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Triptolide inhibits proinflammatory factor-induced over-expression of class II MHC and B7 molecules in renal tubular epithelial cells

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KEY WORDS triptolide; epithelial cells; major histocompatibility complex; antigens CD80; antigens CD86; intercellular adhesion molecule-1

ABSTRACT

AIM: To investigate the effects of triptolide on proinflammatory factor-induced over-expression of class II major histocompatibility complex (class II MHC), B7-1, B7-2, and intercellular adhesion molecule-1 (ICAM-1) in human proximal tubular epithelial (HKC) cells. **METHODS:** HKC cells were exposed to both interferon γ (IFN-γ, 200 μg/L) and tumor necrosis factor α (TNF-α, 20 μg/L) and cultured in media containing different concentrations of triptolide for 24 h. Class II MHC, B7-1, B7-2, and ICAM-1 levels in HKC cells were evaluated by flow cytometry. ICAM-1 mRNA level was measured by semi-quantitative RT-PCR method. **RESULTS:** HKC cells expressed quite high level of ICAM-1 and very low levels of class II MHC, B7-1, and B7-2 molecules. Class II MHC, B7-1, B7-2, and ICAM-1 levels in HKC cells were significantly increased by the costimulation of IFN-γ and TNF-α. Triptolide inhibited the over-expression of class II MHC, B7-1, and B7-2 molecules in a concentration-dependent manner. The up-regulations of ICAM-1 molecules and ICAM-1 mRNA level were not altered by triptolide. **CONCLUSION:** Triptolide can significantly inhibit proinflammatory factors induced over-expression of class II MHC, B7-1, and B7-2 costimulatory factors in tubular epithelial cells. These results may contribute to the therapeutic effects of triptolide in some renal diseases.

INTRODUCTION

Renal tubular epithelial cell is one of the major cell kinds in tubulointerstitium, and always thought to be the "victim" of tubulointersitial injuries, but recent works suggested that renal tubular epithelial cells played an important role in renal inflammatory damages. Renal tubular epithelial cells have been proved to up-regulate class II major histocompatibility complex (class II MHC) molecules in inflammatory renal diseases and *in vitro*, when stimulated by IFN-γ and lipopolysaccharide (LPS)[1-3]. Moreover, *in vitro* and *in vivo* studies

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demonstrated that renal tubular epithelial cells could be induced to express costimulatory molecules, such as B7-1 and ICAM-1, under distinct experimental conditions[2-4]. The relevance of these cell-surface markers to immune-mediated events in the kidney has been further supported by their increased expression on renal tubular epithelial cells *in vivo*, in settings such as chronic-graft-versus-host disease, allograft rejection, and lupus nephritis[1,2,5,6]. These results suggest that renal tubular epithelial cells may play an important role in initiating or propagating renal damage through interactions with T lymphocytes.

Tripterygium wilfordii Hook f (TWHf), a member of celastraceae plant family, has been used as anti-inflammatory agent in traditional Chinese medicine for centuries[7]. The extract of the herb was proved to have potent immunosuppressive activity *in vivo* and *in vitro*[8,9]. Studies have shown that TWHf extract is effective in the therapy of rheumatoid arthritis, systemic lupus erythematosus, glomerular nephritis, and acute rejection after transplantation[10,11]. Hundreds of patients who suffered in inflammatory renal diseases had taken the TWHf extract therapy and got obvious improvement in our institute. Among the large number of components that have been isolated from the extract of TWHf, triptolide is one of the major active components with most potent immunosuppressive activity[12]. To further understand the mechanism of the immunosuppressive activity of triptolide, the effects of triptolide on the regulation of class II MHC, B7-1, B7-2, and ICAM-1 molecules in human proximal tubular epithelial cells were investigated in this study.

MATERIALS AND METHODS

Reagents Triptolide (purity >99 %) was obtained from Institute of Dermatology, Chinese Academy of Medical Sciences (Nanjing, China)[13]. Human IFN-γ was purchased from Jingmei Biotech Co Ltd. (Shenzhen, China). Human recombinant TNF-α was purchased from Promega (Madison, WI, USA). Monoclonal mouse anti-human HLA-DP, DQ, and DR antigen antibody (Clone: CR3/43) which was able to anti HLA-DP, DQ, and DR at the same time, monoclonal mouse anti-human ICAM-1 (CD54) antibody (Clone: 6.5B5) and fluorescein isothiocyanate (FITC) labeled rabbit anti-mouse immunoglobulins were purchased from DAKO (Glostrup, Denmark). Monoclonal mouse anti-human CD80 (B7-1, Clone: BB1) and CD86 (B7-2, Clone: FUN-1) antibody were got from PharMingen (San Diego, CA, USA).

