

TREM-1 modulates dendritic cell maturation and dendritic cell-mediated T cell activation induced by ox-LDL

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Abstract

Background: Triggering receptor expressed on myeloid cells (TREM)-1 is identified as a major upstream proatherogenic receptor. However, the cellular processes modulated by TREM-1 in the development of atherosclerosis and plaque destabilization has not been fully elucidated. In this study, we investigated the effects of TREM-1 on dendritic cell maturation and dendritic cell-mediated T-cell activation induced by oxidized low-density lipoprotein (ox-LDL) in atherogenesis.

Methods: Human peripheral blood monocytes were differentiated to dendritic cells and stimulated by ox-LDL. Naive autologous T cells were co-cultured with pretreated dendritic cells. The expression of TREM-1 and the production of inflammatory cytokines were assessed by real-time PCR, western blot and ELISA. The expression of immune factors was determined with FACS to evaluate dendritic cell maturation and T-cell activation.

Results: Stimulation with ox-LDL promoted dendritic cell maturation, TREM-1 expression and T-cell activation, and exposure of T cells to ox-LDL-treated dendritic cells induced production of interferon- γ and IL-17. Blocking TREM-1 suppressed dendritic cell maturation with low expression of CD1a, CD40, CD86 and HLA-DR, decreased production of TNF- α , IL-1 β , IL-6 and MCP-1, and increased secretion of TGF- β and IL-10. In addition, stimulation of ox-LDL induced miR-155, miR-27, Let-7c and miR-185 expression, whereas inhibition of TREM-1 repressed miRNA-155. Silencing TREM-1 or miRNA-155 increased SOCS1 expression induced by ox-LDL. T cells derived from carotid atherosclerotic plaques or healthy individuals showed similar result patterns.

Conclusion: These data suggest that TREM-1 modulates maturation of dendritic cells and activation of plaque T cells induced by ox-LDL, a pivotal player in atherogenesis.

Background

Atherosclerosis is characterized by lipid accumulation and chronic inflammation of the arterial wall, involving both innate and adaptive immune responses. Dendritic cells have been found within atherosclerotic lesions, and serve a key player in atherogenesis via antigen presentation, lipid internalization, and secretion of inflammatory cytokines^[1-3]. These cells also have compact adhesion to endothelium, causing plaque rupture and thrombosis, and finally resulting in acute cardiovascular events after induction by oxidized low-density lipoprotein (ox-LDL)^[4-6].

Triggering receptor expressed on myeloid cells (TREM)-1 belongs to an immunoglobulin superfamily, and is involved in immune modulation of various diseases^[8-11], particularly through amplifying production of interleukin (IL) -6, tumor necrotic factor - α , monocyte chemoattractant protein (MCP) -1 and other pro-inflammatory cytokines^[12-15]. In addition, TREM-1 participates in the process of innate and adaptive immunity by promoting differentiation of mononuclear cells to dendritic cells and accelerating antigen presentation^[16, 17]. It contributes to the development of atherosclerosis by promoting monocytes,

monocyte/macrophage pro-inflammatory responses, modulation of vascular smooth muscle cell function, and formation of inflammatory foam cells. In contrast, blocking of TREM-1 attenuates atherosclerosis. However, it remains unclear whether TREM-1 induces an amplified inflammatory response through excess immune maturation in ox-LDL-treated dendritic cells. The aim of this study was to explore the role of TREM-1 in the dendritic cell immune maturation, inflammatory amplification and T-cell activation.

Materials And Methods

The study was conducted in accordance with the Helsinki Declaration. The protocol was approved by the local ethics committee of Shanghai East Hospital, and informed consent was given by all participants.

1. Reagents

Lipofectamine® 2000 Transfection Reagent and TREM-1 siRNA were purchased from Invitrogen (Carlsbad, USA). Trizol was purchased from Sigma (St. Louis, USA). LP17 (LQVTDSGLYRCVIYHPP) and a sequence-scrambled control-peptide (TDSRCVIGLYHPPLQVY) were produced by GL Biochem (Shanghai) LTD, as described by Gibot et al^[18]. These peptides were obtained with good yields (>99%), purified and confirmed by preparative chromatography, and free of endotoxin. The anti-CD14 magnetic bead was purchased from Merck Millipore (Darmstadt, Germany). IL-4 and GM-CSF were from Peprotech (New Jersey, USA). The ELISA kits and CD1a-FITC, CD40-FITC, CD86-FITC, HLA-DR-PE antibodies were purchased from BD (Franklin Lakes, USA). The anti-TREM-1 primary antibody was purchased from R&D (HK, China). The anti-SOCS1, GAPDH primary antibodies were purchased from Cell Signaling (Denver, USA). The cDNA Synthesis Kit and Premix Ex Taq SYBR Green PCR Kit were purchased from Takara (Shiga, Japan). Other reagents were purchased from Gibco (Grand Island, USA).

2. Cell culture

Venous blood taken from healthy volunteers was added with Ficoll-Hypaque solution 1.077 and then centrifuged using density gradient. Peripheral blood mononuclear cells (PBMCs) were selected (over 98%) by anti-CD14 magnetic beads. The cell sediments were grown in RPMI-1640 containing GM-CSF (100 ng/mL), IL-4 (40 ng/mL) for 6 days.

3. T cell isolation

Atherosclerotic plaques (type IV) were harvested during carotid endarterectomy in patients with recent clinical events of minor stroke or transitory ischemic attacks^[19]. The specimens were kept in RPMI-medium and separated immediately into cells. The following experiments were done using the methods previously reported^[18]. Briefly, the specimens were dissected into multiple blocks in phosphate-buffered saline and medium RPMI-1640, and digested with enzyme mixture containing collagenase IV (1.25 mg/mL; Invitrogen), Liberase DL (25 µg/mL; Roche Applied Science), and DNase I (0.2 mg/mL; TAKARA Bio Group) for 1.5 hours at 37°C. With a 100 µm Celltrics filter, the tissue ghosts and cells were

strained. PBMCs were then isolated from the supernatant by Ficoll-Paque density gradient. T cells were purified further by Dynabeads® Untouched™ Human T Cells (Invitrogen)^[19,20].

4. T cell–dendritic cellco-culture

CD4⁺ T cells were purified from PBMCs using untouched CD4⁺ T cell isolation kit (Miltenyi Biotech). Dendritic cells and T cells were cocultured with 96-well flat-bottom plates. At day 7, cells were collected and washed, and then resuspended in RPMI/10% fetal calf serum. Proliferation of T cells was measured by (1) carboxy fluorescein succinimidyl ester (CFSE) staining: with a ratio of 10:1 between T cells and dendritic cells, autologous dendritic cells were cocultured with 2×10^5 T cells pre-labeled by CFSE (5 $\mu\text{mol/L}$). Four days later, cells were harvested to test the CFSE expression by flow cytometry, and culture supernatant was collected for measurement of cytokines. Results were expressed as percentage of proliferating CFSE^{low}/CD4⁺ cells. (2) bromodeoxyuridine incorporation assay: autologous dendritic cells were co-cultured with 2×10^5 T cells for 5 days in 200 μL of complete culture medium^[19]. At the last 16 hours, bromodeoxyuridine (10 $\mu\text{mol/L}$) (R&D Systems) was added. The cells were fixed and processed according to the manufacturer's instruction. Proliferative response was assessed as optical density value^[19,20].

