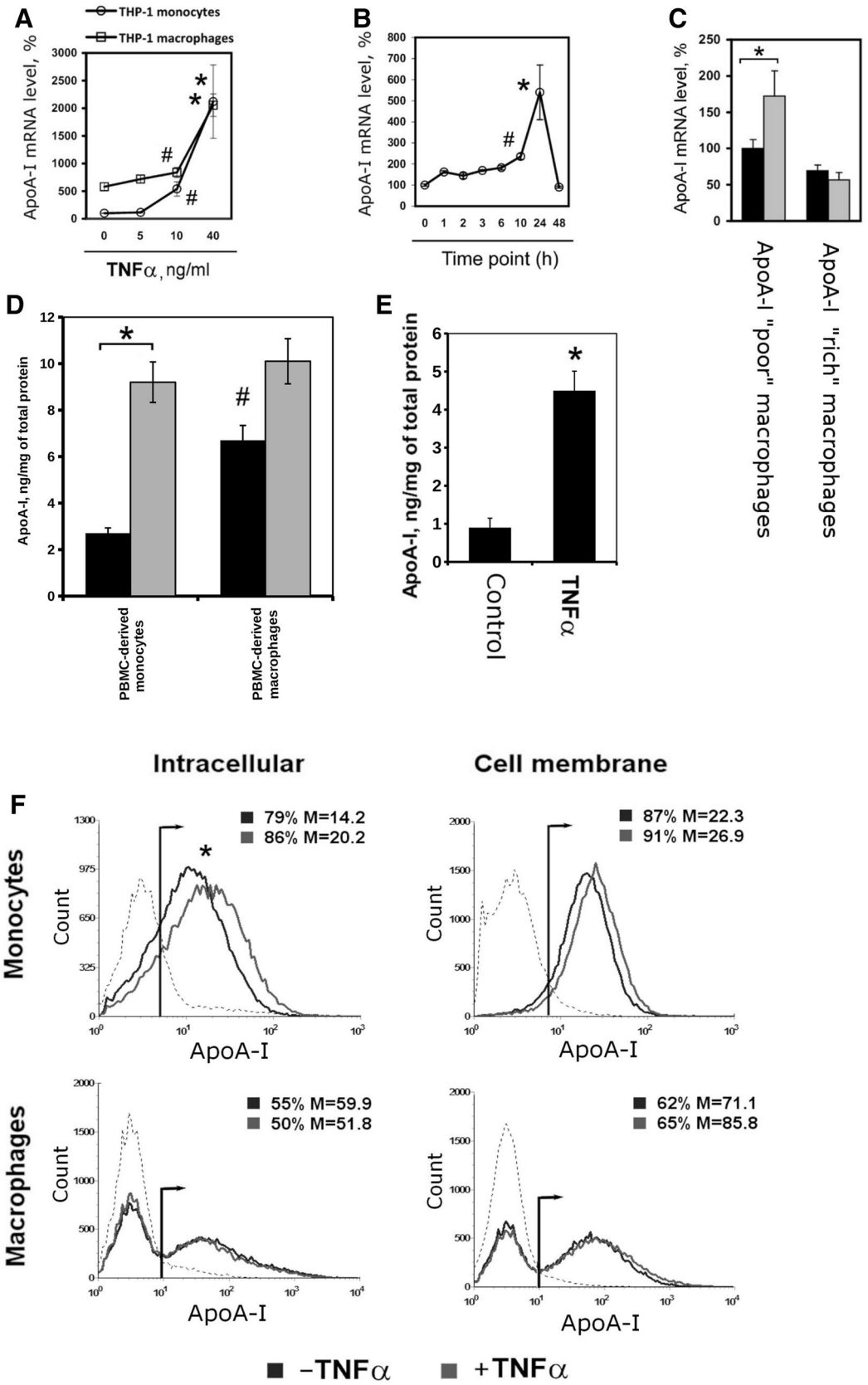

A
THP-1 monocytes
THP-1 macrophages
ApoA-I mRNA level, %
TNFα, ng/ml
B
ApoA-I mRNA level, %
Time point (h)
C
ApoA-I mRNA level, %
ApoA-I "poor" macrophages
ApoA-I "rich" macrophages
D
ApoA-I, ng/mg of total protein
PBMC-derived monocytes
PBMC-derived macrophages
E
ApoA-I, ng/mg of total protein
Control
TNFα
F
Intracellular
Cell membrane
Monocytes
Macrophages
79% M=14.2
86% M=20.2
87% M=22.3
91% M=26.9
55% M=59.9
50% M=51.8
62% M=71.1
65% M=85.8
Count
ApoA-I
−TNFα
+TNFα

◂**Fig. 1** TNFα stimulates ApoA-I gene expression, protein synthesis, and secretion by human monocytes and macrophages. **a** The impact of several concentrations of TNFα on apoA-I gene expression in THP-1 monocytes and macrophages 24 h after treatment of the cells with TNFα. **b** The levels of apoA-I gene expression in THP-1 monocytes 0–48 h after treatment of the cells with TNFα (10 ng/ml). Real-Time RT-PCR. The *Y* axis values correspond to the relative level of apoA-I mRNA (100% in THP-1 monocytes). Values are presented as means ± the standard error of the mean of six independent experiments. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a Dunnett's criterion ($^{\#}p < 0.05$, $^{*}p < 0.01$). **c** The levels of apoA-I gene expression in THP-1 macrophages (sorting of ApoA-I "poor" and ApoA-I "rich" macrophages according with the content of plasma membrane-associated endogenous ApoA-I). Gray columns—macrophages treated with TNFα, black columns—untreated macrophages. Real-Time RT-PCR. The *Y* axis values correspond to the relative level of apoA-I mRNA (100% in ApoA-I "poor" THP-1 macrophages). Values are presented as means ± the standard error of the mean of four independent experiments. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a nonpaired Student's *t* test ($^{*}p < 0.05$). **d** ELISA analysis of ApoA-I protein content in PBMC-derived monocytes and macrophages (control—black columns, TNFα-treated cells—gray columns). **e** ELISA analysis of secreted ApoA-I protein content in media of THP-1 macrophages at third day of differentiation treated with TNFα for 48 h. Values are presented as means ± the standard error of the mean of four independent experiments. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a nonpaired Student's *t* test ($^{*}p < 0.05$). F- FACS analysis of intracellular and plasma membrane localization of ApoA-I in THP-1 monocytes and macrophages with or without TNFα treatment (10 ng/ml, 24 h); the percents of ApoA-I positive cells are indicated, percent of ApoA-I positive cells and median value (M) are shown. Dotted line shows the isotype control. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a nonpaired Student's *t* test ($^{*}p < 0.05$)

