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Interleukin-27 inhibits foam cell formation by promoting macrophage ABCA1 expression through JAK2/STAT3 pathway

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ABSTRACT

The purpose of this study is to determine whether IL-27 regulates macrophage ABCA1 expression, foam cell formation, and also explore the underlying mechanisms. Here, we revealed that IL-27 decreased lipid accumulation in THP-1 derived macrophages through markedly enhancing cholesterol efflux and increasing ABCA1 expression at both protein and mRNA levels. Our study further demonstrated that IL-27 increased ABCA1 level via activation of signal transducer and activator of transcription 3 (STAT3). Inhibition of Janus kinase 2, (JAK2)/STAT3 suppressed the stimulatory effects of IL-27 on ABCA1 expression. The present study concluded that IL-27 reduces lipid accumulation of foam cell by upregulating ABCA1 expression via JAK2/STAT3. Therefore, targeting IL-27 may offer a promising strategy to treat atherosclerotic vascular disease.

1. Introduction

Chronic inflammation in arterial wall and lipid accumulation in macrophages play pivotal roles in the development of atherosclerosis [1]. Several proinflammatory cytokines are known to promote atherosclerosis, for instance, interleukin-1(IL-1), IL-6 and IL-8, but less is known about the roles and mechanisms of anti-inflammatory cytokines in atherosclerosis, such as IL-27 and IL-10 [2–4].

Recent studies have shown that IL-27 is a member of IL-12 family cytokines that is composed of IL-27p28 and Epstein–Barr virus induced gene 3 (EBI3) [1,5–7]. The IL-27 receptor that consists of WSX-1 and gp130 and is required for all established signaling pathways initiated IL-27 [2,5]. The expression of the IL-27 is decreased in atherosclerotic plaque [2], but the mechanisms underlying its anti-atherosclerotic effects, especially its effect on lipid metabolism, remains largely unknown.

It is well known that ATP-binding cassette transporter A1 (ABCA1) plays a critical role in the control of cellular cholesterol homeostasis, ABCA1 is highly expressed in macrophages and mediates cellular cholesterol efflux [8–12]. Regulation of ABCA1 expression impacts the reverse cholesterol transport (RCT) and foam cell formation [13–15]. In detail, IL-27 activates cellular signal pathway [16–19], and induction of anti-inflammatory immune responses [7,20]. Several studies reported a potential role of STAT molecules in the ABCA1 gene regulation [21,22]. It was also reported that STAT3 and STAT4 bind to the CRE site in the first intron of ABCA1 [23]. Currently available data showed that a negative cross-talk between IFN-γ/STAT1 and LXRs-dependent upregulation of ABCA1 [24]. Therefore, we hypothesized that IL-27 may play an protective role in atherosclerosis by upregulating ABCA1 expression.

2. Materials and methods

2.1. Cell culture and foam cell formation evaluated by Oil Red O staining

THP-1 cells were obtained from the Institute of Life Science Research Center, Shanghai (Chinese Academy of Science, China), and maintained in RPMI Medium 1640 supplemented with 10% fetal bovine serum (FBS) at 37 °C. After one week, THP-1 cells were

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treated with phorbol-12-myristate-13-acetate (PMA, 160 nmol/L; Sigma Chemical Co) for 48 h. The medium was then replaced with a serum-free medium containing oxidized low density lipoprotein oxLDL (50 μg/ml) for 48 h to fully differentiate THP-1 cells to foam cells. Foam cells were stained with 0.5% Oil red O. Hematoxylin was used for counterstaining, Cells were photographed at ×400 magnification.

2.2. Cellular cholesterol efflux experiments

Cells were cultured at 60% confluence, and then labeled with 0.2 mCi/ml [^{3}H] cholesterol for 24 h, as previously described [13,25]. Next day, cells were washed with fresh media, followed by addition of IL-27. Equilibrated [^{3}H] cholesterol-labeled cells were washed with PBS and incubated in efflux medium containing RPMI 1640 medium and 0.1% BSA with 25 μg/ml human plasma apolipoprotein A-I (apoA-I) for 6 h. The medium was removed and centrifuged at 14,000*g* for 10 min. Total cell-associated radio-activity was determined by dissolving the cells in isopropanol. Medium and cell-associated [^{3}H] cholesterol was then measured by liquid scintillation counting. Percent efflux was calculated by the following equation: [total media count/(total cellular count + total media count)] × 100%.

2.3. Protein extraction and Western blot analysis

Cells were washed three times in cold PBS and lysed on ice. Cell lysates were boiled at 95 °C for 7 min, in Laemmli SDS-loading buffer and separated by SDS–PAGE and electrophoretically transferred to PVDF membranes (0.45 μm; Minipore). Membranes were blocked in 5% milk in TBST and then incubated with primary antibodies. Antibodies to measure ABCA1 expression were purchased from Abcam. To check for protein loading and transfer, we determined the levels of β-actin using anti-mouse-β-actin antibody (Beyotime). In general, the membranes were washed three times in TBST and then incubated for 4 h with peroxidase-conjugated secondary antibodies. After three washes of 15 min each with TBST, the proteins were visualized using chemiluminescence [13].

2.4. RNA isolation and real-time polymerase chain reaction (PCR) analysis

Total RNA was extracted using TRIzol reagent (Invitrogen) in accordance with the manufacturer's instructions. Relative quantitative real-time PCR (qPCR) was performed, using SYBR Green detection chemistry on a LightCycler Run 5.32 Real-Time PCR System (Roche). Melting curve analyses of all real-time PCR products were performed and shown to produce a single DNA duplex. Quantitative measurements were determined using the Ct method. Within the experiment, β-actin was used as the internal control.