5. T cell differentiation and suppression

With anti-CD45RO-coupled magnetic beads and LD negative selection columns (Miltenyi Biotech), we depleted CD45RO⁺ cells from CD4⁺T cells. In the purified cells, the percentage of CD4⁺CD45RO⁻CD45RA⁺T cells was >90%. In 1 mL of ex vivo 15 medium, 1×10^5 autologous dendritic cells were co-cultured with 1×10^6 CD4⁺CD45RO⁻T cells. After 6 days, 20 U/mL recombinant human IL-2 was added, and then cells were cultured for another 7 days. Thirteen days later, T cells were collected, washed, and assessed for their functions.

To determine proliferation and/or cytokine production of these T cells, autologous CD4⁺CD45RO⁻ T cells pre-labeled by CFSE (5 $\mu\text{mol/L}$) were cultured with mature dendritic cells (10:1; $10^5\text{T}:10^4\text{DC}$), in the presence or absence of T cells + dendritic cells, T cells + dendritic cell+ oxLDL or T cells + dendritic cells + SiTREM-1+oxLDL(1:1 ratio). At day 5, cells were collected and CFSE expression was tested by flow cytometry. Results were expressed as percentage of proliferating CFSE^{low}/CD4⁺ cells^[19,20].

6. Real time-reverse transcription PCR (RT-PCR)

Using Trizol and cDNA Synthesis Kit, total RNA was extracted and converted to cDNA. Real-time PCR was performed using an ABI 7500 system using Premix Ex Taq SYBR Green chemistry.

7. Western blot

After lysed in ice for 45 min, the samples were quantified using Bradford. Then, the samples with equal concentration of protein were subjected to SDS-PAGE, transferred to NC membrane and blocked using 5%

non-fat milk. At 4°C, incubated with primary antibodies overnight and subsequently coated with HRP-conjugated secondary antibodies for 1 hour at room temperature, the immunoblot was visualized using enhanced chemiluminescence.

8. Enzyme-linked immunosorbent assay

The concentration of cytokines in the supernatant was determined using human ELISA kits.

9. Flow cytometry

After stained with CD1a-FITC, CD40-FITC, CD86-FITC or HLA-DR-FITC antibody for 30 min, CD1a-, CD40-, CD86- or HLA-DR- positive cells were analyzed using flow cytometer.

10. siRNA and plasmid transfection

Cells were transfected with either control RNA or siRNA targeted human TREM-1, miR155 mimics, miR155 inhibitors, and the controls (RiboBio, China) using the Lipofectamine® 2000 transfection procedure. The cells were harvested at 48 hours post-transfection, and were treated with LP-17 (100 ng/mL) for 24 hours as well as isotype matched controls in the presence of ox-LDL (50 µg/ml).

11. Enzyme-linked immunosorbent assay

At day 7 after a 24-hour treatment as indicated, supernatants in dendritic cells were collected, and the levels of TNF-α, MCP-1, IL-10, IL-1β, TGF-β, IL-6, CCL5 and CCL17 in cell cultures were determined by enzyme-linked immunosorbent assay according to manufacturer's instructions (R&D Systems).

12. Statistical analysis

Data are expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's HSD test was used to compare the significant differences. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were done using SPSS 20.0.

Results

1. ox-LDL induced TREM-1 expression in dendritic cells

To investigate the effects of ox-LDL on TREM-1 expression, dendritic cells were exposed to ox-LDL (50 µg/ml) for 0, 6, 12 and 24 hours, and then TREM-1 protein and RNA levels were evaluated by western blot and real-time PCR. As shown in Fig 1, incubation with native LDL did not induce any increase of TREM-1 mRNA and protein levels. On the contrary, ox-LDL induced a marked up-regulation of TREM-1 mRNA and protein levels.

2. TREM-1 mediated dendritic cell maturation and inflammation

The expression of typical surface molecules indicated that dendritic cells grew from immature to mature. After stimulation with ox-LDL, the expression of CD1a, CD40, CD86, and HLA-DR in dendritic cells were markedly increased (Fig 3A-3D). Ox-LDL also induced high levels of TNF- α , IL-1 β , IL-6, and MCP-1 (Fig 4).

After treatment with SiTREM-1 or LP-17, the upregulation of dendritic cell maturation induced by ox-LDL was significantly repressed (Fig. 3A-D). Meanwhile, treatment with LP-17 inhibited the production of TNF- α , IL-1 β , IL-6, and MCP-1 in the dendritic cell cultures (Fig 4).

3. T cell activation induced by ox-LDL-treated dendritic cells was alleviated by inhibition of TREM-1

In dendritic cell-T cell co-culture experiments, ox-LDL-treated dendritic cells stimulated proliferation of T cells (Figure 3G). In contrast, after treatment with SiTREM-1 or LP-17, proliferation of T cells stimulated by ox-LDL-treated dendritic cells was markedly suppressed (Figure 3G).

In a 5-day MLR assay, the ability of activating allogeneic T cells was lower for SiTREM-1 or LP-17-stimulated dendritic cells than that for ox-LDL-treated dendritic cells (Fig 4A).

4. TREM-1 engagement on ox-LDL-treated dendritic cells induced Th1/Th17-polarizing pro-inflammatory responses

As shown in Fig 6, T cells were activated with ox-LDL-stimulated dendritic cells. CD86, CCR7 and CXCR4 were significantly increased. Similarly, several proinflammatory Th1/Th17 cell-priming cytokines and chemokines, including IFN- γ , and IL-17, TNF- α , IL-1 β , IL-6, MCP-1, CCL5 and CCL17, were markedly induced in response to ox-LDL. However, treatment with SiTREM-1 or LP-17, T cell costimulatory molecule, Th1/Th17 cell-priming cytokines and chemokines were repressed significantly, meanwhile the secretion of typical Th2 cytokines, IL-10 and TGF- β were promoted markedly. The difference in typical Th2 cytokines and IL-4 secretion was not significant.

5. miR-155 and SOCS1 signaling were involved in the effect of TREM-1

Ox-LDL induced expressions of miR-155, miR-27, Let-7c and miR-185, with miR-155 being most prominent. Induction of miR-155 was abolished after inhibition of TREM-1 (Fig. 7 A). Ox-LDL reduced significantly expression of SOCS-1 mRNA and protein, while cells initially pretreated with mimics or antagomir against miR-155 and next treated with ox-LDL were manifested by a marked decrease or increase in expression of SOCS-1 (Fig. 7B-C). This suggests that miR-155 induced by TREM-1 exerts its effects through down-regulation of SOCS-1, and subsequently mediated production of proinflammatory cytokines.

6. TREM-1 was essential to ox-LDL-induced effect on dendritic cell maturation, cytokine production, and T cell proliferation in patients with atherosclerosis

Dendritic cells differentiated from peripheral blood monocytes of patients who underwent carotid endarterectomy showed higher expression of TREM-1 (Fig 9A), CD1a, CD40, CD86 and HLA-DR (Fig 8A-8D),

and elevated concentrations of IL-6, and IL-10, TNF- α , TGF- β at baseline (Fig 8F), which were further enhanced by ox-LDL stimulation. Pretreatment with LP17 (an inhibitory peptide) or TREM-1 markedly decreased expression of CD1a, CD40, CD86 and HLA-DR and secretion of TNF- α , IL-6, MCP-1, but increased expression of IL-10 and TGF- β (Fig 8A-D,F).

Ox-LDL treated dendritic cells derived from peripheral blood monocytes of patients with carotid atherosclerosis induced autologous plaque T cell proliferation, which was abolished by blocking TREM-1 with LP17 or TREM-1 siRNA (Fig 8

Discussions

The results of this study show that (1) TREM-1 was up-regulated by ox-LDL and orchestrated pivotal molecular events triggering immune maturation of dendritic cells and production of inflammatory factors. Moreover, ox-LDL induced proinflammatory Th1/Th17 type immune activation of dendritic cells and T-cell activation, whereas inhibition of TREM-1 attenuated these effects. (2) TREM-1 induced miR-155 expression via down-regulation of SOCS-1, and subsequently mediated production of proinflammatory cytokines.