be associated with HDL-mediated reverse cholesterol (CS) transport including CS efflux from macrophages to HDL with subsequent transport to liver [5]. ApoA-I activates the lecithin cholesterol acyltransferase [6] and possesses anti-inflammatory [7–10], antioxidant [11–13], protective from mitochondrial injury [14], and antithrombotic [15] activities. ApoA-I protein is mostly produced in liver by hepatocytes and in small intestine by enterocytes [16]. Currently, the mechanism of apoA-I gene transcription regulation is well elucidated in hepatocytes where the minimal promoter (− 41 to + 1 vs. major transcription start site, TSS) and more distant part of the promoter known as the hepatic enhancer (HE) (− 222 to − 110 vs. TSS) of apoA-I gene are sufficient for the liver-specific apoA-I gene transcription [17, 18]. The HE contains three regulatory regions: A (− 214 to − 192), B (− 169 to − 146), and C (− 134 to − 119). The sites A and C are also known as hormone responsive elements (HREs) and contain sequences for binding of some nuclear receptors. HNF4α [19], PPARα [20], LRH-1 [21], and RXRα [22, 23] are described as positive regulators of apoA-I gene transcription while LXRs [24], FXR [25], ARP-1 [20], and PPARγ [26] are negative regulators of apoA-I gene in hepatic and intestinal cells. The site B of the HE interacts with Forkhead family transcription factors FOXA2 [27, 28] and FOXO1 [29, 30]. Mouse models of atherosclerosis have shown that the expression of exogenous human apoA-I in mouse macrophages decreases the level of atherosclerotic lesions of aorta and increases CS efflux from macrophages [31–33], but has no influence on the levels of ApoA-I and HDL-associated CS in serum [32]. Recently, we have shown expression of endogenous ApoA-I gene in human monocytes and macrophages and demonstrated that endogenous ApoA-I stabilizes ATP-binding cassette transporter A1 and modulates TLR4 signaling, inhibiting inflammatory response in human macrophages [34]. In contrast to the hepatocytes or enterocytes, pro-inflammatory cytokine TNFα stimulates apoA-I expression in both human monocytes and macrophages, which suggests the role of endogenous ApoA-I in the possible negative feedback restricting inflammation development [34]. The mechanisms controlling apoA-I expression under TNFα administration in macrophages are currently unknown. Here, we show that TNFα-induced activation of apoA-I gene depends on JNK and MEK1/2 signaling pathways in human monocytes, whereas inhibition of NFκB, JNK, or p38 blocks an increase of apoA-I gene expression in the macrophages treated with TNFα. Nuclear receptor PPARα is a ligand-dependent regulator of apoA-I gene, whereas LXRs stimulate apoA-I mRNA transcription and ApoA-I protein synthesis and secretion by macrophages. Treatment of human macrophages with PPARα or LXR synthetic ligands as well as knock-down of LXRα and LXRβ by siRNAs interfered with the TNFα-mediated activation of apoA-I gene in human monocytes and macrophages.

Materials and methods

Chemical inhibitors, synthetic ligands, and recombinant protein

MAP-kinase inhibitors and NFκB inhibitor were purchased from Biomol, USA: SB203580 (p38 inhibitor)—cat. number EI-286; SP600125 (JNK1/2/3 inhibitor)—cat. number EI-305; U0126 (MEK1/2 inhibitor)—cat. number EI-282; QNZ (NFκB inhibitor)—cat. number EI-352. PPARα ligands were purchased from Sigma, USA: WY-14643—cat. number C7081 and MK-886—cat. number M2692. LXR ligand was purchased from Biomol: TO901317—cat. number GR-232. Human recombinant TNFα was purchased from Sigma—cat. number T0157.

Antibodies

Mouse monoclonal antibodies against human β-actin (cat. number ab3280), against human PPARα (cat. number ab2779), rabbit polyclonal antibodies against human LXRβ (cat. number ab56237), mouse monoclonal antibodies against human LXRα (cat. number ab41902), and goat FITC-labeled secondary antibodies against mouse IgG (cat. number ab6669-1) were purchased from Abcam. Mouse monoclonal antibodies against human ApoA-I (cat. number 0650–0050) were purchased from AbD Serotec. Rabbit polyclonal antibodies against human apolipoprotein E were kindly gifted by Dr. I.A. Oleinik (Institute of Experimental Medicine, Russia). Goat polyclonal antibodies to human ApoA-I were described previously [35].

Cell cultures

Human acute monocytic leukemia cell line THP-1 was obtained from the Cell Culture Bank of the Institute of Cytology, Russian Academy of Sciences. The differentiation of THP-1 cells into macrophages was performed by treatment of the cells with phorbol 12-myristate 13-acetate (PMA) (Sigma, USA) (50 ng/ml, 24 h).

Human PBMC cells were isolated from healthy donor blood and differentiated into macrophages as described [34]. See Supplements for details.

Reverse transcription and Real-Time PCR

RNA isolation, reverse transcription procedures, and Real-Time PCR including multiplexing details were performed as described earlier [26, 36]. Primers and probes for human genes β-actin [36], GAPDH, apoA-I, LXRα, LXRβ, PPARα [37] were described earlier. The levels of mRNA of genes are presented as the results of GAPDH and β-actin normalization as described [38]. The number of cycles (C_t value) required to reach a threshold level of fluorescence that is ~ 10 S.D. values (of fluctuations in the background fluorescence) above the mean background fluorescence was determined for each PCR and primer set by use of the CFX96 Real-Time PCR system and automated software (Bio-Rad). The relative amount of mRNA (as a percentage of the control sample) was calculated by the relation $2^{C_{t(\text{control})}-C_{t(\text{sample})}} \times 100$.

ChIP assay

Chromatin immunoprecipitation (ChIP) was performed as described previously [37, 39].

FACS

FACS was performed as described earlier [34]. See Supplements for details.

Western blotting and ELISA

Western blot assay and ELISA were performed as described [30]. See Supplements for details.