2.5. Transfection of siRNA

We used small interfering RNA (siRNA) to block the expression of STAT3. Control non-silencing siRNAs were synthesized by the Biology Engineering Corporation in Shanghai, China. siRNAs were transfected into THP-1 cells (2×10^6 cells/well) using Lipofectamine 2000. After 48 h, the siRNA of STAT3 suppressed the expression of these proteins by 78% and 84%, respectively, according to Western blot analyses compared with the control siRNA.

2.6. Statistical analysis

Data are expressed as means ± SEM based on at least three independent experiments. Statistical analyses were performed with the GraphPad Prism V.5.01 software using the One-way or two-way analysis of variance (ANOVA). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. $P < 0.05$ was considered significant difference.

3. Results

3.1. IL-27 decreases lipid accumulation in THP-1 macrophage-derived foam cells

To investigate whether anti-inflammatory cytokine IL-27 affects intracellular lipid accumulation, semi-quantitative we performed lipid analysis of cultured THP-1-derived macrophages with Oil Red O staining. We found that macrophage without ox-LDL did not contain high levels of lipid droplets due to less Oil red O staining (Fig. 1A). Treatment of macrophages with 50 μg/mL ox-LDL for 48 h resulted in foam cell formation which was characterized by heavy lipid loading. This effect in ox-LDL-treated macrophages for 48 h was decreased with the presence of IL-27 (10 ng/ml), as measured by Oil-Red-O staining (Fig. 1A) and lipid mass quantification (Fig. 1B). Addition of neutralizing antibody against IL-27 to cell cultures abolished the effects of IL-27 on lipid accumulation, further establishing its role of lipid regulation (Fig. 1).

3.2. IL-27 increases cellular cholesterol efflux

We first examined whether IL-27 inhibited ox-LDL uptake. THP-1-derived macrophages were incubated with fluorescence labeled ox-LDL (DiI-ox-LDL) for different time periods, followed by analysis of by flow cytometry. There was no significant changes in the DiI-ox-LDL uptake rates in control and IL-27 treated macrophages. (Fig. 2A), suggesting that the reduced lipid accumulation in macrophage foam cells might result from an increase in cholesterol efflux.

To test this possibility, we examined the rate of apoA-I-specific cholesterol efflux from THP-1 macrophage-derived foam cells in response to IL-27 treatment. We found that cholesterol efflux was significantly increased in IL-27-treated cells when compared with control cells, and anti-IL-27 treatment led to a significant decrease in [^{3}H]-cholesterol efflux in a time-dependent manner (Fig. 2B). Similar results were observed in the concentration-dependent experiments, in which IL-27 markedly increased cholesterol efflux, especially when IL-27 concentrations reached 10 ng/ml or 20 ng/ml (Fig. 2C). Taken together, our results showed that IL-27 enhanced cholesterol efflux to apoA-I of foam cells, contributing to decreased lipid accumulation.

3.3. ABCA1 expression is induced by IL-27 in THP-1 macrophages

To gain a better understanding of the molecular mechanism underlying the above effect of IL-27, we first focused on the expression of ABCA1. To do so, THP-1 macrophage-derived foam cells were treated with 10 ng/ml IL-27 for 24 h, followed by measuring ABCA1 mRNA and protein levels using RT-qPCR and Western blot analyses, respectively.

Our results showed that the expression of ABCA1 mRNA was dramatically repressed by IL-27, but increased by IL-27 antibody in both time- and concentration-dependent manners (Fig. 3A and B), As expected, IL-27 increased ABCA1 protein expression when compared with control (Fig. 3C and D), but IL-27 antibody decreased ABCA1 protein level in both time- and concentration-dependent manners (Fig. 3E and F). These findings suggest that IL-27 may regulate cholesterol efflux by affecting ABCA1 expression in THP-1 macrophage-derived foam cells.