Abundant evidence supports the concept that atherosclerosis represents a chronic inflammatory disease, in which accumulation of lipid-laden macrophage is critical^[1-3]. Recent researches demonstrated an existence of increased number of matured dendritic cells in vulnerable plaques^[21]. Like the most potent antigen-presenting cells, dendritic cells directly engulf endogenous or exogenous immunogen in atherosclerotic plaques (such as ox-LDL) and produce various cytokines (such as TNF- α , interleukins and MCP-1)^[21-22]. Because these invasive cytokines could mediate plaque rupture and subsequent cause acute coronary events^[2, 3], dendritic cells were traditionally thought to be critical for the pathogenesis of cardiovascular disease. In the present study, we found that stimulation of ox-LDL increased sharply the number of CD1a-, CD40-, CD86- or HLA-DR- positive cells and promoted the secretion of IL-1 β , IL-6, TNF- α and MCP-1. These observations support a notion that ox-LDL induces a harmful change and inflammatory response of dendritic cells^[4-6].

TREM-1 is an immunoreceptor expressed mostly on neutrophils and monocytes/macrophages. Interestingly, TREM-1 is also identified on endothelial cells and regulates thrombin generation^[23, 24]. Previous studies suggest that TREM-1 appears to augment inflammatory responses in infectious disease^[17, 25], and regulate noninfectious cardiovascular disease^[26-28]. In acute myocardial infarction models, TREM-1 was up-regulated in infarcted zone, and inhibiting TREM-1 could effectively reduce myocardial inflammation and improve cardiac function^[26]. Moreover, TREM-1 was expressed in human atherosclerotic lesions, and inhibition of TREM-1 alleviated atherosclerosis in a murine model^[27]. We previously reported that soluble TREM-1 level in serum was associated with all-cause mortality and major adverse cardiac events in patients with acute myocardial infarction, independent of established conventional risk factors^[28]. Likewise, TREM-1 co-localized with dendritic cells in atherosclerotic plaques

from patients with carotid stenosis^[29], suggesting that TREM-1 with dendritic cells may play a key role in the pathogenesis of atherosclerosis. In line with previous findings^[19], we found that ox-LDL induced dendritic cell maturation with promoted expression of CD1a, CD40, CD86 and HLA-DR and increased secretion of inflammatory cytokines, which could be abrogated by using TREM-1 siRNA or LP17. Instead, production of TGF- β and IL-10 was up-regulated.

The present study showed that T cells co-cultured to oxLDL-treated dendritic cells induced secretion of IFN- γ and IL-17, with polarization to Th1 and/or Th17 subsets. Silencing TREM-1 abolished the effects of dendritic cells induced by oxLDL and subsequent T-cell activation, with down-regulation of transcription factor TBX21 and RORC expression, and induced T regulatory cells. T cells obtained from healthy individuals or carotid atherosclerotic plaques showed similar result patterns. In general, human atherosclerosis has a pro-inflammatory T-cell phenotype^[30–32]. Although IL-17 and corresponding Th17 cells are likely to be pro-atherogenic^[33–34], their exact role is controversial. Regulatory T cells (Tregs) regulates the activation of T cells and suppresses pro-inflammatory effects, inhibiting formation of foam cells and subsequently preventing atherosclerosis. Thus, therapy with increasing Tregs may be promising for the treatment of atherosclerotic cardiovascular diseases^[35]. In this study, oxLDL induced T cells activation mediated by dendritic cells from vulnerable atherosclerotic plaques, which was reversed by TREM-1 silencing. This suggests that TREM-1 may play an important role in the development of atherosclerosis and plaque rupture by a novel-specific immune modulatory mechanism.

TREM-1 activation has been shown to augment monocyte/macrophage proinflammatory responses and induced foam cell formation, promoting atherosclerosis^[27]. In this study, TREM-1 amplified inflammation in dendritic cells following ox-LDL treatment, providing further evidence that TREM-1 could aggravate atherosclerosis not only by macrophage response, but at least partly by modulating dendritic cells.

Numerous researches have assessed the role of miR dysregulation in atherosclerotic disease progression^[36]. MiR-155 is significantly upregulated in plasma and plaques in patients with atherosclerosis, whereas miR-155 deficiency attenuated atherogenesis in apoE^{-/-} mice^[37–39]. SOCS1 is one of the target genes of miR-155^[40, 41]. In this study, TREM-1 regulated miR-155 expression and inflammatory response. Furthermore, mimics of miR-155 down-regulated and antagomir against miR-155 up-regulated SOCS-1 expression, respectively. These data substantiate a notion that TREM-1-induced miR-155 exerts its effects through down-regulation of SOCS-1, and subsequently mediates proinflammatory cytokines, including, IL-1 β , IL-6, MCP-1 and TNF- α .

Conclusions

ox-LDL induces an augmented inflammatory response via TREM-1 activation in dendritic cells. Deficiency of TREM-1 attenuates ox-LDL-mediated inflammation and T cell activation. MiR-155/SOCS1 pathway may play a key role in TREM-1 mediated inflammation and T-cell activation in ox-LDL-induced dendritic cells. Although many questions regarding the molecular network need be answered, our findings

provide biochemical insights that inhibition of TREM-1 could be a novel strategy and promising target for the treatment of atherosclerosis.

Abbreviations

TREM-1 Triggering receptor expressed on myeloid cells 1

ox-LDL oxidized low-density lipoprotein

IL-6 interleukin - 6

TNF - α tumor necrotic factor - α

MCP-1 monocyte chemoattractant protein-1

PBMCs Peripheral blood mononuclear cells

CFSE carboxy fluoresce insuccinimidyl ester

Tregs regulatory T cells

Declarations

Acknowledgements

Not applicable.

Authors' contributions

W-YK, WJ , HL, and S-YK performed the experiments and analyzed the data. W-YK, Y-JY, Z-CY, L-XD, and ZQ designed the study and wrote the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used during the study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was conducted in accordance with the Helsinki Declaration. The protocol was approved by the local ethics committee of Shanghai East Hospital, and informed consent was given by all participants.

Consent for publication

Not applicable.

Competing interests

Not applicable.