Cell culture and treatment Human renal proximal tubular epithelial (HKC) cells was a gift from Prof ZHENG Fa-Lei (Peking Union Medical College, Beijing, China)[14]. Cells in passages 10-15 were cultured, and treated in RPMI-1640 (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10 % heat-inactivated fetal calf serum (FCS). Cells at 80 %-90 % confluent were cultured in serum free medium for 24 h before treated with IFN-γ (200 μg/L), TNF-α (20 μg/L), and different concentrations of triptolide (0.4, 2.0, 10 μg/L) for another 24 h. Cells cultured in RPMI-1640/10 % FCS or treated with IFN-γ and TNF-α only served as controls. Human spleen cells were primary cultured from donor spleen and served as positive control for measurement of class II MHC, B7-1, B7-2, and ICAM-1 molecules by flow cytometry. In brief, donor spleen tissue was sliced into 1 cm^3 cubes, and squeezed through a 200-hole/cm^2 steal mesh. Single nuclear cells were collected by centrifugation and cultured in RPMI-1640 containing 10 % FCS and lipopolysaccharide (LPS, 10 μg/L) for 72 h.

Flow cytometry HKC cells (1×10^6) were harvested by versene solution (0.02 %), washed twice in 1 mL PBS (0.01 mol/L, pH 7.4) solution, fixed in 0.1 % paraformaldehyde for 15 min, and then washed and incubated in 200 μL PBS containing 0.5 % bovine serum albumin (BSA) and 0.02 % Na_3N for 40 min at 4 ℃ with proper antibodies (mouse anti-human HLA-DP, DQ, DR, ICAM-1, CD80, and CD86 antibody). Then the cells were washed and incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG in dark at 4 ℃ for 30 min. The antibodies were diluted to recommend concentrations according to the manufactures' instructions. Background fluorescence was adjusted with cells labeled with mouse control antibodies as the first antibody. LPS-activated human spleen

cells served as positive controls. Analyses were done on a Coulter flow-cytometer (Miami, Florida, USA).

RT-PCR Total cellular RNA was isolated using Trizol reagent (Sangon, Shanghai, China) in accordance with the manufacturer's instructions. After assessing purity of final products by *A* value ratios at 260/280 nm, RNA samples were reverse-transcribed into cDNA by a reverse-transcription kit (Promega). The human ICAM-1 primers were 5'-TATGGCAACGACTCC TTCT-3' (sense) and 5'-CATTCAGCGTCACCTTGG-3' (antisense). A pair of porcine β-actin primers was used at the same time as control: 5'-CTACAA-TGAGCTGCGTGTGG-3' (sense) and 5'-TAGCTCTTCTTCAGGGAGGA-3' (antisense). After amplification, PCR products were electrophoresed through a 2 % agarose gel with ethidium bromide (0.5 mg/L) to visualize the DNA bands. The expected lengths of PCR products were 237 bp (ICAM-1) and 450 bp (β-actin). The results were analyzed by BF-300 image system and its software provided by Sixing Biotech (Shanghai, China).

Statistical analysis Data were expressed as mean±SD. Analysis of variance (ANOVA) and *F* test were used to evaluate the statistical significance. $P<0.05$ was considered statistically significant.

RESULTS

IFN-g and TNF-a induced over-expression of class II MHC, B7-1, B7-2, and ICAM-1 in HKC cells The LPS-stimulated spleen cells expressed high levels of class II MHC, B7-1, B7-2, and ICAM-1 molecules when analyzed by flow cytometry. HKC cells cultured in RPMI-1640/10 % FCS expressed quite high levels of ICAM-1 and very low levels of class II MHC, B7-1, and B7-2 molecules. When stimulated with IFN-γ (200 μg/L) and TNF-α (20 μg/L) together for 24 h, the expression of class II MHC, B7-1, B7-2, and ICAM-1 in HKC cells are all significantly increased (Tab 1, Fig 1). The expression of ICAM-1 mRNA also increased significantly by the costimulation of IFN-γ and TNF-α (Fig 2).