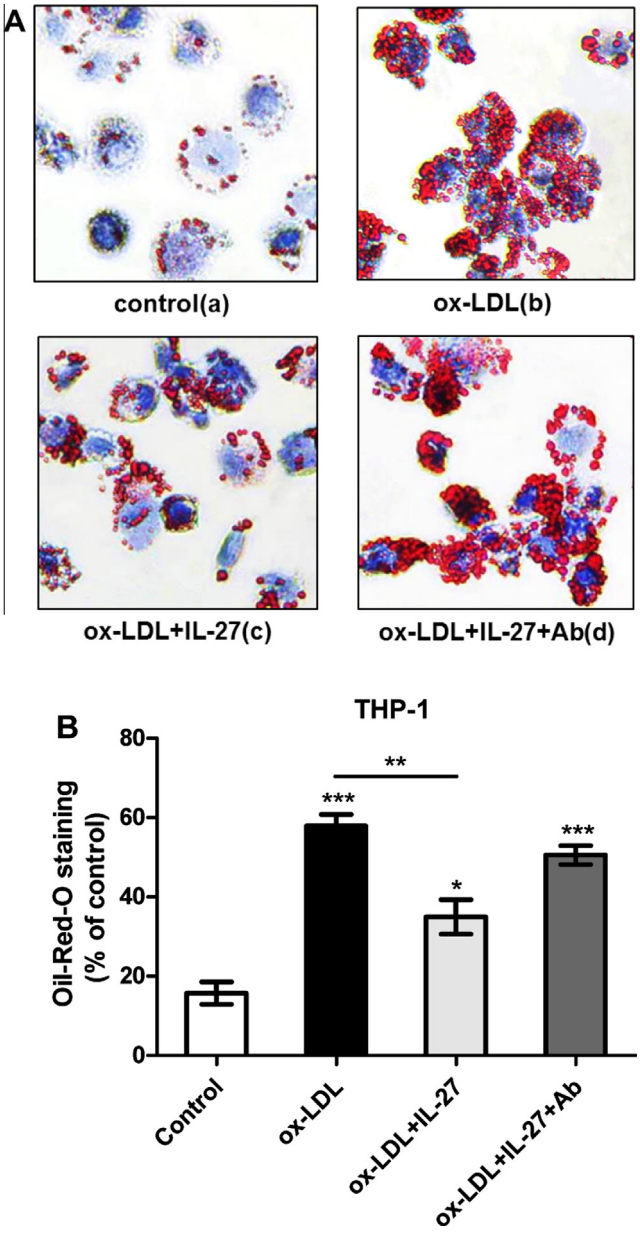


Fig. 1. Effects of IL-27 on intracellular lipid accumulation in macrophages. (A) THP-1-derived macrophages were stained for lipids with Oil red O without (a) or with (b–d) the presence of ox-LDL (50 μg/ml). (b) ox-LDL alone; (c and d) ox-LDL with IL-27 (10 ng/ml) and antibody against IL-27 (1.5 μg/ml) respectively. Cells were viewed under a Nikon microscope (magnification ×400). (B) Bar graph data based on Image-Pro Plus software: data of the area of lipid droplets/the area of total cells, are expressed as the mean ± SEM values ($n = 3$). $^{***}P < 0.0001$ ox-LDL vs control; $^{**}P < 0.01$, ox-LDL vs ox-LDL + IL-27; $^{*}P < 0.05$, control vs ox-LDL + IL-27.

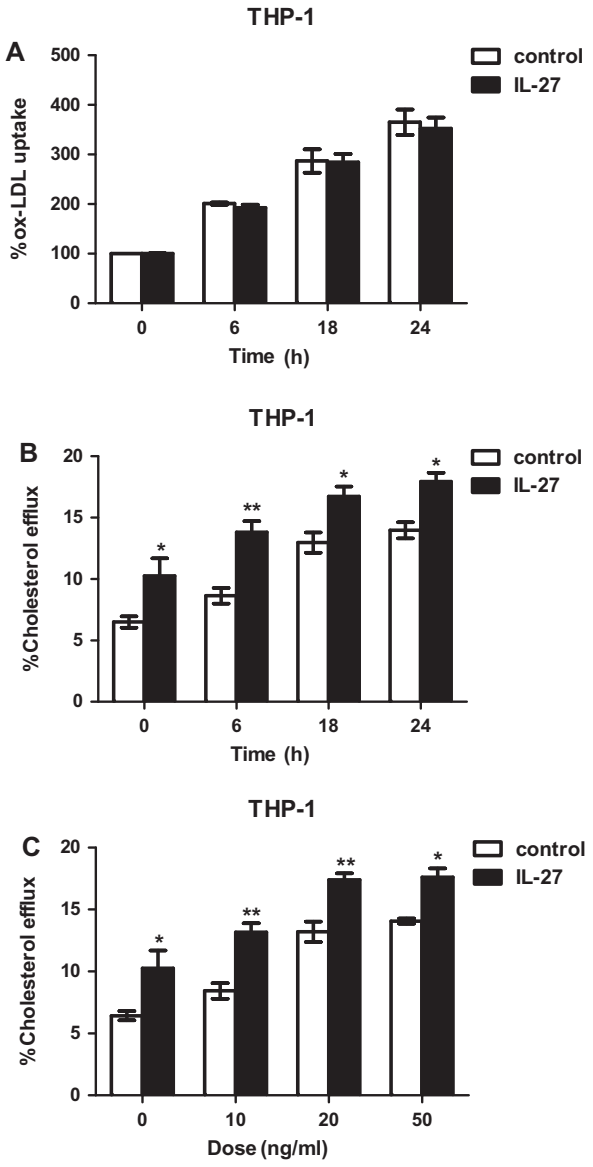


Fig. 2. IL-27 effects on oxLDL uptake and cholesterol efflux in macrophages. (A) Ox-LDL uptake. DiI-oxLDL (1 μg/ml) was added to THP1 cells in the presence and absence of IL-27 for the different times (0, 6, 18 and 24 h), and the uptake was analyzed by flow cytometry. Untreated control values were set to 100%, and then uptake data were calculated as the percentage of DiI-ox-LDL uptake versus the control. (B) Time-dependent effects of IL-27 on cholesterol efflux: Foam cells were treated with 10 ng/ml of IL-27 for indicated times, and apoA-I-mediated cholesterol efflux was analyzed as described in Section 2. (C) Concentration-dependent effects of IL-27 on cholesterol efflux: Foam cells were incubated with indicated concentrations of IL-27 for 24 h. ApoA-I-mediated cholesterol efflux was analyzed by liquid scintillation counting assays as described in Section 2. All the results are expressed as mean ± SEM from 3 independent experiments. $^{*}P < 0.05$ vs control group, $^{**}P < 0.01$ vs control group.

3.4. The JAK2/STAT3 pathway is involved in the upregulation of ABCA1 induced by IL-27

Analysis of the human ABCA1 promoter using Transcription Element Search online website revealed 63 putative STAT binding sites. The scores of five positions of STAT binding sites within the ABCA1 promoter are very high (Table 1). Indeed, transfection with STAT3 resulted in repression of the ABCA1 3′UTR reporter, but STAT3 siRNA had no effect, when compared with the control (Fig. 4A).