Nuclear extract preparation, EMSA, and DNA-affinity precipitation

Nuclear extracts were prepared from THP-1 cells as described previously [40] with slight modifications. The following synthetic oligonucleotides together with their 5′-end-biotin-labeled derivatives were purchased from Syntol, Russia (sequences of upper strands only are shown): HRE-A: 5′-CATGAACCCTTGACCCCTGCCCTG-3′ and HRE-C: 5′-CTTGAGCTGATCCTTGAACTCTTAAG-3′. The synthetic oligonucleotides were end-labeled with [γ^{32}P] ATP using T4 polynucleotide kinase (Scientific Industrial Corp. SibEnzyme, Russia) and annealed to form double-stranded probes as described [40]. EMSA was performed as described [40]. For DNA-affinity precipitation assay, biotin-labeled probes were incubated with nuclear extracts as described [41] and precipitated with streptavidin-coated magnetic particles (Roche Applied Science). Precipitated proteins were analyzed by Western blotting.

siRNA-mediated knock-down

Scrambled control RNA oligonucleotides (sc-37007), siRNA against LXRα (sc-38828), and siRNA against LXRβ (sc-45316) were purchased from Santa Cruz Biotechnology. siRNA against PPARα was described earlier [42]. PMBC-derived macrophages were cultivated in RPMI media, containing 10% FCS during 48 h, and then were transfected by siRNAs using DharmaFECT 4 reagent (Thermo Fisher Scientific) for 72 h in accordance with the manufacturer's instructions.

Statistical analysis

Results are presented as mean ± SEM. The statistical analyses of differences between compared groups were performed using a nonpaired *t* test and Dunnett test for multiple comparisons. Differences were considered statistically significant at the $p < 0.05$ level. All statistical analyses were performed using the program Statistica 5.0, StatSoft, USA.

Results

TNFα stimulates apoA-I gene expression in human monocytes and macrophages

Treatment of THP-1 monocytes and macrophages with TNFα resulted in a dose-dependent stimulation of apoA-I gene expression (Fig. 1a). A significant increase of apoA-I mRNA in macrophages was found 10–24 h after treatment with TNFα. Expression of apoA-I was reduced to the basal level at 48 h after treatment of macrophages with TNFα (Fig. 1b). Recently, we have shown that human macrophages can be divided to two populations based on intracellular and membrane-associated ApoA-I content—ApoA-I "poor" and ApoA-I "rich" cells [34]. Sorting of viable THP-1 macrophages based on membrane surface-associated ApoA-I content showed that TNFα stimulated apoA-I expression in ApoA-I "poor" but not in ApoA-I "rich" macrophages (Fig. 1c). These data suggest that membrane-associated endogenous ApoA-I appears to down-regulate TNFα signaling in macrophages; however, further studies are needed to find the mechanism of such effect.

At the protein level, TNFα stimulated ApoA-I about threefolds over the level in untreated PBMC-derived monocytes and slightly (~ 1.5-folds) increased ApoA-I content in PBMC-derived macrophages (Fig. 1d). At the same time,