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Figures

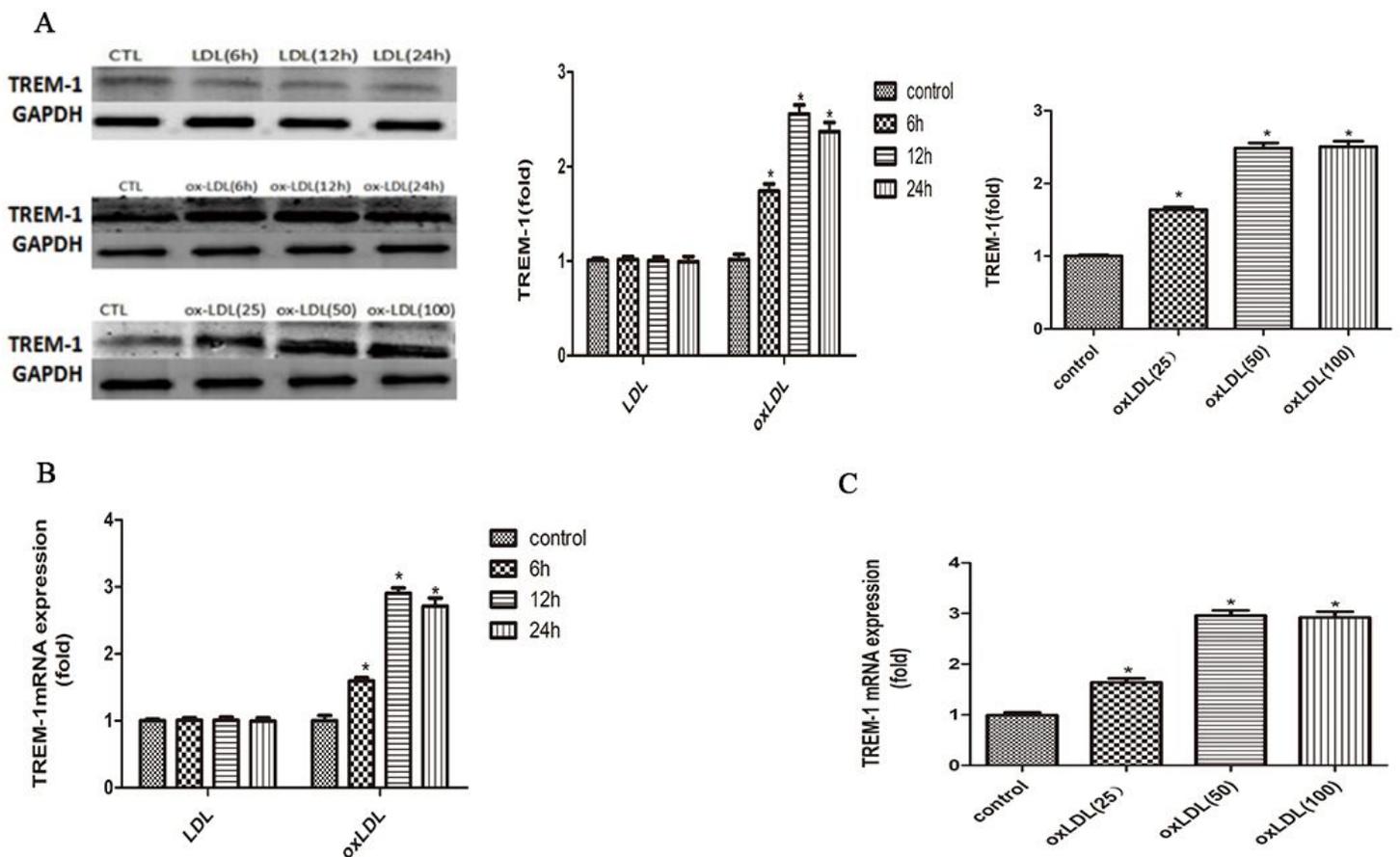


Figure 1

ox-LDL induced DCs TREM-1 expression. DCs were treated with LDL or ox-LDL, protein and mRNA levels of TREM-1 were determined. (a) DCs at day 6 were treated with LDL at 6, 12, 24h, DCs at day 6 were treated with oxLDL at 6, 12, 24h, and DCs at day 6 were treated with oxLDL at 25, 50 or 100 $\mu\text{g mL}^{-1}$ for 24 h. Then, protein levels of TREM-1 were determined. (b) DCs at day 6 were treated with LDL or oxLDL at 50 $\mu\text{g mL}^{-1}$ at 0, 6, 12, 24 h, then, mRNA levels of TREM-1 were determined. (c) DCs at day 6 were treated with oxLDL at 25, 50 or 100 $\mu\text{g mL}^{-1}$ for 24h, then, mRNA levels of TREM-1 were determined. (N=6), * $p < 0.05$, compared with control cells; # $p < 0.05$, compared with ox-LDL treated cells.

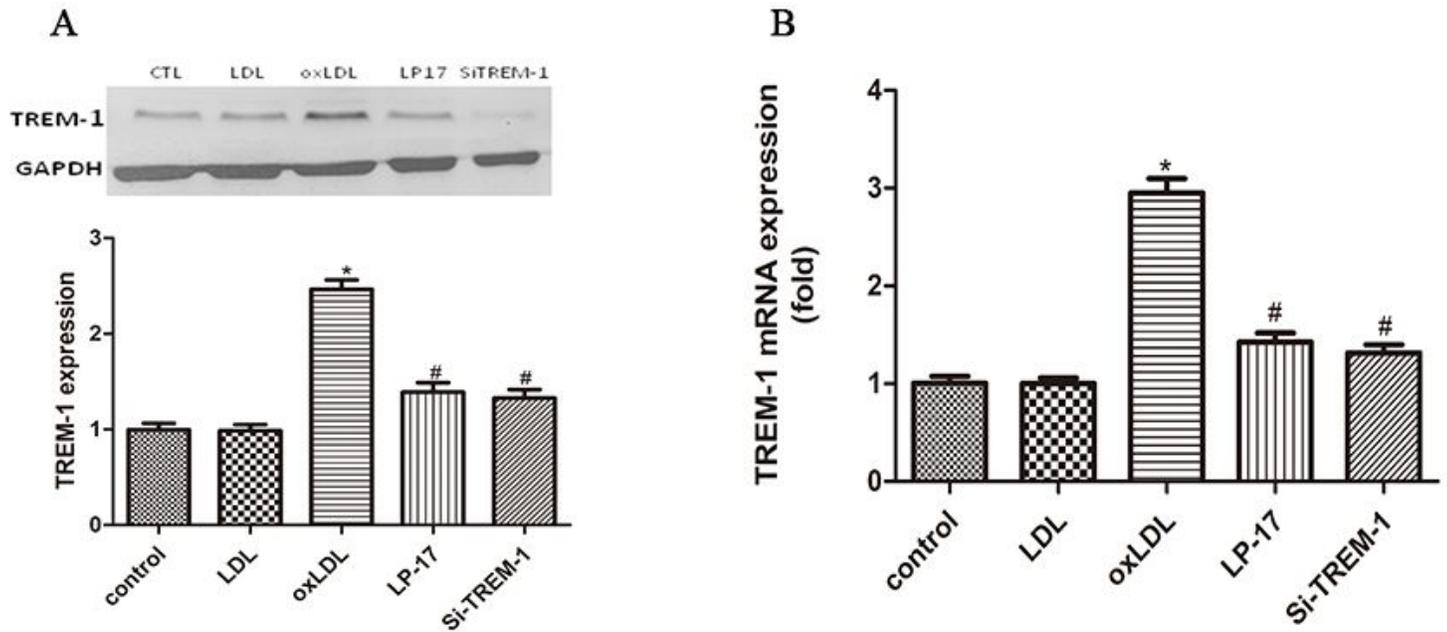


Figure 4

LP17 and SiTREM-1 regulated ox-LDL-induced DCs TREM-1 expression. For downregulation of TREM-1, DCs was transfected with TREM-1 Silencer (SiTREM-1), or treated with LP17 at day 6. oxLDL was added 6 h after transfection and further incubated for 24 h. Then, mRNA levels and protein levels of TREM-1 were determined. (N=6). * $p < 0.05$, compared with control cells; # $p < 0.05$, compared with ox-LDL treated cells.