We then analyzed whether IL-27 induced ABCA1 expression though JAK2/STAT3 pathway. Our results showed that IL-27 activated JAK2 in THP-1 cells (Fig. 4B). Moreover, to test whether IL-27 induces activation of STAT3, we incubated THP-1 cells with or without IL-27, followed by evaluating phosphorylated STAT3 (pSTAT3), or the active form of STAT3 (Fig. 4C). Addition of IL-27 significantly increased the levels of pSTAT3, suggesting that IL-27 induces activation of STAT3. These results provide evidence to support that activation of JAK2/STAT3 is required in the IL-27 -mediated induction of ABCA1 gene in THP-1 cells.

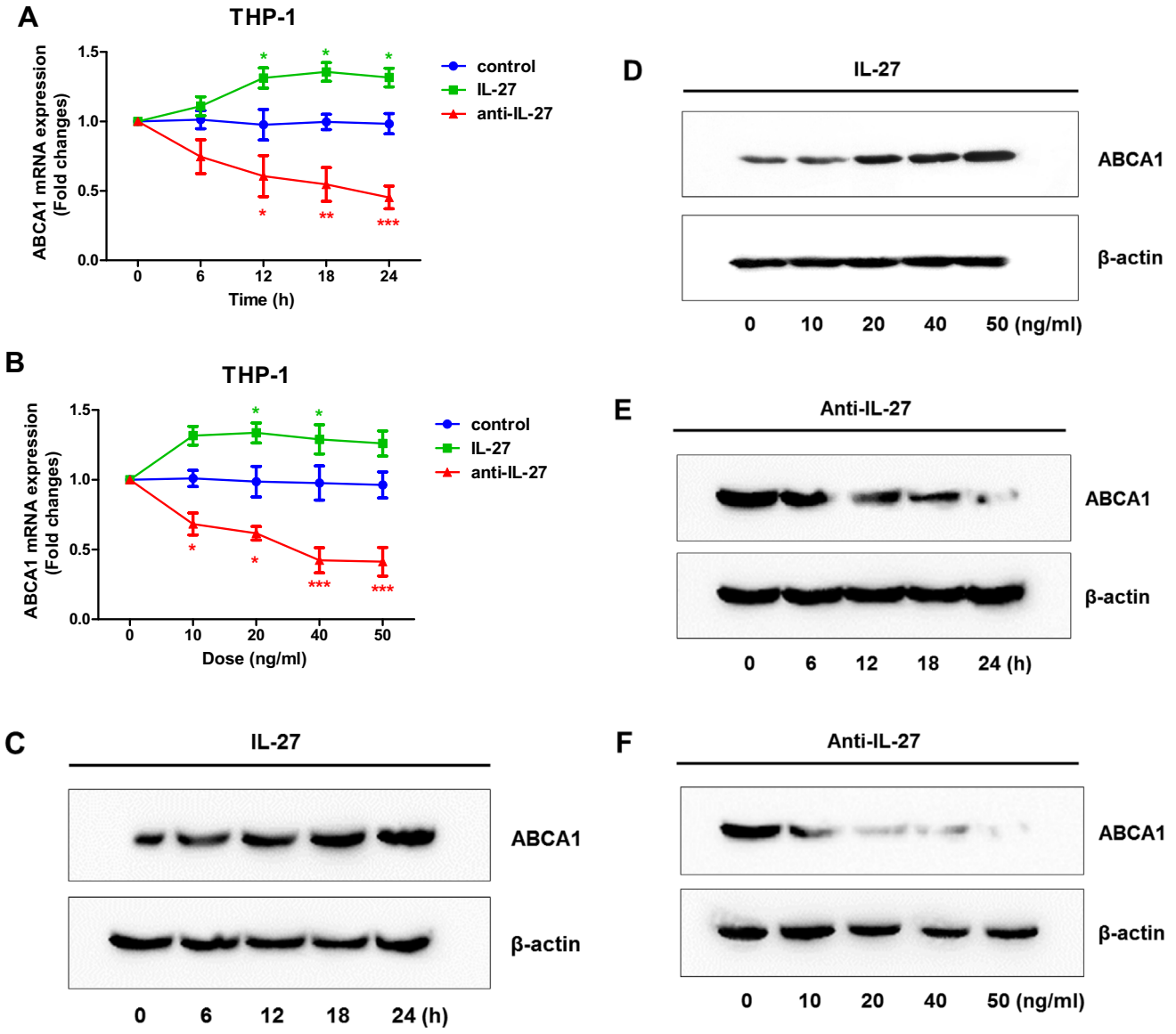
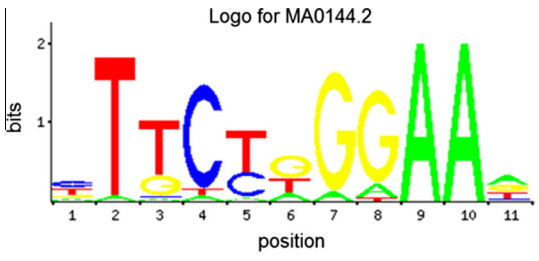


Fig. 3. IL-27 increases ABCA1 expression in THP-1 macrophage-derived foam cells. (A and B) Effects of IL-27 on ABCA1 mRNA levels: total RNA was extracted and RT-qPCR was performed as described in Methods. All results are expressed as mean ± SEM from 3 independent experiments. $^{*}P < 0.05$ vs control group, $^{**}P < 0.01$ vs control group, $^{***}P < 0.001$ vs control group. (C and D) Effects of IL-27 on ABCA1 protein levels: foam cells were incubated with 10 ng/ml IL-27 for various time periods or treated with various concentrations of IL-27 as indicated for 24 h. (E and F) Effects of anti-IL-27 on ABCA1 protein levels: foam cells were incubated with 10 ng/ml anti-IL-27 for various time periods or treated with various concentrations of anti-IL-27 as indicated for 24 h.

