

## Effects of *Tripterygium wilfordii* saponins and interleukin-10 on dendritic cells from human peripheral blood<sup>1</sup>

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**KEY WORDS** *Tripterygium wilfordii*; dendritic cells; cell culture; HLA-DR antigens; CD80 antigens; interleukin-10; interleukin-12; flow cytometry; reverse transcriptase polymerase chain reaction; enzyme-linked immunoabsorbent assay

### ABSTRACT

**AIM:** To study the effects of *Tripterygium wilfordii* (T<sub>II</sub>) and IL-10 on human leukocyte antigen-DR (HLA-DR) and CD80 expressions and IL-12 p40 subunit production and transcription of dendritic cells (DC) in human peripheral blood from healthy volunteers *in vitro*.

**METHODS:** DC were generated by culturing plastic-adherent peripheral blood mononuclear cells with GM-clony stimulating factor (GM-CSF), IL-4, and TNF $\alpha$ . The expressions of HLA-DR and CD80 were examined by flow cytometry after the cells were stained with immunofluorescence antibodies. Enzyme-linked immunosorbent assay and reverse transcriptase polymerase chain reaction analysis were used to detect IL-12 p40 protein level and mRNA transcription, respectively.

**RESULTS:** T<sub>II</sub> 5 - 20 mg/L and IL-10 50 - 200  $\mu$ g/L greatly down-regulated the membrane expressions of HLA-DR and CD80 on DC in a concentration-dependent manner. IL-12 p40 production and mRNA transcription were also inhibited in DC both by T<sub>II</sub> and by IL-10.

**CONCLUSION:** T<sub>II</sub> and IL-10 exert immunosuppressive role via inhibiting membrane expressions of HLA-DR and CD80 and synthesis of IL-12 p40 subunit in DC.

### INTRODUCTION

*Tripterygium wilfordii* (T<sub>II</sub>) is a Chinese traditional immunosuppressive drug which has been used to treat autoimmune diseases and transplantation rejection by inhibiting the function of T cell<sup>[1,2]</sup>. But it is not well known as to how it plays an inhibitory role on T cell function. Interleukin-10 (IL-10) is a potent immunosuppressive cytokine that inhibits T cell response *in vitro* and *in vivo*. This inhibitory effect is related to its actions on antigen presenting cells (APC). It has been reported that IL-10 suppresses the activation of T cell by inhibiting major histocompatibility complex (MHC) and co-stimulatory molecules expressions and cytokines productions of APC<sup>[3-5]</sup>. Dendritic cells (DC) are professional APC that are specialized in initiation of T cell-dependent immune responses. The property of mature DC to act as the most potent APC is due to their high expression of MHC and co-stimulatory molecules and also due to their production of cytokines, especially interleukin-12<sup>[6-8]</sup>. In the present study, we generated DC from human peripheral blood mononuclear cells (PBMC) and then analyzed the effects of T<sub>II</sub>, compared with that of IL-10, on the expressions of relevant surface molecules [human leukocyte antigen-DR (HLA-DR) and CD80] and the production and transcription of IL-12 p40 during the maturation of DC *in vitro*.

### MATERIALS AND METHODS

**Culture medium and reagents** RPMI-1640 (Gibco/BRL) supplemented with penicillin G 100 kU/L, streptomycin 0.1 g/L, and fetal calf serum 10 % (Gibco/BRL) was used as the culture medium. Recombinant human IL-4, IL-10, and TNF $\alpha$  were purchased from Promega Co (USA). Recombinant human GM-clony stimulating factor (GM-CSF) was a generous gift from Prof HOU Yun-De (Institute of Virology, Chinese Academy of Preventive Medicine, Beijing). T<sub>II</sub> was

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purchased from Taizhou Pharmaceutical Factory (purity > 90 %, China). The following monoclonal antibodies (mAb) were used for immunostaining assays: anti-CD1a, anti-CD14, anti-HLA-ABC, anti-HLA-DR (DAKO, Denmark), anti-CD40 (PharMingen, USA), anti-CD80 (Southern Biotechnology, USA), and anti-CD83 (Ansell, USA). FITC-conjugated rabbit anti-mouse Ig was purchased from DAKO.

**Preparation of DC from peripheral blood**  
PBMC of healthy volunteers (Nanjing Blood Center) were isolated by Ficoll-Hypaque, washed twice, and allowed to adhere to 24-well plates (Nunc) at a density of  $3 \times 10^6$  cells per dish in 1 mL of complete culture medium. After 2 h at 37 °C in 5 % CO<sub>2</sub>, the nonadherent cells were removed and the adherent cells were cultured in a complete culture medium containing GM-CSF (200 µg/L) and IL-4 (500 kU/L). Every other day, 300 µL of medium was removed and fresh medium with full dose of cytokines was added. TNFα (10 µg/L) was added to induce maturation of DC on d 7 while T<sub>H</sub> 5, 10, and 20 mg/L, and IL-10 50, 100, or 200 µg/L were added respectively, and cells were harvested on d 9.

