

Full-length article

Triptolide suppresses CD80 and CD86 expressions and IL-12 production in THP-1 cells¹Jing LIU, Qing-li WU, Yong-hong FENG, Yi-fu YANG, Xiao-yu LI, Jian-ping ZUO²*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China***Key words**

triptolide; CD80 antigens; CD86 antigens; interleukin-12

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Abstract

Aim: To investigate the effects of triptolide, a diterpenoid triepoxide from *Tripterygium wilfordii* Hook F (TWHF), on the co-stimulatory molecule expression and interleukin-12 (IL-12) production from THP-1 cells. **Methods:** THP-1 cells were differentiated into macrophage-like cells by Me₂SO, and then cultured with IFN- γ (500 kU/L) and lipopolysaccharide (LPS) (1 mg/L) with or without triptolide. The surface molecule expressions were analyzed on a FACScan flow cytometer. IL-12p40, IL-12p70 were assayed by ELISA. **Results:** Triptolide suppressed CD80 and CD86 expressions on IFN- γ (500 kU/L) and LPS (1 mg/L) activated THP-1 cells at nontoxic dosages of 2.5–0.625 μ g/L. Furthermore, the production of IL-12p40 and IL-12p70 were also significantly reduced in THP-1 cells exposed to triptolide. **Conclusion:** Triptolide impairs the antigen-presenting function by inhibiting CD80 and CD86 expressions and decreased IL-12p40 and IL-12p70 (bioactive form) productions from the activated THP-1 cells.

Introduction

Human cells of monocytic lineage, including monocytes, macrophages, and monocyte-derived dendritic cells, play an essential role in initiating and maintaining immune responses by acting as antigen-presenting cells (APC). They process and present antigenic peptides via the major histocompatibility complex (MHC) molecules and the expression of costimulatory molecules^[1,2]. The best-characterized costimulatory molecules to date are two structurally related proteins, CD80 and CD86. Both provide costimulation to T-cells, leading to their proliferation, cytokine production, and development^[2,3]. *In vivo* studies using anti-CD80 and anti-CD86 monoclonal antibodies or genetically manipulated animals have demonstrated that a blockade of CD80/CD86 costimulatory molecules can prolong allograft survival and reduce the severity of autoimmune diseases^[4,5]. Preliminary clinical studies in human subjects also support this concept^[6]. Therefore, pharmacological intervention in the upregulation of CD80/CD86 may therefore be useful in the treatment of autoimmune diseases or in transplantation.

Tripterygium wilfordii Hook F (TWHF) is a herb used in traditional Chinese medicine for the treatment of rheumatoid arthritis and other autoimmune diseases^[7,8]. Triptolide, a highly oxygenated diterpenoid triepoxide, is the major component responsible for the immunosuppressive and anti-inflammatory effects of TWHF^[9]. It is effective for the treatment of autoimmune diseases, prevention of allograft rejection, and graft-versus-host disease in animal experiments^[10–13]. The inhibitory effect of triptolide on T-cell activation is stronger than cyclosporine^[14] and FK-506^[15] *in vitro*. Furthermore, it can induce activated T-cell apoptosis^[16]. Thus, T-cells are considered to be the main target for triptolide. Recently, Hong *et al*^[17] and Li *et al*^[18] demonstrated that triptolide inhibited upregulation of B7 on activated human proximal tubular epithelial cells. These results suggest that triptolide may have suppressive effects on APC. In a further analysis of this theory, we used a human monocytic leukemia cell line (THP-1) that had been differentiated into macrophage-like cells by treatment with Me₂SO. The macrophage-like THP-1 cells were used to examine the influences of triptolide on the expression of CD80 and CD86 molecules and the production

of IL-12, an important cytokine that biases CD4⁺ T-cells toward Th1 differentiation^[19].