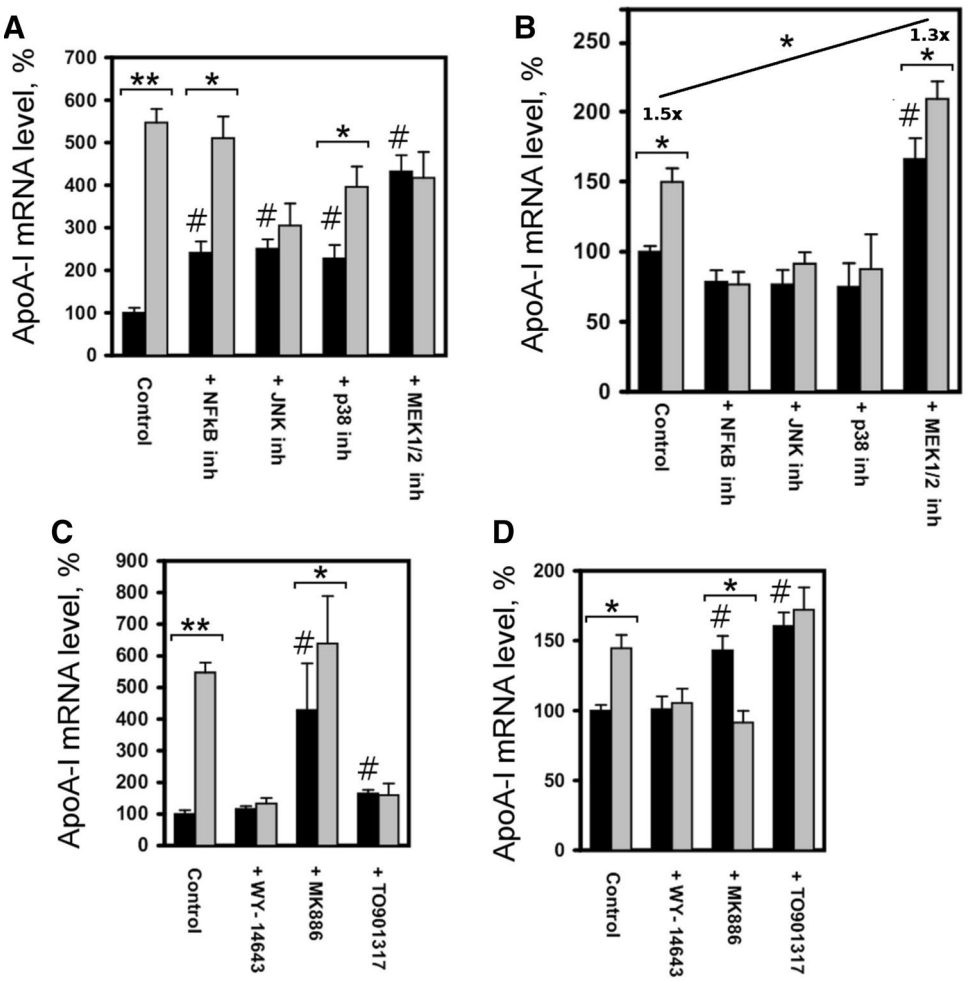


Fig. 2 Regulation of endogenous apoA-I gene expression under the impact of TNFα in THP-1 monocytes and macrophages. Role of MAP-kinase cascades, NFκB, PPARα, and LXRs. Real-Time RT-PCR. Expression of apoA-I gene in THP-1 monocytes (**a, c**) and macrophages (**b, d**). The *Y* axis values correspond to the relative level of gene expression (100% in control THP-1 monocytes). Control, THP-1 cells without inhibitors and nuclear receptor ligands; p38 inh, SB203580 (25 μM); JNK inh, SP600125 (10 μM); MEK inh, U0126 (10 μM); NFκB inh, QNZ (10 nM). WY-14643 (10 μM); MK886 (10 μM); TO901317 (5 μM). THP-1 cells were administered by TNFα (10 ng/ml) for 24 h. The inhibitors and nuclear receptor ligands had been added 1 h before TNFα. Black columns represent data for untreated cells, and gray columns represent data for cells treated with TNFα. Values are presented as means ± the standard error of the mean of six independent experiments. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a nonpaired Student's *t* test (*$p < 0.05$; **$p < 0.01$) and a Dunnett's criterion (#$p < 0.05$)

TNFα activated ApoA-I protein secretion by THP-1 macrophages (Fig. 1e). FACS analysis showed that treatment of THP-1 cells with TNFα led to an significant increase of the intracellular ApoA-I protein level in THP-1 monocytes but not in macrophages (Fig. 1f). However, TNFα did not alter the levels of membrane-associated ApoA-I in THP-1 monocytes and macrophages (Fig. 1f).

MAP-kinases and NFκB are involved in TNFα-mediated activation of apoA-I gene expression in human monocytes and macrophages

Treatment of THP-1 monocytes with inhibitors of NFκB, p38, or JNK resulted in an twofolds increase of apoA-I mRNA, whereas inhibition of MEK1/2 increased apoA-I expression

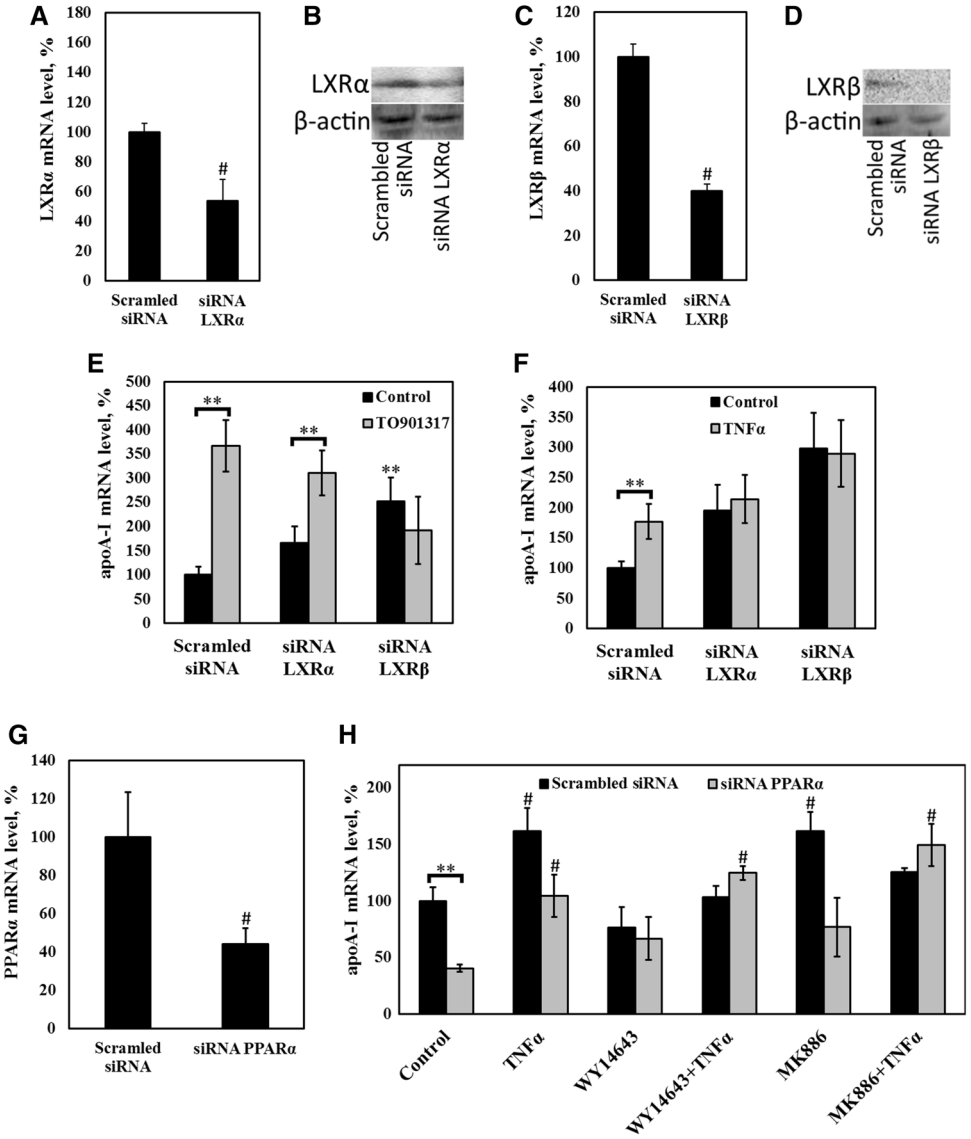


Fig. 3 Knock-down of nuclear receptors PPARα, LXRα, or LXRβ: the influence on nuclear receptor ligand and TNFα effects on the apoA-I gene expression. **a, b** Effect of siRNA against LXRα on the LXRα expression in human PBMC-derived macrophages; **a** Real-Time RT-PCR, **b** Western assay. **c, d** Effect of siRNA against LXRβ on the LXRβ expression in PBMC-derived macrophages; **c** Real-Time RT-PCR, **d** Western assay. **e** effects of LXRα or LXRβ knock-down on the TO901317-dependent stimulation of apoA-I gene expression in PBMC-derived macrophages: Real-Time RT-PCR. F-effects of LXRα or LXRβ knock-down on the TNFα-dependent stimulation of apoA-I gene in PBMC-derived macrophages: Real-Time RT-PCR; G- effect of siRNA against PPARα on the PPARα expression in human PBMC-derived macrophages: Real-Time RT-PCR. H-effect of PPARα knock-down on the WY14643, MK886, or TNFα-mediated regulation of apoA-I gene in PBMC-derived macrophages: Real-Time RT-PCR. Values are presented as means ± the standard error of the mean of four independent experiments. The statistical analyses of differences between compared groups were performed using a nonpaired Student's *t* test ($^{\#}p < 0.05$) and a Dunnett's test ($^{**}p < 0.05$)