Table 1
5-Putative sites were predicted with these settings (80%) in sequence of ABCA1.

Model ID	Model name	Score	Relative score	Start	End	Strand	Predicted site sequence
MA0144.2	STAT3	10.451	0.924163936564185	421	431	1	CTTTCTGGAAG
MA0144.2	STAT3	6.548	0.876880831684594	590	600	1	CTTCTGGGGAT
MA0144.2	STAT3	5.844	0.868352185557197	638	648	1	GTTCTGGGACG
MA0144.2	STAT3	5.153	0.859981028633856	1843	1853	1	TTCCAAGGAAG
MA0144.2	STAT3	5.560	0.864911652176258	2004	2014	1	CTTCCTTGAAG



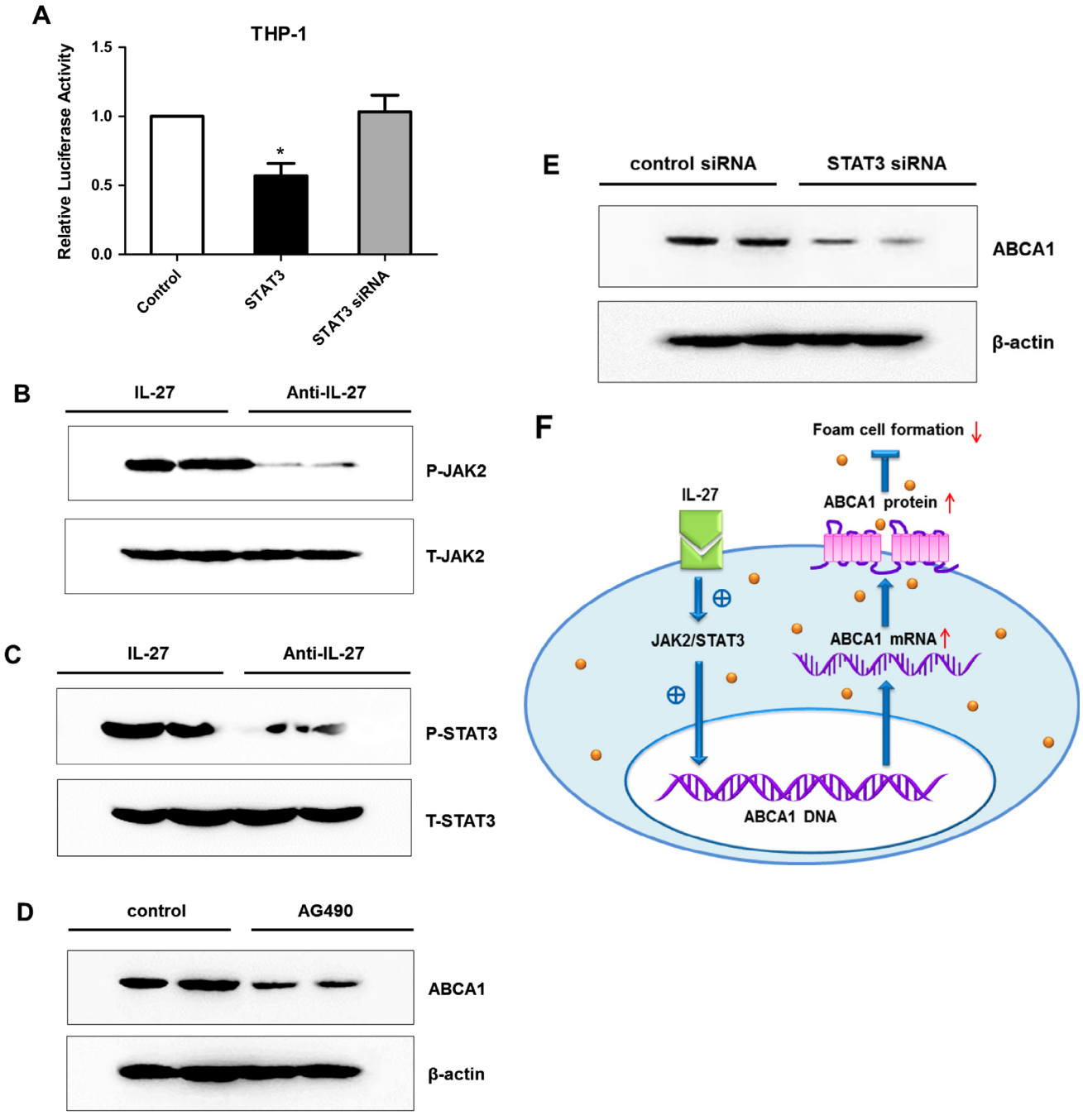


Fig. 4. IL-27 activates JAK2/STAT3 in THP1 macrophages. (A) Transfection with STAT3 or STAT3 siRNA: expression of STAT3 causes repression of the Luciferase-ABCA1 3′UTR reporter. Data are presented as mean ± SEM. *Denotes $P < 0.05$. (B) THP1 cells were transformed using PMA for 48 h and incubated without or with IL-27 (10 ng/ml) for 24 h. Isolated protein was immunoprecipitated using antibody against JAK2 and blotted against phosphorylated and total JAK2. (C) THP1 cells were transformed using PMA for 48 h and incubated without or with IL-27 (10 ng/ml) for 24 h. Isolated protein was immunoprecipitated using antibody against STAT3 and blotted against phosphorylated and total STAT3. (D) Cells were incubated with AG-490 or transfected siSTAT3 for 48 h, followed by examination of ABCA1 expression by Western blot analysis. (E) Cells were transfected siSTAT3 for 48 h, followed by examining ABCA1 expression by Western blot analysis. (F) Model of the mechanism underlying IL-27-induced upregulation of ABCA1 protein levels and cellular cholesterol efflux. IL-27 treatment in THP-1 macrophage-derived foam cells increases JAK2/STAT3 phosphorylation and induced ABCA1 protein expression. Consequently, cholesterol efflux is promoted. ABCA1, ATP-binding cassette transporter A1; IL-27, interleukin-27; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3.

3.5. The effects of IL-27 on lipid content and ABCA1 expression through JAK2/STAT3 pathway

We tested the effects of JAK2 and STAT3 on cholesterol content in response to treatment with IL-27. HPLC was then conducted to determine cellular cholesterol content. As demonstrated, the concentrations of total cellular cholesterol, free cholesterol and cholesterol ester in IL-27-treated cells were significantly lower than those in the control cells. Treatment with AG-490 (JAK2 inhibitor) and siSTAT3 did not decrease cholesterol content any further

Table 2
Role of JAK2/STAT3 on the cholesterol content of THP-1 macrophage-derived foam cells.

	Control	IL-27	AG-490 + IL-27	siSTAT3 + IL-27
TC (mg/g)	498 ± 41	295 ± 34*	480 ± 38	483 ± 25
FC (mg/g)	196 ± 22	144 ± 16*	192 ± 14	197 ± 32
CE (mg/g)	301 ± 26	167 ± 23*	298 ± 28	294 ± 18
CE/TC (%)	60.4	56.6	62.0	60.8

THP-1 macrophage-derived foam cells were divided into 4 groups and cultured in medium at 37°C containing 10 ng/ml IL-27. HPLC was performed to determine the levels of cellular total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE). The results are expressed as mean ± SEM from 3 independent experiments, each performed in triplicate.