**Flow cytometry** DC were washed in PBS and incubated for 30 min at 4 °C with the panel of mAbs listed above. Cells were washed and stained with FITC-conjugated rabbit anti-mouse Ig. The populations were analyzed on the FACScan cytometer (Becton Dickinson).

**Determination of IL-12 p40 protein** Control and T<sub>H</sub>-treated or IL-10-treated DC ( $1 \times 10^6$ ) were cultured in 24-well plates in 1 mL of culture medium. After 3 d, supernatants were harvested for determination of IL-12 p40 levels by enzyme-linked immunoabsorbent assay (ELISA) (ELISA kit purchased from Jingmei Biotech Co, Shenzhen).

**Reverse transcriptase-polymerase chain reaction** RNA was extracted from untreated and T<sub>H</sub> (20 mg/L)-treated or IL-10 (200 µg/L)-treated DC ( $5 \times 10^6$ ) and CV-1 cell line (monkey kidney cell, as negative control) using TRIzol reagents (Gibco/BRL) according to the manufacturer's guidelines. After preparation of cDNA, PCR was performed essentially as previously described<sup>[9]</sup>. Reactions were incubated in a DNA thermal cycler for 35 cycles (denaturation: 1 min, 94 °C; annealing: 1 min, 60 °C; extension: 1 min, 72 °C). β<sub>2</sub>-Microglobulin mRNA amplification was performed on the cDNA as positive control of reaction efficiency. IL-12 p40 primers used were as follows; sense primer 5'-GGA TGC CCC TGG AGA AAT GG-3', antisense

primer 5'-AGG TGG AGG TCA GCT GGG AG-3' (amplified fragment of 655 bp).

## RESULTS

### Generation of human peripheral blood DC

DC were generated by culturing with GM-CSF, IL-4, and TNFα and had typical dendritic morphology and high stimulatory capacity for T cell. DC expressed CD1a (94.2 %) and CD83 (94.2 %), two DC-specific markers, and lost the expression of monocytic marker CD14 (7.9 %). These cells also expressed HLA-ABC (98.3 %) and HLA-DR (96.0 %), CD40 (97.7 %) and CD80 (98.2 %), co-stimulatory molecules involved in T cells activation.

**Expressions of HLA-DR and CD80 on the surface of DC** In the first set of experiments, DC were generated from PBMC cultured in GM-CSF and IL-4 and then stimulated for 48 h by TNFα 10 µg/L in the absence or presence of T<sub>H</sub> and IL-10. The effects of T<sub>H</sub> on the induction of HLA-DR and CD80 were analyzed. After treatment with T<sub>H</sub> at 5, 10, or 20 mg/L, the membrane expression of HLA-DR on DC declined to 56.1 %, 71.2 %, and 80.8 %, and the membrane expression of CD80 on DC declined to 47.9 %, 71.2 %, and 78.0 %, respectively.

IL-10 50, 100, or 200 µg/L greatly decreased the membrane expression of HLA-DR by 46.1 %, 56.3 %, or 75.3 %, respectively, and the expression of CD80 by 42.3 %, 61.1 %, or 73.4 %, respectively. Similar results were observed by repeating the test with the PBMC derived from other two volunteers.

**Production and transcription of IL-12 p40 subunit** DC secreted high levels of IL-12 p40 protein, and the addition of T<sub>H</sub> 5 - 20 mg/L or IL-10 50 - 200 µg/L strongly inhibited the production of this cytokine subunit (Fig 1, 2). DC expressed mRNA for chain of IL-12 p40 in DC control, while little of this expression was detected in DC treated with T<sub>H</sub> 20 mg/L or IL-10 200 µg/L (Fig 3).

## DISCUSSION

Dendritic cells play a critical role in the immune response<sup>[6]</sup>. It is well known that the induction of primary responses relies on DC that have the capacity to express high levels of antigenic and co-stimulatory signals, and to sensitize naive T cells *in vitro* and *in vivo*. Antigens that have entered the endocytic pathway of the DC are

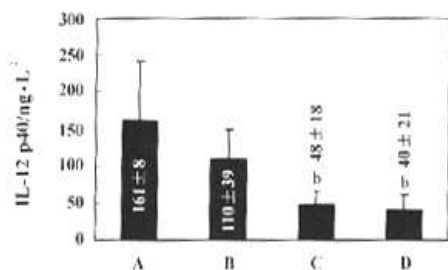


Fig 1. Effects of T<sub>1</sub> on production of IL-12 p40 protein in dendritic cells. (A) 0 mg/L; (B) 5 mg/L; (C) 10 mg/L; (D) 20 mg/L. *n* = 6 independent experiments.  $\bar{x} \pm s$ . <sup>b</sup>*P* < 0.05 vs group A.