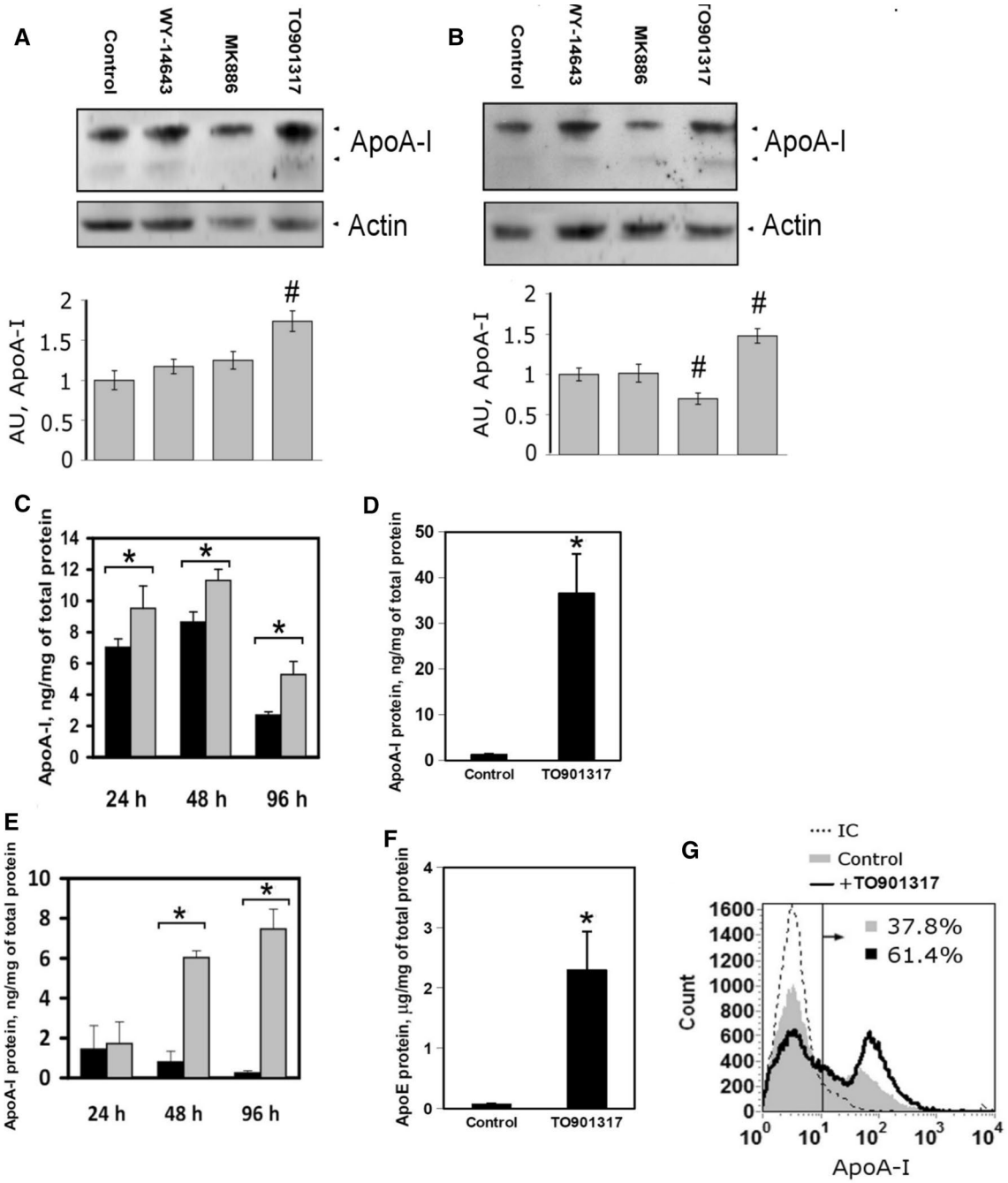
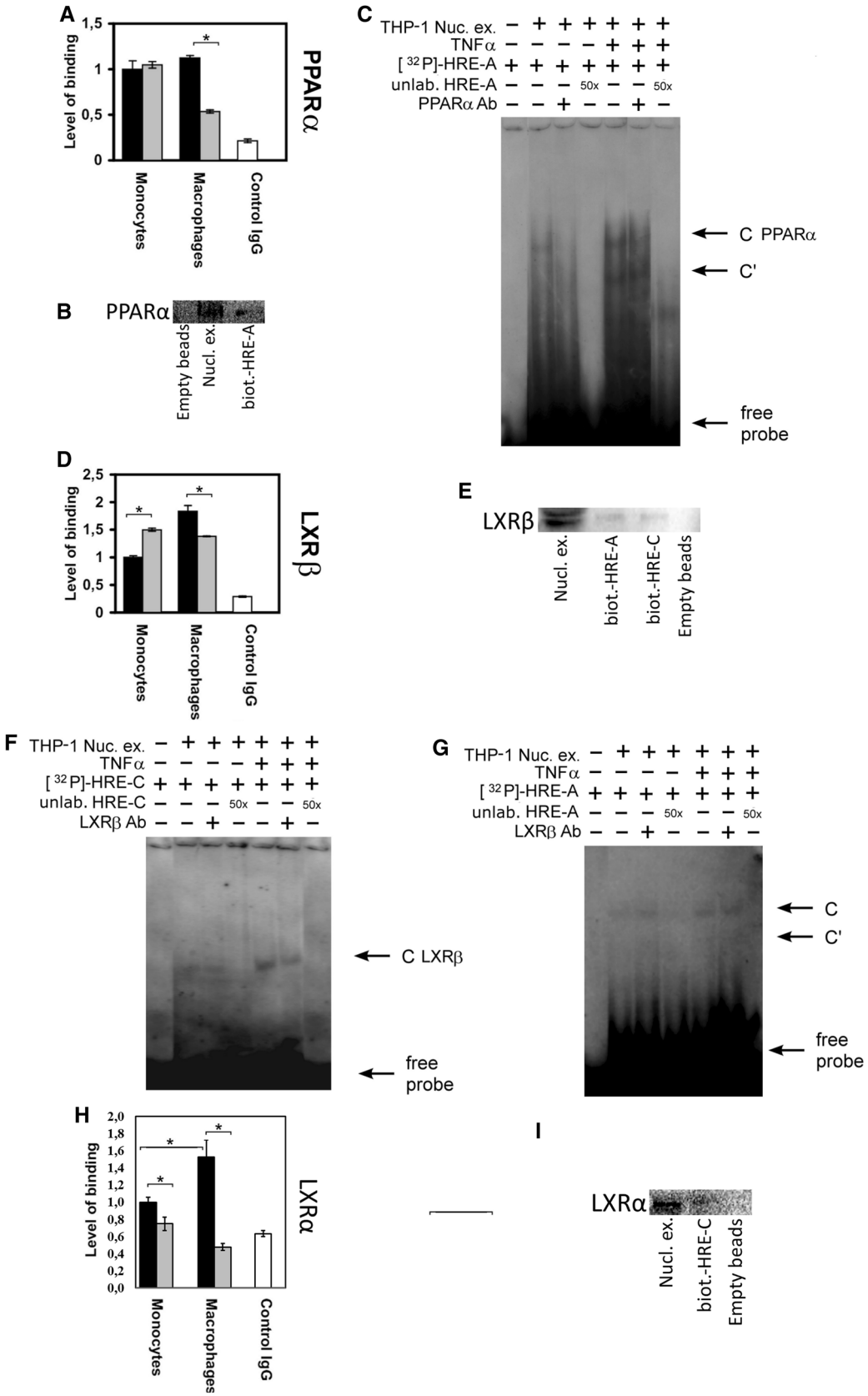