Triptolide inhibited the up-regulation of class II MHC, B7-1, and B7-2 Triptolide inhibited the over-expression of class II MHC, B7-1, and B7-2 molecules in a dose-dependent manner. But triptolide has no significant influence on the proinflammatory factors induced up-regulation of ICAM-1 molecules and ICAM-1 mRNA level. (Tab 1, Fig 2, 3)

DISCUSSION

Tripterygium wilfordii Hook f (TWHf) has been used in traditional Chinese medicine for centuries. A large number of compounds have been identified from the extract of TWHf, including diterpenoids (such as triptolide and tripdiolide), alkaloids, glycosides, β-sitosterol, and triptoquinones[15]. Previous studies have shown that triptolide (PG490) is the most efficient constituents of TWHf with potent immunsuppessive and anti-inflammatory abilities. Some other ingredients of TWHf, such as tripdiolide and tripcholorolide, also have been proved to have immunosuppressive effect by some experimental systems, but their effects seems not as strong as triptolide's[16]. Although triptolide is often used as a replacer of corticoid in the patients who can not

Tab 1. Effect of triptolide (Trip) on the expression (mean fluorescence intensity) of class II MHC, B7-1, B7-2, and ICAM-1 in human tubular epithelial cells. *n*=3. Mean±SD. $^cP<0.01$ *vs* control. $^eP<0.05$, $^fP<0.01$ *vs* IFN-g+TNF-a group.

Group	Class II MHC	B7-1	B7-2	ICAM-1
Control	1.7±0.4	0.93±0.15	0.92±0.24	99±18
IFN-γ (200 μg/L) + TNF-α (20 μg/L)	7.3±1.5^c	1.69±0.18^c	2.3±0.3^c	155±24^c
IFN-γ + TNF-α + Trip (0.4 μg/L)	5.1±1.0	1.7±0.3	1.9±0.4	161±21
IFN-γ + TNF-α + Trip (2 μg/L)	4.9±1.0^e	1.42±0.25	1.58±0.16^f	166±31
IFN-γ + TNF-α + Trip (10 μg/L)	2.94±0.23^f	1.34±0.19^e	1.45±0.28^f	154±31

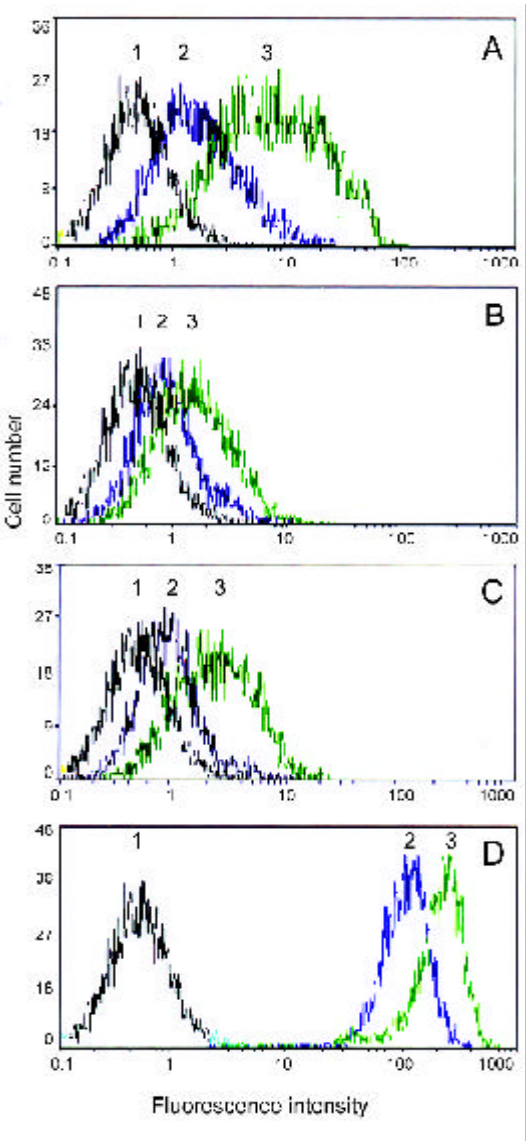

Fig 1. IFN-g and TNF-a induced up-regulation of the expression of class II MHC, B7-1, B7-2, and ICAM-1 in HKC cells. A: class II MHC; B: B7-1; C: B7-2; D: ICAM-1. Line 1 (black): Negative control; Line 2 (blue): Cells without IFN-g and TNF-a treatment; Line 3 (green): Cells treated with IFN-g (200 mg/L) and TNF-a (20 mg/L).