* $P < 0.05$ vs control.

(Table 2). These result have clearly demonstrated that IL-27 decreased cholesterol content likely via the JAK2/STAT3 signaling pathway.

We further investigated the role of the JAK2/STAT3 pathway in ABCA1 expression in THP-1 macrophage-derived foam cells in response to IL-27 stimulation. Our Western blot results showed that AG-490 and siSTAT3 can reverse the effects of IL-27 on ABCA1 expression (Fig. 4D and E). Taken together, the above results indicated that JAK2/STAT3 signaling pathway plays an important role in IL-27-induced decrease in lipid accumulation.

4. Discussion

The progression of atherosclerosis is mainly induced by foam cell formation, it is a complex process impacted by multiple factors. Several studies have shown that IL-27 is the dominant negative factor in both inflammation response and atherosclerosis, the higher levels of IL-27 are associated with protect against cardiovascular disease [26–28], but the underlying molecular mechanism by which IL-27 exerts its anti-atherosclerotic effect remains unclear. Promotion of cholesterol efflux from macrophages reduces foam cell formation and protects against the development of atherosclerosis [29]. ABCA1 mediates the efflux of cellular cholesterol and phospholipids to apoA-I, and to form high density lipoprotein (HDL), eventually increases the RCT [30,31]. It indicates that upregulating the expression of ABCA1 would probably inhibit the formation of foam cells and the development of atherosclerosis. In this study, IL-27 as a novel issue regulates ABCA1. We provide experimental evidence that IL-27 enhances ABCA1-mediated cholesterol efflux in THP-1 derived foam cells through the JAK2/STAT3 signaling pathway (Fig. 4F). In addition to anti-inflammatory role, our findings in THP-1 derived macrophages suggest that the protective mechanism of IL-27 may result from its prevention of cellular cholesterol accumulation. These data keep consistent with the reported properties of IL-27, including notable anti-atherosclerotic roles.

Both lipid uptake and cholesterol efflux can impact on foam cell formation. We found that IL-27 did not inhibit ox-LDL uptake, but induced cholesterol efflux to apoA-I from human macrophages, as a result of the stimulation of ABCA1 expression. Our data showed that the treatment of THP-1 derived macrophages with IL-27 activates JAK2/STAT3 phosphorylation. Specifically, inhibition of JAK2 with AG-490 reduced IL-27-induced ABCA1 expression. Additionally, transfection of THP-1 derived macrophages with siRNA of STAT3 attenuated IL-27-induced ABCA1 expression. Those data are concordant with previous studies showing several well conserved STAT3 binding sites located in the promoter of the human ABCA1 gene as an essential mechanism for its regulation [23]. Although IL-27 can efficiently activate both STAT1 and STAT3, our results have revealed that induces ABCA1 expression via activation of STAT3, but not STAT1, the specific mechanism and whether other signal molecules or proteins are the targets of IL-27 remains to be explored in the future studies.