Fig. 4 Regulation of ApoA-I protein synthesis and secretion in THP-1 monocytes and macrophages. Role of nuclear receptors PPARα and LXRs. **a, b** Western assay of ApoA-I in THP-1 monocytes (**a**) and macrophages (**b**). The *Y* axis values correspond to the relative level of ApoA-I protein content (actin normalized) defined using densitometry (1 AU—arbitrary unit corresponds to the level of ApoA-I in untreated THP-1 cells). **c** ELISA analysis of intracellular ApoA-I protein at 24, 48, and 96 h after treatment THP-1 macrophages with TO901317 (gray columns) or DMSO (black columns). Control, THP-1 cells without nuclear receptor ligands; WY-14643, an agonist of PPARα (10 µM); MK886, an antagonist of PPARα (10 µM); TO901317, an agonist of LXRs (5 µM). **d** ELISA analysis of secreted ApoA-I protein at 48 h after treatment of PBMC-derived macrophages with or without TO901317. **e** ELISA analysis of secreted ApoA-I protein at 24, 48, and 96 h after treatment THP-1 macrophages with TO901317 (gray columns) or DMSO (black columns). **f** ELISA analysis of secreted ApoE protein at 48 h after treatment PBMC-derived macrophages with or without TO901317. **g** FACS analysis of plasma membrane surface-associated ApoA-I in PBMC-derived macrophages at 48 h after treatment with or without TO901317. *IC* isotype control. Percent of plasma membrane ApoA-I positive cells is shown. Values are presented as means ± the standard error of the mean of four independent experiments. The statistical analyses of differences between compared groups were performed using a nonpaired Student's *t* test ($^{*}p < 0.05$) and a Dunnett's criterion ($^{\#}p < 0.05$)


A
Level of binding
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PPARα
Monocytes
Macrophages
Control IgG
B
PPARα
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LXRα
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biot.-HRE-C
Empty beads

◂**Fig. 5** Effect of TNFα on PPARα, LXRα, or LXRβ binding to the apoA-I HE in THP-1 cells. **a** PPARα ChIP assay: level of binding, relative abundance of PPARα bound to the apoA-I HE (given 1 in the control probe); black columns correspond to untreated THP-1 cells; gray columns correspond to THP-1 cells treated with TNFα (10 ng/ml) for 24 h; white columns (control IgG)—ChIP with an unspecific human serum IgG fraction (negative control). **b** DNA-affinity precipitation of nuclear extracts from THP-1 macrophages with antibodies against PPARα. **c** EMSA analysis of PPARα binding to site A. **d** LXRβ ChIP assay: level of binding, relative abundance of LXRβ bound to the apoA-I HE (given 1 in the control probe); black columns correspond to untreated THP-1 cells; gray columns correspond to THP-1 cells treated with TNFα (10 ng/ml) for 24 h; white columns (control IgG)—ChIP with an unspecific human serum IgG fraction (negative control). **e** DNA-affinity precipitation of nuclear extracts from THP-1 macrophages with antibodies against LXRβ. F, G- EMSA analysis of LXRβ binding to site C (**f**) or site A (**g**). **h** LXRα ChIP assay: level of binding, relative abundance of LXRα bound to the apoA-I HE (given 1 in the control probe); black columns correspond to untreated THP-1 cells; gray columns correspond to THP-1 cells treated with TNFα (10 ng/ml) for 24 h; white columns (control IgG)—chromatin immunoprecipitation with an unspecific human serum IgG fraction (negative control). **i** DNA-affinity precipitation of nuclear extracts from THP-1 macrophages with antibodies against LXRα. Values are presented as means ± the standard error of the mean of four independent experiments. The statistical analyses of differences between compared groups (with or without TNFα) were performed using a nonpaired Student's *t* test (*$p < 0.05$). THP-1 Nuc. ex.—nuclear extracts of THP-1 macrophages, TNFα—treatment of the cells with TNFα (10 ng/ml, 24 h), [^{32}P]- HRE-A and [^{32}P]-HRE-C—radioactively labeled oligonucleotides site A and site C, unlab. HRE-A and unlab. HRE-C—unlabeled oligonucleotides site A or site C added in a 50-fold excess, PPARα Ab and LXRβ Ab—antibodies against PPARα and LXRβ. C and C′ indicate DNA–protein complexes. nuc. ex.—input nuclear extracts (positive control), biot.-HRE-A—precipitation of nuclear protein/biotinylated site A oligonucleotide complexes, biot.-HRE-C—precipitation of nuclear protein/biotinylated site C oligonucleotide complexes, empty beads—precipitation without specific oligonucleotides (negative control)

fourfolds as compared to the baseline level. The TNFα-dependent stimulation of apoA-I gene was abolished after treatment of THP-1 monocytes with JNK or MEK1/2 but not NFκB or p38 inhibitors (Fig. 2a). Differentiation of THP-1 cells into macrophages abrogated apoA-I gene activation upon NFκB, p38, or JNK inhibition (Fig. 2b). Treatment of THP-1 macrophages with MEK1/2 inhibitor led to a 1.6-folds increase of apoA-I expression (Fig. 2b). Inhibition of NFκB, p38, JNK blocked the TNFα-dependent activation of apoA-I gene, whereas MEK1/2 inhibition decreased the level of TNFα-mediated stimulation of apoA-I gene in THP-1 macrophages (Fig. 2b). The similar results were obtained on PBMC-derived monocytes and macrophages (data not shown).