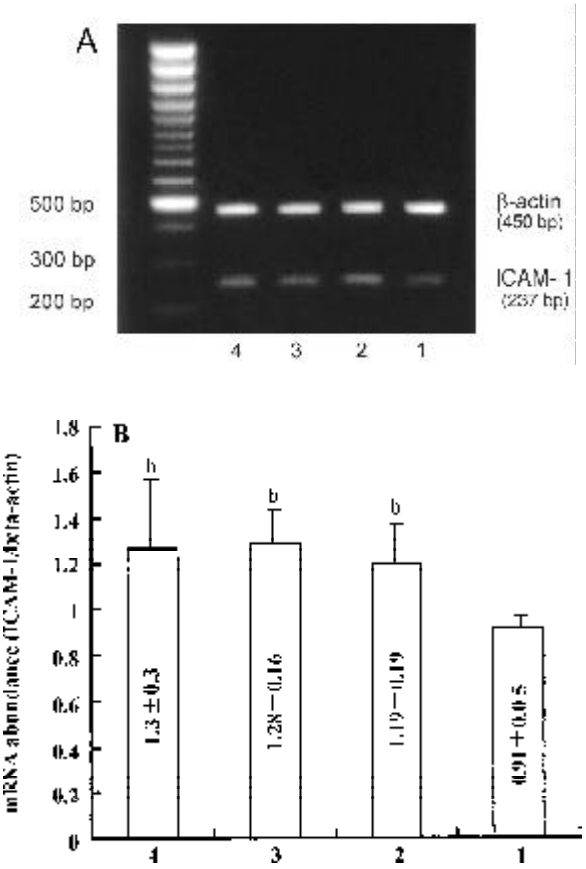

Fig 2. ICAM-1 mRNA expression in HKC cells. A: ICAM-1 mRNA were reverse-transcribed, amplified with PCR, and electrophoresed through a 2 % agarose gel. B: The result was analyzed by BF-300 Image System. Lane 1: Control; Lane 2: Treated with IFN-g (200 mg/L) and TNF-a (20 mg/L); Lane 3: Treated with triptolide (2 mg/L), IFN-g, and TNF-a; Lane 4: Treated with triptolide (10 mg/L), IFN-g, and TNF-a. *n*=4. Mean±SD. $^{b}P<0.05$ *vs* control.

stand corticoid-therapy, our clinical experience and some experimental results showed that the effects of triptolide were different from some other immunosuppressants. Triptolide is useful in some patients who suffered in IgA nephropathy to whom corticoid was not effective[17], and is more effective in preventing T cell proliferation and interferon-gamma production than is FK506[18]. Lots of works have been done to investigate the mechanisms of Triptolide's potent immunosuppressive effects. Studies have shown that triptolide inhibits mitogen-induced lymphocyte proliferation, mixed lymphocyte reaction, generation of cytotoxic T cells and induces apoptotic death of T lymphocyte[13,19,20]. Triptolide can also markedly increase cAMP/PKA activity in T lymphocyte[21], and is a potent inhibitor of NF-κB activation in T lymphocyte[22]. However, the previous works on triptolide focused on its effects on lymphocytes, the effects of triptolide on the immune abilities of renal tubular epithelial cells are still not understood.