Materials and methods

Reagents Triptolide was provided by the Department of Chemistry, Shanghai Institute of Materia Medica. Lipopolysaccharide (LPS, Escherichia coli 055: B5), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and 3,3',5,5'-tetramethylbenzidine (TMB) were from Sigma (St Louis, MO, USA). Recombined human IFN- γ was purchased from Shanghai Clonbiotech (Shanghai, China). PE-conjugated mAbs were used to detect cell surface expression of CD14, HLA-ABC, HLA-DR, CD80 and CD86 by flow cytometry, as well as isotype-matched mAbs, which were purchased from PharMingen (San Jose, CA, USA).

Cells The human monocytic THP-1 line (ATCC, Manassas, VA, USA) was maintained in suspension culture in RPMI1640 medium, supplemented with 10% FBS. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO₂ and were subcultured at 1/10 dilution every 5–6 d. Cells were used to differentiate when seeded at a density of 5 × 10⁸ cells/L in a 10-cm² culture dish for 24 h.

Flow cytometry analysis THP-1 cells were cultured in a screw cap container 2.4 × 10⁶ cells in 4 mL of RPMI1640 medium with 10% FBS per-well. Cells were differentiated with 1.2% Me₂SO for 24 h, then pre-treated with IFN- γ (500 kU/L) for 16 h and stimulated by LPS (1 mg/L) for another 24 h. Various concentrations of triptolide were added at the initiation of the culture. Cells were collected and washed in an ice cold staining buffer (PBS containing 0.1% w/v sodium azide, 1% FBS, pH 7.2). Cells were then resuspended at 4.0–5.0 × 10⁹/L in an ice-cold staining buffer. Optimal concentrations of each fluorochrome-labeled antibody were added. Cells were incubated at 4 °C for 30 min, then washed twice and analyzed by flow cytometry using FACSCalibur (Becton Dickinson, San Jose, CA, USA). Data were illustrated by CELLQuest.

Cytokine analysis THP-1 cells were cultured in 24-well plates at 6.0 × 10⁵ cells in 2 mL of RPMI1640 medium with 10% FBS and other treatments were the same as in the surface molecules analysis. Culture supernatants were harvested and stored at -20 °C. Human IL-12p70 and IL-12p40 productions were measured using ELISA kits (PharMingen) according to the manufacturer's instructions.

MTT assay Cytotoxicity was assessed by MTT assay^[20]. Me₂SO-differentiated THP-1 cells were incubated in a 96-well plate at 5.4 × 10⁴ cells in 180 μ L of RPMI1640 medium with 10% FBS per-well for 72 h with and without various concentrations of triptolide. MTT (5 g/L) 18 μ L was added 5 h prior

to the end of the culture, and then 90 μ L solvent (10% SDS, 50% *N,N*-dimethyl formamide, pH 7.2) was added. The cells were then incubated for another 7 h and value was measured at 570 nm by a microplate reader (Bio-Rad Model 550, Japan).

Statistical analysis Results were expressed as mean \pm SD. An independent two-tailed *t*-test was performed and *P* < 0.05 was considered to be statistically significant.

Results

Triptolide influenced the surface molecule expressions on Me₂SO-differentiated THP-1 cells THP-1 cells are known to be activated by LPS stimulation and have been used as a surrogate for investigation of human monocytes^[21]. When THP-1 cells are treated with Me₂SO, the cells differentiate into macrophage-like cells and proliferation is profoundly inhibited. IFN- γ pretreatment and LPS stimulation enhanced the expression of a variety of surface molecules such as HLA-ABC, HLA-DR, and B7 molecules on THP-1 cells. Triptolide could suppress CD80 and CD86 expressions on IFN- γ (500 kU/L) and LPS (1 mg/L)-activated THP-1 cells at 2.5–0.625 μ g/L (Figure 1). Triptolide also weakly down-regulated the HLA-ABC expression, but it did not change the expressions of HLA-DR and CD14 on THP-1 cells.