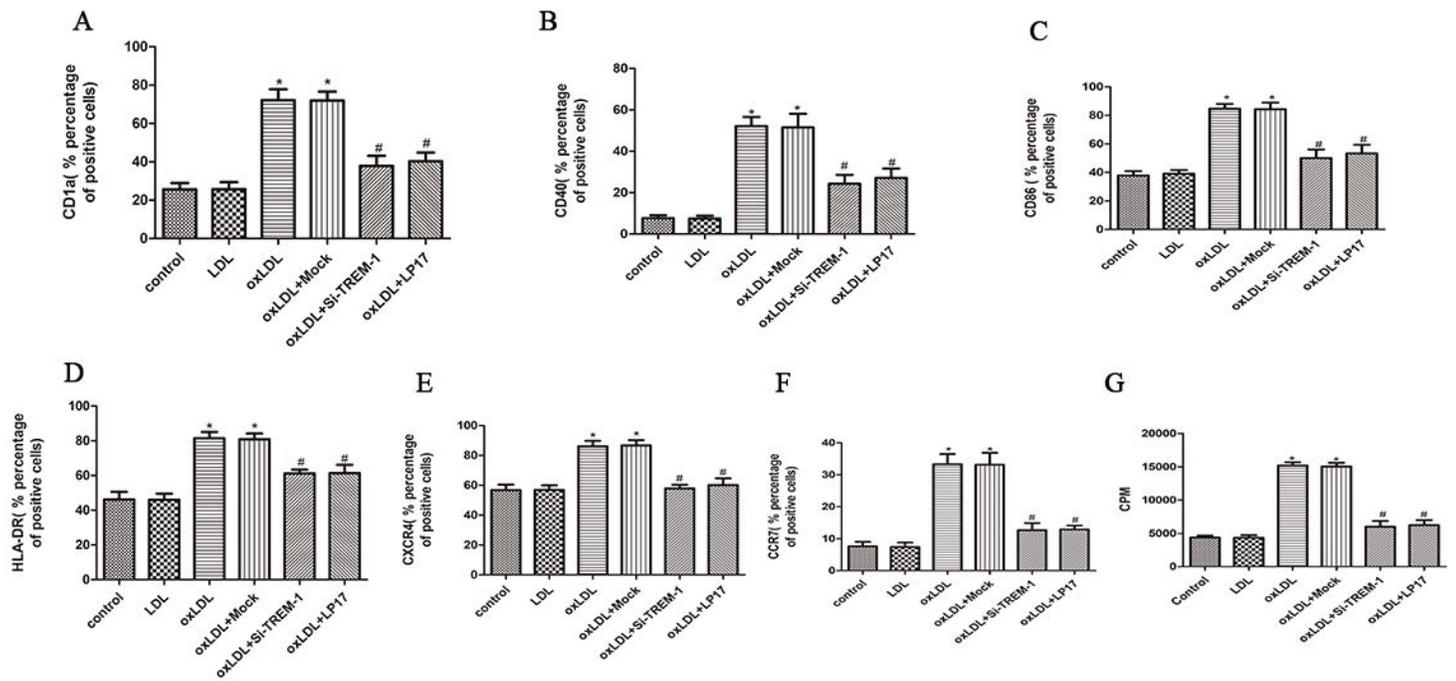


Figure 7

TREM-1 play a important role in DCs maturation and subsequent T-cell proliferation induced by ox-LDL. The expressions of CD1a, CD40, CD86, HLA-DR, CXCR4 and CCCR7 on day 7 DCs were analysed by flow cytometry. A. ox-LDL increased CD1a-positive DCs from $25.65 \pm 3.72\%$ to $72.15 \pm 4.46\%$. B. ox-LDL increased CD40-positive DCs from $7.62 \pm 2.23\%$ to $52.15 \pm 1.17\%$. C. ox-LDL increased CD86-positive DCs from $37.91 \pm 1.64\%$ to $84.72 \pm 3.96\%$. D. ox-LDL increased HLA-DR-positive DCs from $46.2 \pm 0.96\%$ to $81.44 \pm 3.55\%$. (E) ox-LDL increased CXCR4-positive DCs from $56.77 \pm 1.89\%$ to $86.15 \pm 3.96\%$. (F) ox-LDL increased CCR7-positive DCs from $7.62 \pm 0.69\%$ to $33.3 \pm 2.16\%$. (G) T-cell proliferation determined by BrdU incorporation assay. After the indicated treatment, DCs were washed and cocultured in triplicates for 5 days with autologous T cells. $10 \mu\text{mol L}^{-1}$ BrdU was present in the last 16 h. Proliferative response was evaluated as OD value. Results represent the Mean \pm SD of six experiments. $N=6$, * $p < 0.05$, compared with control cells; # $p < 0.05$, compared with ox-LDL treated cells.

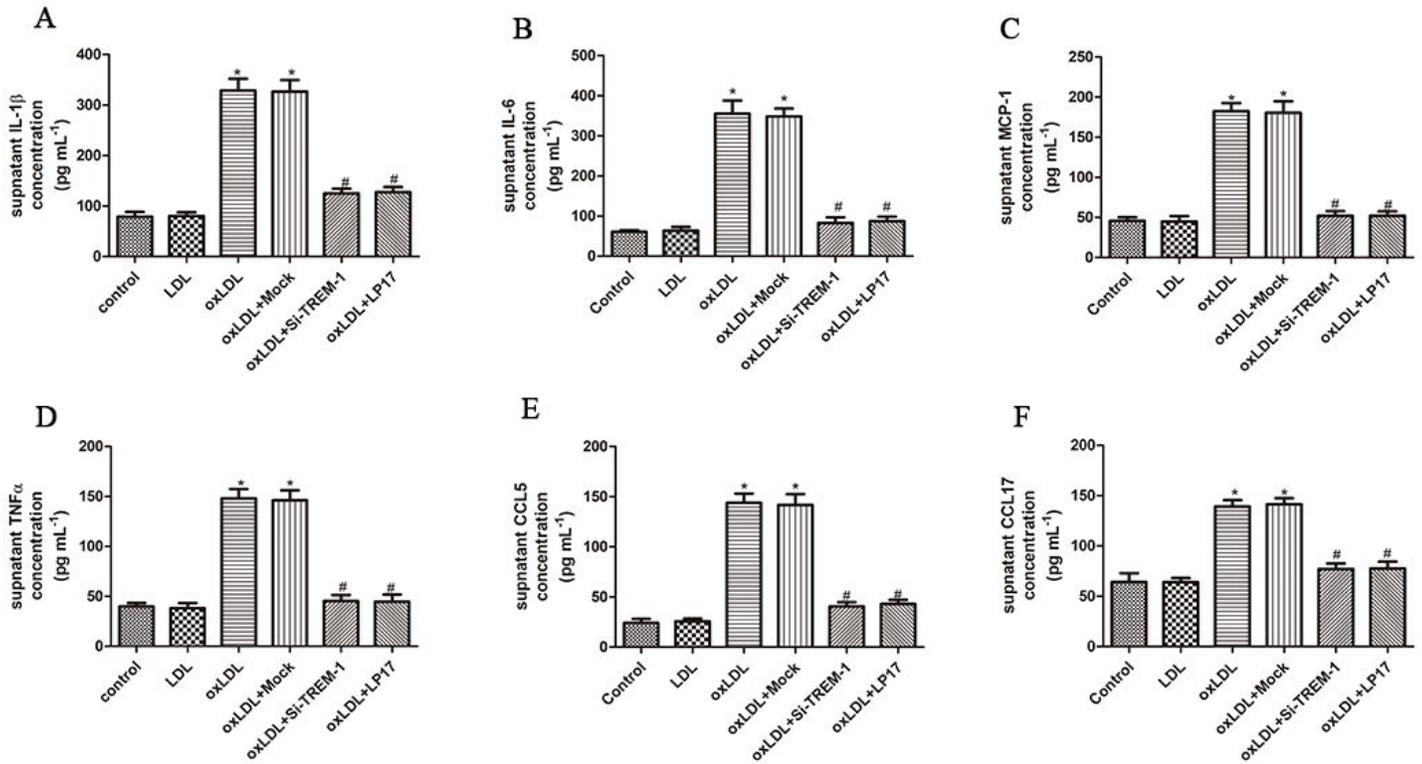


Figure 10

TREM-1 regulated ox-LDL-induced DCs inflammation. DCs at day 6 were treated with ox-LDL, At 24 h after cells were transfected with TREM-1 siRNA or relevant scrambled siRNAs or treated with LP-17. Cytokine production tested by ELISA. DC supernatants were collected at day 7.(N=6). * $p < 0.05$, compared with control cells; # $p < 0.05$, compared with ox-LDL treated cells.