Nuclear receptors PPARα and LXRs regulate expression of apoA-I gene and interfere with TNFα-mediated activation of apoA-I gene expression in monocytes and macrophages

Treatment of THP-1 cells with synthetic PPARα antagonist MK886 or synthetic LXR agonist TO901317 stimulated expression of apoA-I gene in both THP-1 monocytes and macrophages (Fig. 2c, d). Treatment of THP-1 monocytes and macrophages with synthetic PPARα agonist WY-14643 did not alter the level of apoA-I gene expression but abolished the TNFα-dependent activation of apoA-I gene transcription in these cells (Fig. 2c, d). The stimulation of apoA-I gene expression under the impact of TNFα was also blocked in the presence of TO901317 in THP-1 monocytes and macrophages (Fig. 2c, d). Treatment of THP-1 monocytes with MK886 led to a decrease of the level of TNFα-dependent activation of apoA-I gene expression (Fig. 2c). TNFα did not stimulate but slightly repressed apoA-I gene activity in the MK886 treated THP-1 macrophages (Fig. 2d). To verify that the nuclear receptor ligand-induced interference with TNFα signaling (Fig. 2c, d) was indeed mediated by nuclear receptors, we performed knock-down experiments (Fig. 3). The transfection of macrophages with specific siRNAs led to an substantial decrease in both mRNA and protein levels of studied transcription factors in comparison with cells transfected by scrambled siRNA (Fig. 3a–d, g). Depletion of LXRα did not abrogate the stimulatory effect of TO901317 on apoA-I gene expression in macrophages while depletion of LXRβ abolished this effect (Fig. 3e). These data are in keeping with the previously published results about different effects of TO901317 on the LXRα and LXRβ activities [43]. Both LXRα and LXRβ were essential for the TNFα-mediated activation of apoA-I gene in PBMC-derived macrophages (Fig. 3f). Transfection of PBMC-derived macrophages with siRNA against PPARα downregulated apoA-I gene, but did not abolish the TNFα-mediated stimulation of apoA-I gene activity. Nevertheless, PPARα knock-down restored the TNFα-mediated stimulation of apoA-I gene, which was blocked by WY1463 or MK886 PPARα ligands. (Fig. 3h). Moreover, downregulation of PPARα led to the abrogation of the stimulatory effect of MK886 on apoA-I gene (Fig. 3h). Thereby, PPARα is a ligand-dependent regulator of apoA-I gene in human macrophages while LXRs positively regulate apoA-I gene expression in these cells.

PPARα and LXRs alter ApoA-I protein synthesis in THP-1 monocytes and macrophages

Treatment of THP-1 cells with WY-14643 did not alter the content of ApoA-I protein in THP-1 monocytes and macrophages (Fig. 4a). MK886 decreased the level of ApoA-I protein in THP-1 macrophages but not in monocytes (Fig. 4a, b). Treatment of THP-1 cells with TO901317 resulted in an increase of the content of ApoA-I protein in both THP-1 monocytes and macrophages (Fig. 4a, b). These results were confirmed by ELISA (Fig. 4c).

LXR activation stimulates ApoA-I protein secretion by human macrophages

Treatment of PBMC-derived macrophages with TO901317 resulted in an increase of ApoA-I protein secretion by 36.5 ± 8.7-folds after 48 h (Fig. 4d). TO901317 also led to a significant increase of the secreted ApoA-I protein 48–96 h after treatment of THP-1 macrophages (Fig. 4e). The similar level of the TO901317-induced secretion was shown for ApoE, which is known as a target for LXR-dependent regulation in human macrophages [44, 45], but the content of ApoE was significantly higher than ApoA-I in media from TO901317-treated macrophages (Fig. 4f). LXR activation resulted in an increase of membrane-associated ApoA-I in PBMC-derived macrophages (Fig. 4g).

TNFα alters the levels of PPARα and LXR binding to the HE of apoA-I gene in THP-1 monocytes and macrophages

Next, we showed that PPARα, LXRα, and LXRβ bound to the HE of apoA-I gene. The level of PPARα binding with the apoA-I HE did not alter after differentiation of THP-1 cells to macrophages (Fig. 5a). In contrast, the level of LXRα and LXRβ binding to the HE increased 1.5-folds (LXRα) or 1.8-folds (LXRβ) in THP-1 macrophages as compared to monocytes (Fig. 5d, h). Treatment of THP-1 cells with TNFα did not alter the level of PPARα binding with the HE in THP-1 monocytes but led to a twofold decrease of PPARα binding in macrophages (Fig. 5a). The level of LXRβ binding with the HE of apoA-I gene increased 1.5-folds in monocytes and decreased 1.3-folds in macrophages after treatment of cells with TNFα (Fig. 5d). The level of LXRα binding slightly decreased in monocytes (about 1.3-folds) and further dropped in macrophages (about 2.5-folds) in the presence of TNFα (Fig. 5h). As it was shown by EMSA, the site A formed two different complexes with nuclear proteins from THP-1 macrophages. Treatment of THP-1 macrophages with TNFα led to an increase of both complexes. Antibody against PPARα abolished the slow migrating complex (indicated as C PPARα in Fig. 5c) in untreated THP-1 macrophages suggesting that this complex contained PPARα (Fig. 5c). These data suggest that PPARα binds to the site A of the HE of apoA-I gene in THP-1 macrophages. The same results were obtained in DNA-affinity precipitation experiments (Fig. 5b). EMSA showed the presence of one complex in THP-1 macrophages formed by site C, which was increased upon treatment of cells with TNFα. The depletion of this complex by LXRβ antibody was similar in THP-1 macrophages treated with TNFα or not (Fig. 5f). EMSA analysis revealed that LXRβ can bind the site A in THP-1 macrophages (C′ complex) (Fig. 5g). DNA-affinity precipitation showed that LXRβ was capable to interact with the site A and C (Fig. 5e). Interestingly, in HepG2 cells LXRβ interacts with the site C and B and does not bind the site A [30]. LXRα also bound the site C of the HE (Fig. 5i).