Triptolide inhibited IL-12 production of Me₂SO-differentiated THP-1 cells IFN- γ pretreatment and LPS stimulation induced IL-12p40 and IL-12p70 production from Me₂SO-differentiated THP-1 cells. The productions of IL-12p40 and IL-12p70 in response to IFN- γ (500 kU/L) and LPS (1 mg/L) induction were significantly reduced in THP-1 cells exposed to triptolide in the concentration of 2.5–0.625 μ g/L (Figure 2). IL-10 production was too low to be detected in this experimental system.

Cytotoxicity of triptolide on Me₂SO-differentiated THP-1 cells We examined whether the above suppressive effects of triptolide were due to its cytotoxicity to THP-1 cells. When Me₂SO-differentiated THP-1 cells were cultured with and without different concentrations of triptolide for 72 h, the cytotoxicity was monitored by MTT assay. As shown in Figure 3, triptolide did not exert any cytotoxic effect on the THP-1 cells from 2.5 μ g/L to 0.625 μ g/L in the present study.

Discussion

The immunosuppressive and therapeutic activity of triptolide upon systemic administration has been demonstrated in animal models for human-like diseases, such as in experimental autoimmune uveitis^[22], collagen-induced arthritis^[11], diabetes^[12], nephrotic syndrome^[13]. It also improves

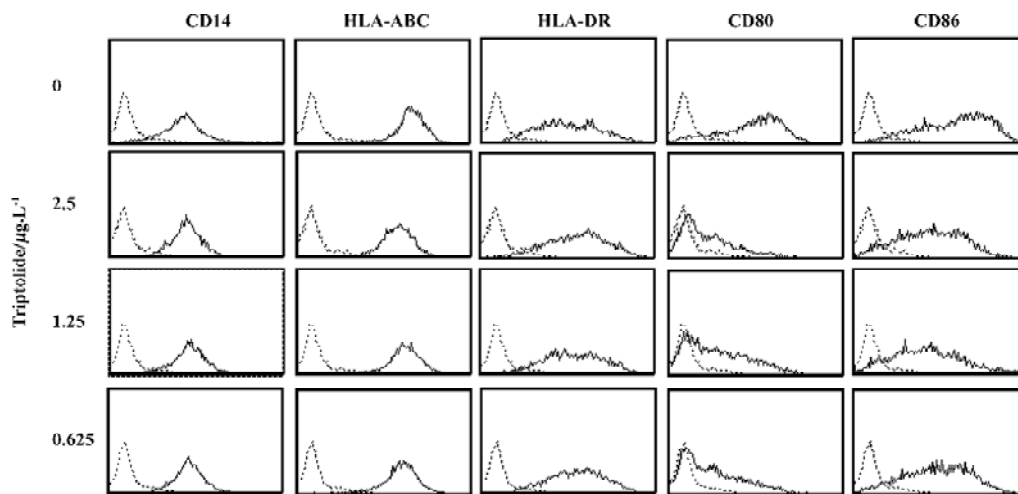


Figure 1. Suppressive effects of triptolide on the expression of CD80 and CD86 in THP-1 cells. Dotted line represents cells incubated with isotype-matched antibodies and solid line represents cells incubated with specific antibodies. Results were obtained from one of the three experiments performed.

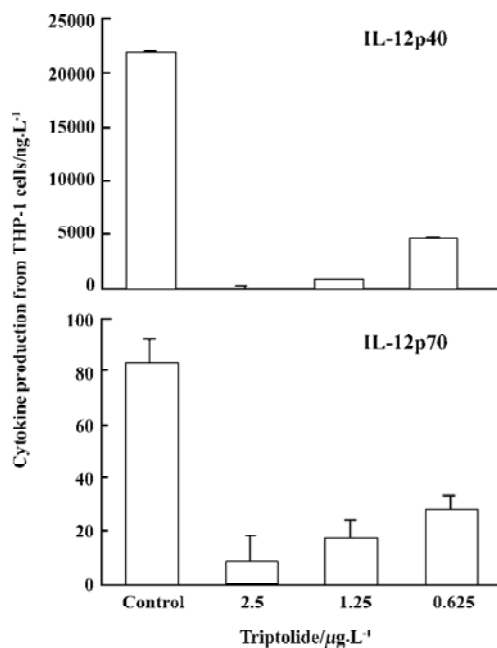


Figure 2. Analysis of IL-12p40 and IL-12p70 productions in THP-1 cells. Mean \pm SD. Results were obtained from one of the three experiments performed.