In this study, the impact of triptolide on class II

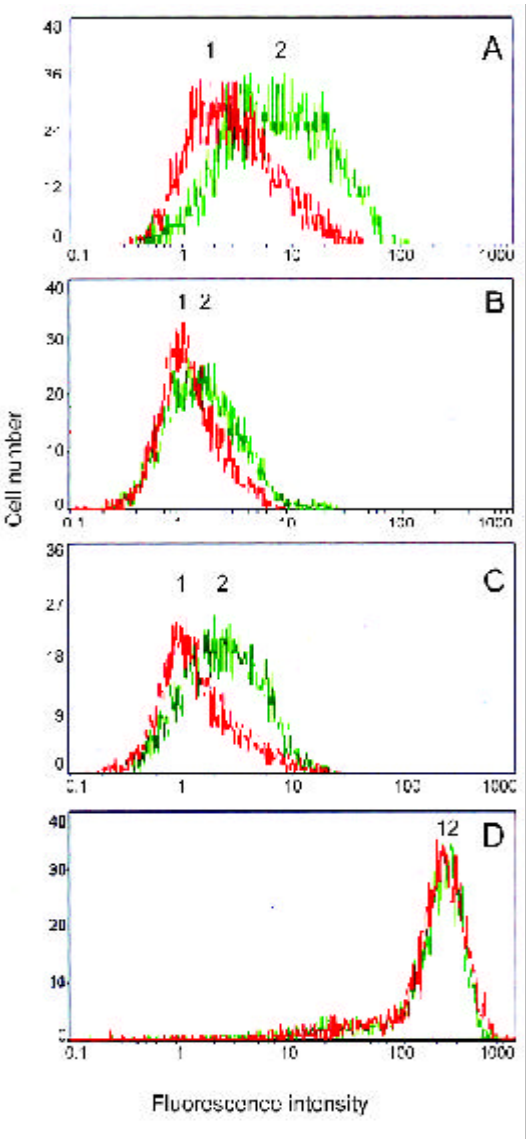

Fig 3. The modulatory effects of triptolide on the expression of class II MHC, B7-1, B7-2, and ICAM-1 in HKC cells. A: class II MHC; B: B7-1; C: B7-2; D: ICAM-1. Line 1 (red): Treated with triptolide (10 mg/L), IFN-g, and TNF-a; Line 2 (green): Treated with IFN-g and TNF-a.

MHC, B7-1, B7-2, and ICAM-1 expression on renal tubular epithelial cells was investigated. Class II MHC plays a major role in the antigen-specific T-cell activation by presenting antigen and peptide antigen to the T-cell receptor (TCR) on T lymphocyte[23]. But this trimolecular interaction is insufficient for complete triggering of T-cell activation. Costimulatory molecules, such as B7-1, B7-2, and CD28 (one of B7-1 and B7-2's counter receptors) contribute to T-cell activation by increasing or stabilizing adhesion between T cell and antigen presenting cell and by transmitting signals that augment TCR-mediated activation signals[24]. CD28 is expressed almost exclusively on T cells, but B7-1 and B7-2 are expressed or could be induced to express on a variety of cell types, including renal tubular epithelial cells in this study. Renal tubular epithelial cells which have been induced to express class II MHC and B7 costimulatory factors have been proved to have T-cell-activating functions[2]. ICAM-1 is another type of costimulatory factor, and is abundantly expressed on tubular epithelial cells. ICAM-1 by itself does not elicit an inflammatory response and more likely functions as an inflammation-enhancing molecule[25]. In this study, we found triptolide could significantly inhibit proinflammatory factors induced up-regulation of class II MHC, B7-1, and B7-2 on tubular epithelial cells, but had no significant influence on ICAM-1 expression. These results suggest that inhibition of class II MHC and B7 costimulatory factors expressions in tubular epithelial cells, and further inhibition of the antigen-presenting activity of tubular epithelial cells may also contribute to the immunosuppressive effects of triptolide.

The effect of IFN-γ or TNF-α alone on the expression of those factors on HKC cells was observed too. We found that IFN-γ could up-regulate the expression of class II MHC and ICAM-1 but not B7-1 and B7-2, and that TNF-α could up-regulate the expression of B7-1, B7-2, and ICAM-1 but not class II MHC (data not shown). But the results show that triptolide can inhibit the over-expressions of class II MHC, B7-1, and B7-2 but not ICAM-1. The molecular mechanisms underlying these manifestations are still not clear now.

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雷公藤内酯醇抑制炎症因子引起的肾小管上皮细胞主要组织相容性II类抗原和B7共刺激分子过度表达

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关键词 雷公藤内酯醇；上皮细胞；主要组织相容性复合物；抗原 CD80；抗原 CD86；细胞间粘附分子-1

目的：观察雷公藤内酯醇对炎症因子引起的人肾小管上皮细胞(HKC)主要组织相容性 II 类抗原（class

II MHC)、B7 共刺激分子及细胞间粘附分子-1 (ICAM-1)过量表达的影响. 方法：采用干扰素γ (IFN-γ)和肿瘤坏死因子α (TNF-α)联合刺激人肾小管上皮HKC细胞，诱导细胞过度表达class II MHC、B7-1、B7-2和ICAM-1. 同时在细胞培养液中加入不同浓度的雷公藤内酯醇，观察其对上述过程的影响. 采用流式细胞术检测细胞内class II MHC、B7-1、B7-2和ICAM-1等细胞因子的表达，采用逆转录PCR技术检测ICAM-1 mRNA表达的变化. 结果：(1) IFN-γ和TNF-α能显著上调class II MHC、B7-1、B7-2共刺激分子和ICAM-1在人肾小管上皮细胞中的表达. (2) 雷公藤内酯醇剂量依赖性地抑制IFN-γ和TNF-α引起的class II MHC和B7共刺激分子过度表达，但对ICAM-1的过度表达无显著影响. 结论：雷公藤内酯醇能抑制炎症因子引起的人肾小管上皮细胞class II MHC和B7共刺激分子过度表达. 这也许是雷公藤内酯醇在一些免疫相关性肾脏疾病中的作用机制之一.

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