Discussion

Here, we show that pro-inflammatory cytokine TNFα activates ApoA-I mRNA and protein expression in human monocytes and macrophages. ApoE expression has been shown to be TNFα-dependent in human macrophages at the early stage of monocyte–macrophage differentiation [46]. Since both ApoA-I and ApoE participate in the reverse CS transport from macrophages [47], coordinated up-regulation of these genes may result in a cooperative effect on the CS efflux from macrophages and/or anti-inflammatory effect.

In contrast to hepatocytes which are the main producers of plasma ApoA-I [16], secretion of ApoA-I by human monocytes and macrophages is TNFα-dependent and is very limited in the resting cells. ApoA-I is involved in the inhibition of local production of TNFα and IL-1β due to blocking of activated T-cells–macrophage interactions [9, 48]. Recently, we have shown that endogenous ApoA-I down-regulates TLR4 surface expression and attenuates LPS-induced activation of TNFα, IL-1β, and NOS2 genes in human macrophages [34]. Interestingly, in mice the capacity of ApoA-I attenuating the LPS-induced activation of TNFα seems to depend on ApoE [49]. Simultaneous induction of endogenous ApoA-I and ApoE expression and secretion in macrophages by TNFα may reflect a crosstalk between these proteins in macrophages during inflammatory processes. Our results suggest that monocyte-macrophage cells may produce ApoA-I in response to TNFα, thereby controlling development of inflammation in a feedback-like manner.

In contrast to hepatocytes [24, 37, 50], LXRs stimulate apoA-I expression, protein synthesis, and secretion by human macrophages which is consistent with the observation that LXRs activate apoE gene expression in macrophages but not in liver cells [44]. Treatment of mice in an atherosclerosis model with an LXR agonist results in

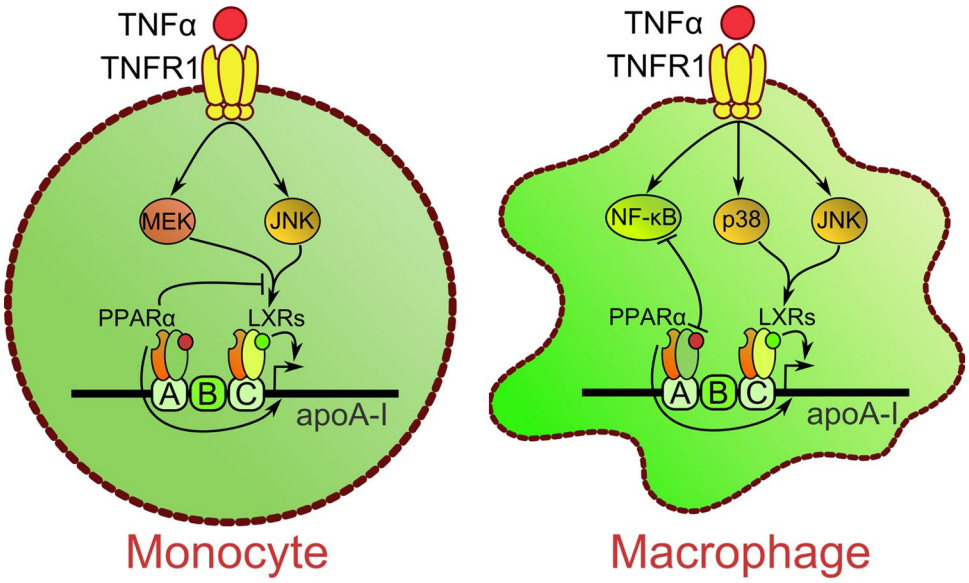

Fig. 6 Hypothetical scheme illustrating a possible mechanism of TNFα-dependent activation of apoA-I gene expression in human monocytes and macrophages. See text for details

a reduction of the atherosclerotic lesion area [51] despite apoA-I gene repression in hepatocytes by LXR ligand [24, 37, 50]. In clinical trial fibrates (gemfibrozil and fenofibrate), which are agonists of PPARα, decreased triglycerides and increased HDL cholesterol levels and plasma ApoA-I [52]. Although we show here that PPARα agonist WY-14643 down-regulates ApoA-I production in macrophages, the expression level of ApoA-I in macrophages seems to have no significant impact on HDL and ApoA-I plasma levels [32]. Our results fit with earlier observed anti-atherogenic properties of LXR agonists. It is possible that the LXR-dependent co-regulation of ApoA-I, ApoE, and ABCA1 genes may be involved in the mechanism controlling cholesterol efflux or modulation of inflammatory responses in macrophages.

Ligand activation of PPARα and LXRs has been shown to inhibit inflammatory responses in macrophages [53, 54]. Our results show that treatment of human macrophages with LXR or PPARα synthetic ligands abolishes TNFα-mediated activation of apoA-I gene expression. Since LXRs and PPARα are also involved in TNFα-dependent inhibition of apoA-I gene activity in HepG2 cells [37, 50], we have suggested that these nuclear receptors are the important components of tissue-specific TNFα-mediated regulation of apoA-I gene expression.

Thus, this study shows positive regulation of endogenous apoA-I gene in human monocytes and macrophages by TNFα. NFκB, MEK1/2, JNK, and p38 signaling pathways and nuclear receptors PPARα and LXRs are involved in the TNFα-mediated activation of apoA-I gene expression in human monocytes and macrophages (Fig. 6). The TNFα-induced up-regulation of ApoA-I gene in macrophages may be important for anti-inflammatory effects of ApoA-I at the early stages of inflammation during atherosclerotic lesion development. Taking into account the antiatherogenic effects of exogenous ApoA-I overexpression in murine macrophages [31–33], our results might be used for development of antiatherogenic approaches based on induction of apoA-I expression in macrophages.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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