Acknowledgments

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References

[1] T. Hirase, H. Hara, Y. Miyazaki, N. Ide, A. Nishimoto-Hazuku, H. Fujimoto, C.J. Saris, H. Yoshida, K. Node, Interleukin 27 inhibits atherosclerosis via immunoregulation of macrophages in mice, Am. J. Physiol. Heart Circ. Physiol. 305 (2013) H420–H429.
[2] E.K. Koltsova, G. Kim, K.M. Lloyd, C.J. Saris, S. von Vietinghoff, M. Kronenberg, K. Ley, Interleukin-27 receptor limits atherosclerosis in Ldlr-/- mice, Circ. Res. 111 (2012) 1274–1285.
[3] C.M. Hedrich, A. Ramakrishnan, D. Dabitao, F. Wang, D. Ranatunga, J.H. Bream, Dynamic DNA methylation patterns across the mouse and human IL10 genes during CD4+ T cell activation; influence of IL-27, Mol. Immunol. 48 (2010) 73–81.
[4] M.T. de Aquino, P. Kapil, D.R. Hinton, T.W. Phares, S.S. Puntambekar, C. Savarin, C.C. Bergmann, S.A. Stohlman, IL-27 limits central nervous system viral clearance by promoting IL-10 and enhances demyelination, J. Immunol. 5 (2014) 56.
[5] W. Jin, Y. Zhao, W. Yan, L. Cao, W. Zhang, M. Wang, T. Zhang, Q. Fu, Z. Li, Elevated circulating interleukin-27 in patients with coronary artery disease is associated with dendritic cells, oxidized low-density lipoprotein, and severity of coronary artery stenosis, Mediators Inflamm. 2012 (2012) 506283.
[6] N. Ouaked, P.Y. Mantel, C. Bassin, S. Burgler, K. Siegmund, C.A. Akdis, C.B. Schmidt-Weber, Regulation of the foxp3 gene by the Th1 cytokines: the role of IL-27-induced STAT1, J. Immunol. 182 (2009) 1041–1049.
[7] A. Fraternale, R. Crinelli, A. Casabianca, M.F. Paoletti, C. Orlandi, E. Carloni, M. Smietana, A.T. Palamara, M. Magnani, Molecules altering the intracellular thiol content modulate NF-kB and STAT-1/IRF-1 signalling pathways and IL-12 p40 and IL-27 p28 production in murine macrophages, PLoS ONE 8 (2013) e57866.
[8] K. Yin, X. Deng, Z.C. Mo, G.J. Zhao, J. Jiang, L.B. Cui, C.Z. Tan, G.B. Wen, Y. Fu, C.K. Tang, Tristetraprolin-dependent post-transcriptional regulation of inflammatory cytokine mRNA expression by apolipoprotein A-I: role of ATP-binding membrane cassette transporter A1 and signal transducer and activator of transcription 3, J. Biol. Chem. 286 (2011) 13834–13845.
[9] G.J. Zhao, S.L. Tang, Y.C. Lv, X.P. Ouyang, P.P. He, F. Yao, Y.Y. Tang, M. Zhang, Y.L. Tang, D.P. Tang, F.S. Cayabyab, G.P. Tian, C.K. Tang, NF-kappaB suppresses the expression of ATP-binding cassette transporter A1/G1 by regulating SREBP-2 and miR-33a in mice, Int. J. Cardiol. 171 (2014) e93–e95.
[10] Q. Lu, S.L. Tang, X.Y. Liu, G.J. Zhao, X.P. Ouyang, Y.C. Lv, P.P. He, F. Yao, W.J. Chen, Y.Y. Tang, M. Zhang, D.W. Zhang, K. Yin, C.K. Tang, Tertiary-butylhydroquinone upregulates expression of ATP-binding cassette transporter A1 via nuclear factor E2-related factor 2/heme oxygenase-1 signaling in THP-1 macrophage-derived foam cells, Circ. J. 77 (2013) 2399–2408.
[11] G.J. Zhao, Z.C. Mo, S.L. Tang, X.P. Ouyang, P.P. He, Y.C. Lv, F. Yao, Y.L. Tan, W. Xie, J.F. Shi, Y. Wang, M. Zhang, D. Liu, D.P. Tang, X.L. Zheng, G.P. Tian, C.K. Tang, *Chlamydia pneumoniae* negatively regulates ABCA1 expression via TLR2-nuclear factor-kappa B and miR-33 pathways in THP-1 macrophage-derived foam cells, Atherosclerosis 235 (2014) 519–525.
[12] S. Bekkering, J. Quintin, L.A. Joosten, J.W. van der Meer, M.G. Netea, N.P. Riksen, Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes, Arterioscler. Thromb. Vasc. Biol. (2014).
[13] M. Zhang, J.F. Wu, W.J. Chen, S.L. Tang, Z.C. Mo, Y.Y. Tang, Y. Li, J.L. Wang, X.Y. Liu, J. Peng, K. Chen, P.P. He, Y.C. Lv, X.P. Ouyang, F. Yao, D.P. Tang, F.S. Cayabyab, D.W. Zhang, X.L. Zheng, G.P. Tian, C.K. Tang, MicroRNA-27a/b regulates cellular cholesterol efflux, influx and esterification/hydrolysis in THP-1 macrophages, Atherosclerosis 234 (2014) 54–64.
[14] C.M. Ramirez, N. Rotllan, A.V. Vlassov, A. Davalos, M. Li, L. Goedeke, J.F. Aranda, D. Cirera-Salinas, E. Araldi, A. Salerno, A. Wanschel, J. Zavadil, A. Castrillo, J.