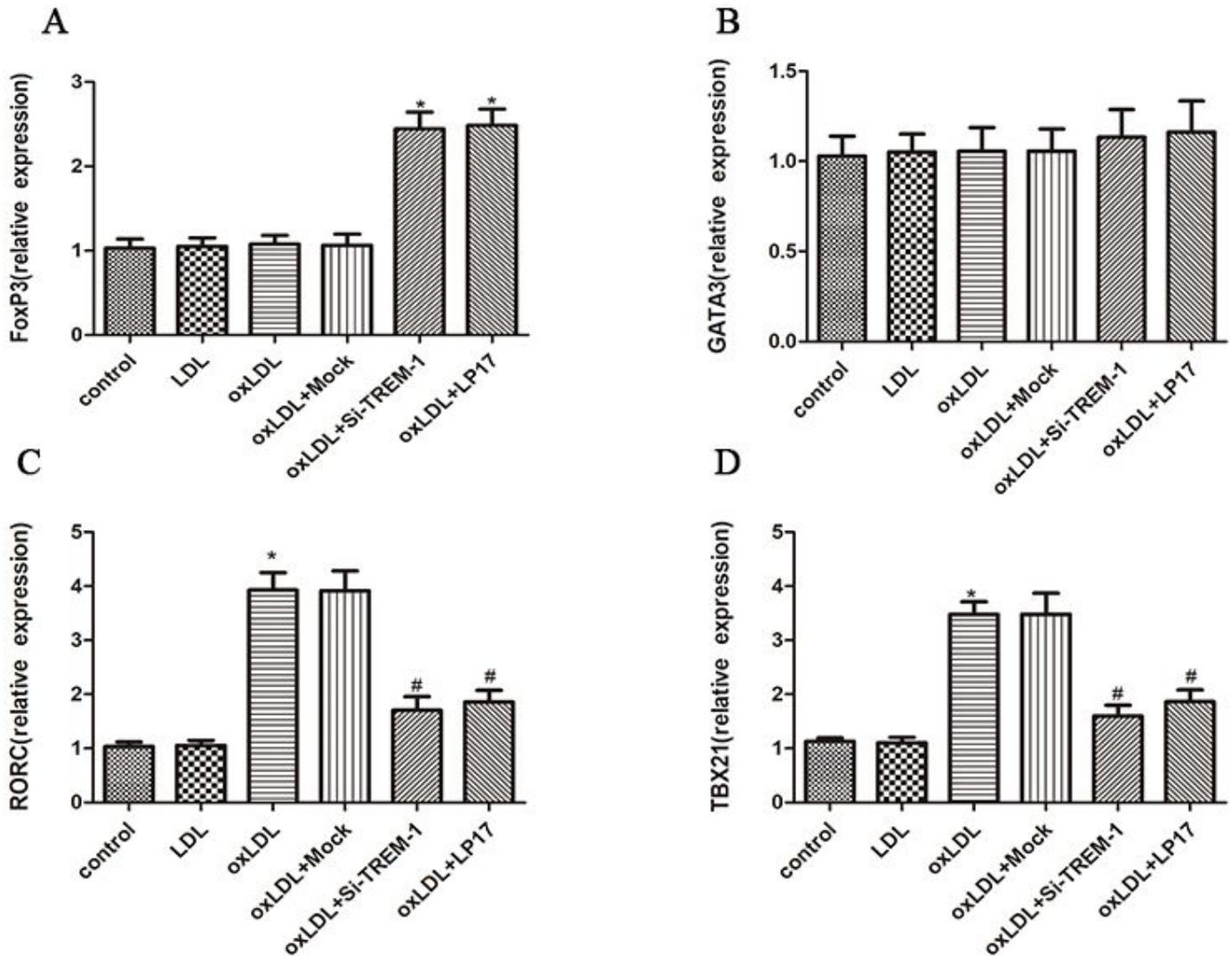


Figure 13

TBX21, GATA3, RORC and FOXP3 gene expressions were evaluated by real-time PCR. oxLDL-treated DCs strongly induced TBX21 and/or RORC expression in naïve T-cell populations. However, TREM-1 inhibition suppressed the expression of TBX21 and RORC while induced expression of FoxP3. (N=6). * $p < 0.05$, compared with control cells; # $p < 0.05$, compared with ox-LDL treated cells.