the survival rate of the heart^[23] and kidney^[24] allograft in the rat transplantation model and graft-versus-host disease in a murine allogeneic bone marrow transplantation model^[25]. However the immunosuppressive mechanism of triptolide has not been completely delineated^[26]. In the present study, we demonstrated a new finding that triptolide, an active component of TWHF, directly down-regulates the costimulation

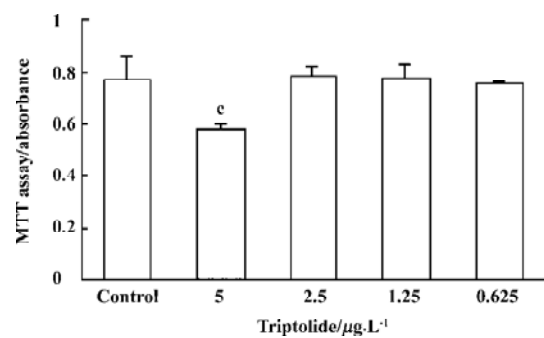


Figure 3. Cytotoxicity of triptolide on THP-1 cells. THP-1 cells ($6.0\times 10^8/\text{L}$) were differentiated with 1.2% Me_2SO . Compounds were added at the initiation of culture. Mean \pm SD. ^c $P<0.01$ vs control. Results were obtained from one of the three experiments performed.

molecule CD80 and CD86 expressions on macrophages-like cells (Me_2SO -differentiated THP-1 cells), and inhibits the bioactive form of IL-12p70 production from Me_2SO -differentiated THP-1 cells by a dose-dependent manner.

CD80 and CD86 on APC delivered the critical costimulatory signals for optimal T-cell activation^[3]. Blockade of CD80 and CD86 signaling resulted in ineffective T-cell activation. The implication is that reduced and blocked CD80/CD86 costimulations can prevent the induction of pathogenic T-cell responses in autoimmune disease and allow for prolonged acceptance of allografts in organ transplantation^[4-6]. In addition to the processing and presentation of antigenic peptides via MHC molecules and the expression of costimulatory molecules, APC played a central role in driving CD4^+ T helper cell differentiation through the

production of critical cytokines, such as IL-12, IL-10, TGF- β and possibly IL-4. The production of bioactive form IL-12p70 by host antigen-presenting cells was a primary determinant in the differentiation of naive CD4⁺ T cells into Th1 effector cells^[19]. These suppressive effects of triptolide on CD80 and CD86 expressions, and IL12p70 production suggest an important therapeutic implication for multiple sclerosis and other abnormal immune response mediated diseases associated with the increased expression of CD80/CD86 and the production of IL-12^[27].

Triptolide has been proven to be a potent immunosuppressive agent, although information on the molecular mechanism of triptolide is scarce. Recent studies have shown that triptolide inhibits T-cell activation and early cytokine gene transcription in T-cell at the purine-box/ARRE/NF-AT and NF- κ B target sequence after specific DNA binding^[28,29]. NF- κ B is a family of transcription factors central to immunity and inflammation. It regulates expression of a number of genes, including cytokines, costimulatory molecules, nitric oxide, and susceptibility to apoptosis^[30]. Therefore, the suppressive effects of triptolide on the co-stimulatory molecular expression and IL-12 production from THP-1 cells can result from the inhibition of NF- κ B transcriptional activation.

In conclusion, we have demonstrated that triptolide down-regulates the levels of CD80 and CD86 expressions in THP-1 cells. Production of bioactive form IL-12p70 in THP-1 cells was effectively inhibited by triptolide. These immunosuppressive activities of triptolide contribute, at least in part, to the therapeutic effect and mechanism of triptolide in the treatment of autoimmune diseases.

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