Kim, Y. Suarez, C. Fernandez-Hernando, Control of cholesterol metabolism and plasma high-density lipoprotein levels by microRNA-144, Circ. Res. 112 (2013) 1592–1601.
[15] T. Jakobsson, N. Venteclef, G. Toresson, A.E. Damdimopoulos, A. Ehrlund, X. Lou, S. Sanyal, K.R. Steffensen, J.A. Gustafsson, E. Treuter, GPS2 is required for cholesterol efflux by triggering histone demethylation, LXR recruitment, and coregulator assembly at the ABCG1 locus, Mol. Cell 34 (2009) 510–518.
[16] H. Wang, R. Meng, Z. Li, B. Yang, Y. Liu, F. Huang, J. Zhang, H. Chen, C. Wu, IL-27 induces the differentiation of Tr1-like cells from human naive CD4+ T cells via the phosphorylation of STAT1 and STAT3, Immunol. Lett. 136 (2011) 21–28.
[17] Y. Kambayashi, T. Fujimura, S. Aiba, Comparison of immunosuppressive and immunomodulatory cells in keratoacanthoma and cutaneous squamous cell carcinoma, Acta Derm. Venereol. 93 (2013) 663–668.
[18] J. Diegelmann, T. Olszak, B. Goke, R.S. Blumberg, S. Brand, A novel role for interleukin-27 (IL-27) as mediator of intestinal epithelial barrier protection mediated via differential signal transducer and activator of transcription (STAT) protein signaling and induction of antibacterial and anti-inflammatory proteins, J. Biol. Chem. 287 (2012) 286–298.
[19] T. Owaki, M. Asakawa, S. Kamiya, K. Takeda, F. Fukai, J. Mizuguchi, T. Yoshimoto, IL-27 suppresses CD28-mediated [correction of medicated] IL-2 production through suppressor of cytokine signaling 3, J. Immunol. 176 (2006) 2773–2780.
[20] Y. Chiba, I. Mizoguchi, K. Mitobe, K. Higuchi, H. Nagai, C. Nishigori, J. Mizuguchi, T. Yoshimoto, IL-27 enhances the expression of TRAIL and TLR3 in human melanomas and inhibits their tumor growth in cooperation with a TLR3 agonist poly(I:C) partly in a TRAIL-dependent manner, PLoS ONE 8 (2013) e76159.
[21] S. Vuletic, W. Dong, G. Wolfbauer, C. Tang, J.J. Albers, PLTP regulates STAT3 and NFkappaB in differentiated THP1 cells and human monocyte-derived macrophages, Biochim. Biophys. Acta 2011 (1813) 1917–1924.
[22] E. Frisdal, P. Lesnik, M. Olivier, P. Robillard, M.J. Chapman, T. Huby, M. Guerin, W. Le Goff, Interleukin-6 protects human macrophages from cellular cholesterol accumulation and attenuates the proinflammatory response, J. Biol. Chem. 286 (2011) 30926–30936.
[23] W. Le Goff, P. Zheng, G. Brubaker, J.D. Smith, Identification of the cAMP-responsive enhancer of the murine ABCA1 gene: requirement for CREB1 and STAT3/4 elements, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 527–533.
[24] M.A. Blahoianu, A.A. Rahimi, M. Kozlowski, J.B. Angel, A. Kumar, IFN-gamma-induced IL-27 and IL-27p28 expression are differentially regulated through JNK MAPK and PI3K pathways independent of Jak/STAT in human monocytic cells, Immunobiology 219 (2014) 1–8.
[25] Y.C. Lv, Y.Y. Tang, J. Peng, G.J. Zhao, J. Yang, F. Yao, X.P. Ouyang, P.P. He, W. Xie, Y.L. Tan, M. Zhang, D. Liu, D.P. Tang, F.S. Cayabyab, X.L. Zheng, D.W. Zhang, G.P. Tian, C.K. Tang, MicroRNA-19b promotes macrophage cholesterol accumulation and aortic atherosclerosis by targeting ATP-binding cassette transporter A1, Atherosclerosis 236 (2014) 215–226.
[26] T. Rubic, R.L. Lorenz, Downregulated CD36 and oxLDL uptake and stimulated ABCA1/G1 and cholesterol efflux as anti-atherosclerotic mechanisms of interleukin-10, Cardiovasc. Res. 69 (2006) 527–535.
[27] M. McLean, M. Hanson, B. Gold, Y. Golubeva, M. Anver, X. Wu, D. Sun, L. Steidler, S. Durum, PTU-103 intra-luminal interleukin (IL)-27 is a potential future therapeutic for inflammatory bowel disease, Gut 63 (Suppl. 1) (2014) A84.
[28] C. Wynick, C. Petes, K. Gee, Interleukin-27 mediates inflammation during chronic disease, J. Interferon Cytokine Res. (2014).
[29] X. Zhou, Z. Yin, X. Guo, D.P. Hajjar, J. Han, Inhibition of ERK1/2 and activation of liver X receptor synergistically induce macrophage ABCA1 expression and cholesterol efflux, J. Biol. Chem. 285 (2010) 6316–6326.
[30] D. Duffy, D.J. Rader, Update on strategies to increase HDL quantity and function, Nat. Rev. Cardiol. 6 (2009) 455–463.
[31] Y.R. Su, D.E. Dove, A.S. Major, A.H. Hasty, B. Boone, M.F. Linton, S. Fazio, Reduced ABCA1-mediated cholesterol efflux and accelerated atherosclerosis in apolipoprotein E-deficient mice lacking macrophage-derived ACAT1, Circulation 111 (2005) 2373–2381.