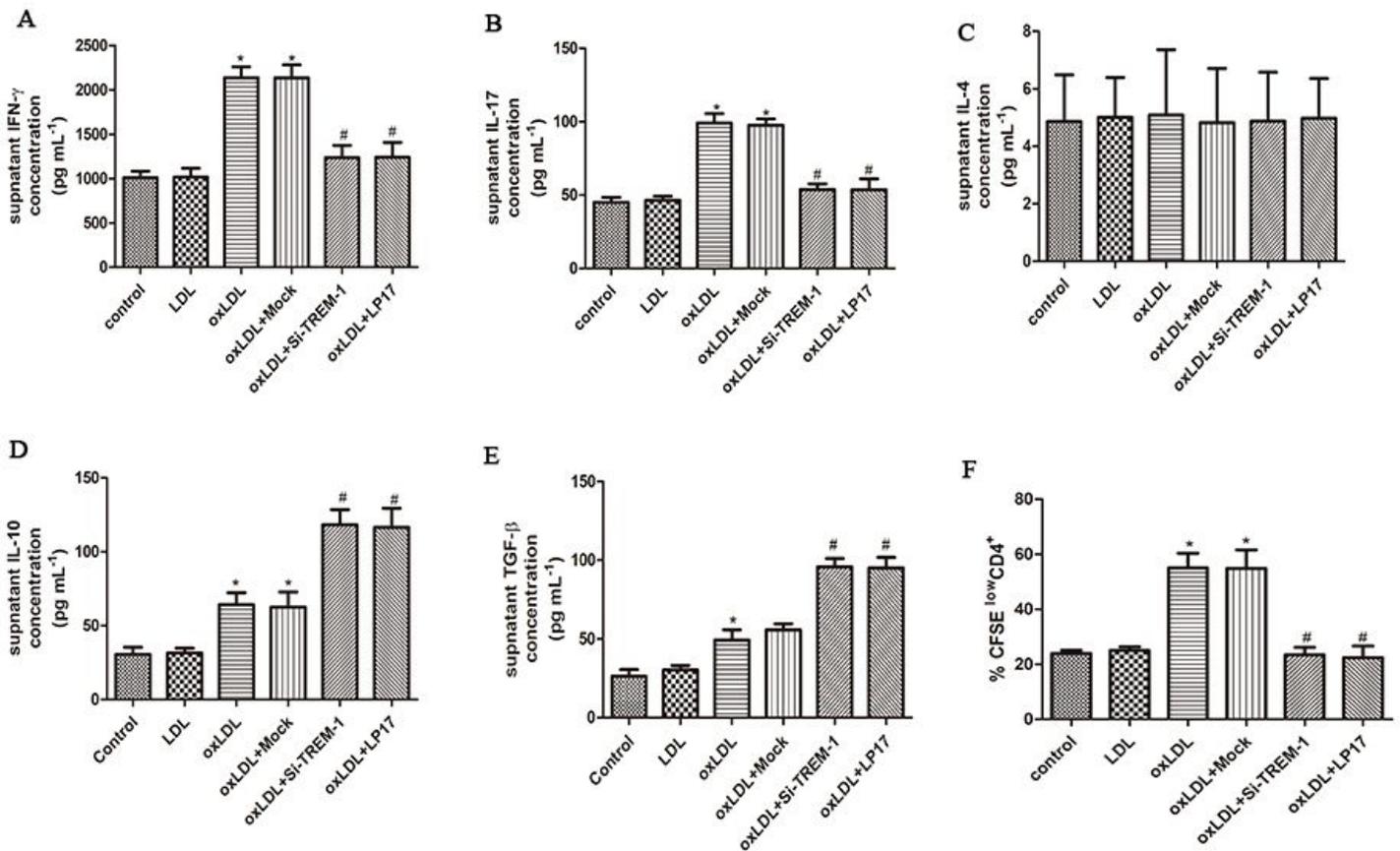


Figure 16

TREM-1 mediated the Th1/Th17-priming induced by oxLDL-treated DCs in MSL. (A-D) Supernatants were collected at day 7 to determine IFN- γ , IL-17, IL-4, TGF- β , and IL-10 content by ELISA. (E) T [DC (siR-TREM-1+oxLDL)] cells suppressed primary T-cell proliferation. Naïve CD4⁺ T cells were cultured with oxLDL- or SiTREM-1 + oxLDL-treated autologous DCs for 13 days. Naïve CD4⁺T cells labelled with 5 μ mol L-1CFSE were stimulated with mature DCs(mDC) alone or in the presence of T [DC], T [DC (oxLDL)], or T [DC (Si-TREM-1+oxLDL)], T [DC (Mock+oxLDL)] cells at a 1:1ratio. T-cell proliferation was measured by CFSE dilution. (N=6). * p <0.05, compared with control cells; # p <0.05, compared with ox-LDL treated cells.

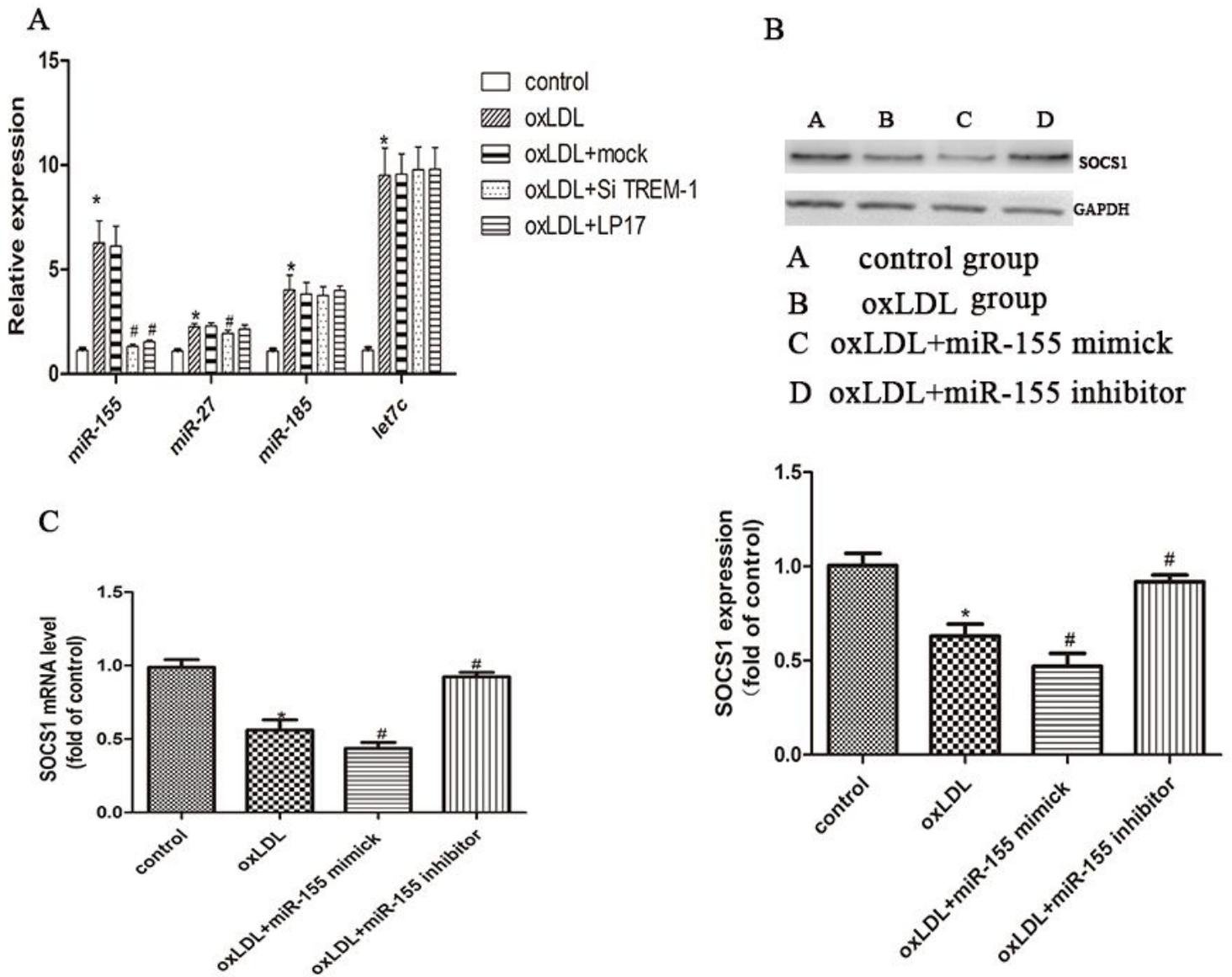


Figure 19

miRNA-155/SOCS1 signalling pathways were involved in the effect of TREM-1 and oxLDL. (a) Expression of miRNAs in DCs was tested by qRT-PCR. The value was normalized as fold change to that of non-treated DCs samples. $n=6$. * $p<0.05$, compared with control cells; # $p<0.05$, compared with ox-LDL treated cells. (b) SOCS1 was induced in oxLDL-treated DCs, and it was enhanced by silencing of TREM-1. $N=6$. * $p<0.05$, compared with control cells; # $p<0.05$, compared with ox-LDL treated cells.

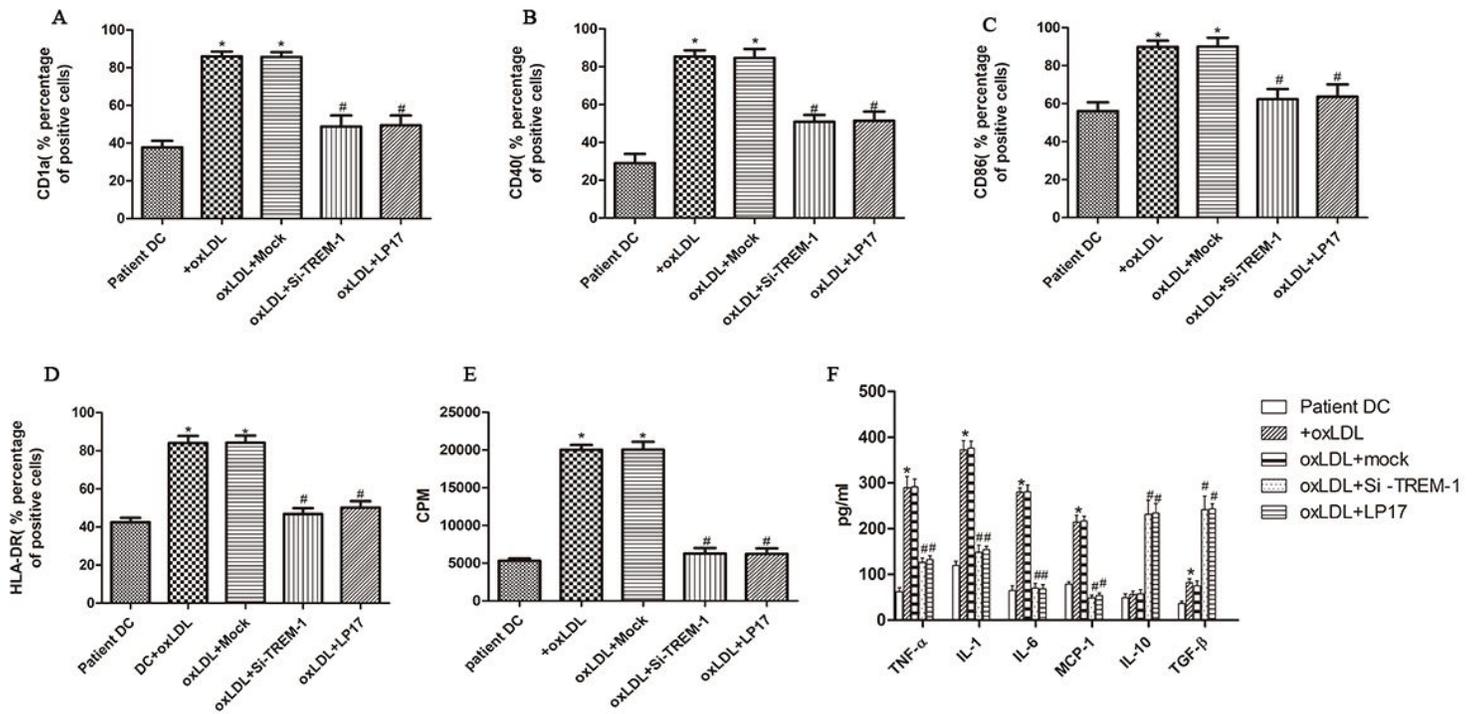


Figure 22

TREM-1 was involved in DC maturation, pro-inflammatory cytokine production and T-cell proliferation from plaquepatients induced by oxLDL. DCs were generated from peripheral blood monocytes of patients who underwent carotidendarterectomy. The same protocol used as that for DCs of normal donors. Briefly, DCs at day 6 were treated with $50\mu\text{g mL}^{-1}$ oxLDL for 24 h. (A-D)The expressions of CD1a, CD40, CD86, HLA-DR, CXCR4 and CCCR7 on day 7 DCs were analysed by flow cytometry. (E) T-cell proliferation determined by BrdU incorporation assay. After the indicated treatment, DCs were washed and cocultured in triplicates for 5 days with autologous T cells. $10\mu\text{mol L}^{-1}$ BrdU was present in the last 16 h. Proliferative response was evaluated as OD value. (F) Cytokine production tested by ELISA. N=6.* $p<0.05$, compared with control cells; # $p<0.05$, compared with ox-LDL treated cells.

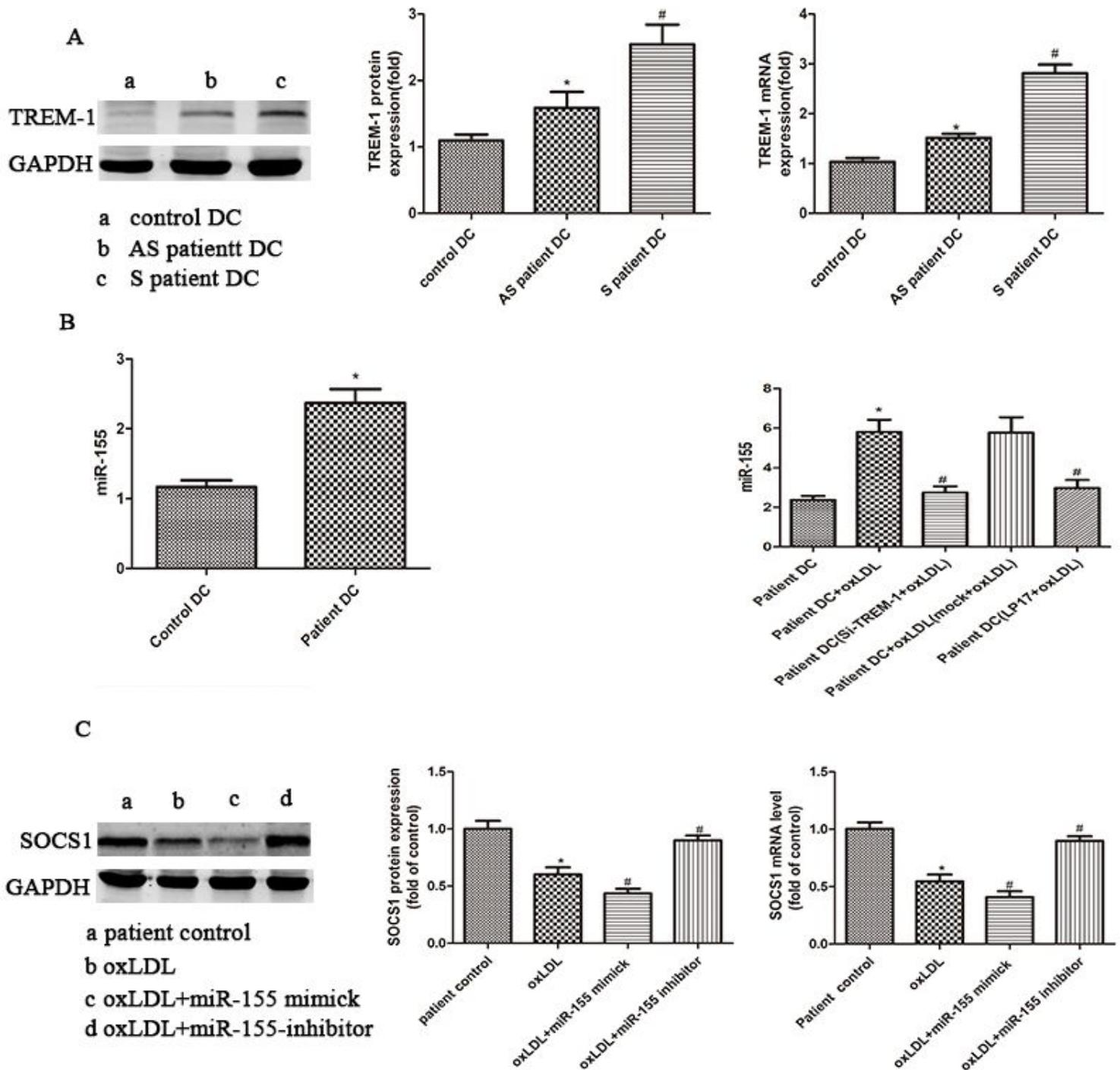


Figure 25

TREM-1 expression in DCs from symptomatic and asymptomatic patients with Carotid stenosis, and SOCS1 signaling pathways were involved in the effect of TREM-1 and oxLDL on DCs from Patients with Carotid stenosis. (A) The mRNA and protein of TREM-1 expression in DCs from symptomatic is higher than from asymptomatic patients with carotid stenosis; (B) DCs were generated from peripheral blood monocytes of patients who underwent carotid endarterectomy. miR-155 expression were determined. (C) SOCS1 was induced in oxLDL-treated DCs, and it was enhanced by silencing of TREM-1. N=6. *p<0.05, compared with control cells; #p<0.05, compared with ox-LDL treated cells.