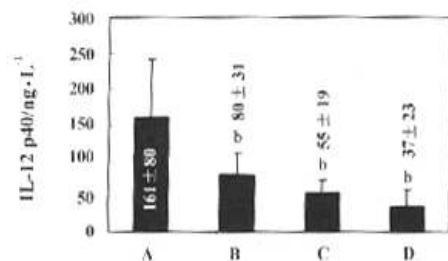


Fig 2. Effects of IL-10 on production of IL-12 p40 protein in dendritic cells. (A) 0 µg/L; (B) 50 µg/L; (C) 100 µg/L; (D) 200 µg/L. *n* = 6 independent experiments.  $\bar{x} \pm s$ . <sup>b</sup>*P* < 0.05 vs group A.

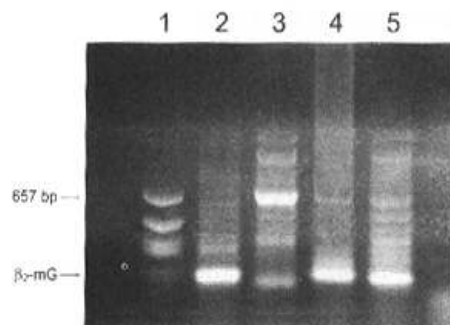


Fig 3. Effects of T<sub>1</sub> and IL-10 on IL-12 p40 mRNA transcription in dendritic cells. 1) Marker; 2) Negative control; 3) Dendritic cell control; 4) T<sub>1</sub> 20 mg/L; 5) IL-10 200 µg/L.

processed there and generally presented by MHC class II molecules to helper T cell. The T cell antigen receptors recognize fragments of antigens bound to molecules of the MHC class II on the surface of DC. In particular,

DC have been shown to up-regulate the expressions of CD80 and CD86 molecules, which provide a co-stimulatory signal upon ligation to CD28 or CTLA-4 on T cell and play a central role in the activation of T cell. T cell are activated when they obtain both the antigenic and co-stimulatory signals. The diminished expressions of MHC class II molecules and co-stimulatory molecules may lead to T cell anergy. This has been confirmed by the gene knock-out mice<sup>(10)</sup>.

As a potent immunosuppressive and anti-inflammatory cytokine, IL-10 is known to interfere with the initiation of human T cell responses. The immunosuppressive properties of IL-10 are mainly related to its effects on APC. As far as antigen presenting cells are concerned, IL-10 has been shown to inhibit CD80 and CD86 expressions on Langerhans cells from mice or on human dendritic cells purified from peripheral blood by the isolation methods without IL-4 and GM-CSF in culture medium<sup>(11,12)</sup>. The results of the present study showed that IL-10 concentration-dependently inhibited the membrane expressions of HLA-DR and CD80 on DC from human PBMC induced by GM-CSF and IL-4.

There are increasing evidences that T<sub>H</sub> is an immunosuppressive drug which inhibits T cell and T cell-mediated delayed-type hypersensitivity. The data presented here indicates for the first time that T<sub>H</sub> greatly down-regulates expressions of HLA-DR and CD80 on the surface of DC in a concentration-dependent manner. T<sub>H</sub> 10 mg/L could induce approximately similar effects to those induced by IL-10 200 µg/L on the HLA-DR and CD80 expressions of DC.

DC spontaneously expresses IL-12, which is a heterodimeric molecule (p35 and p40) that appears to be central in promoting Th1 cell differentiation through induction of IFN- $\gamma$ <sup>(9,13)</sup>. IL-12 p40 is likely to be mainly produced by DC because it can be hardly detected in other APC, ie, macrophages or B cells<sup>(14,15)</sup>. Our results showed that DC secreted IL-12 p40 protein and expressed mRNA for IL-12 p40, and T<sub>H</sub> and IL-10 inhibited production and transcription of the IL-12 p40 subunit. This indicates that T<sub>H</sub> and IL-10 can interfere with T cell differentiation by inhibiting production and transcription of IL-12.

From the above data, we come to the conclusion that T<sub>H</sub>, similar to IL-10, has an important effect on antigen presenting function on DC by down-regulating the membrane expressions of HLA-DR and CD80 and inhibiting the production and transcription of IL-12 p40 by den-

dritic cells. It may be one of the mechanisms by which  $T_H$  is acting as an immunosuppressive drug to treat some autoimmune diseases or transplantation rejections.

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## 雷公藤多甙与白介素-10 对人外周血树突细胞的影响<sup>1</sup>

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**关键词** 雷公藤; 树突细胞; 细胞培养; HLA-DR 抗原; CD80 抗原; 白介素-10; 白介素-12; 流式细胞术; 逆转录聚合酶链反应; 酶联免疫吸附测定

**目的:** 体外研究雷公藤多甙与白介素-10(IL-10)对人外周血树突细胞表面 HLA-DR、CD80 抗原表达及 IL-12 p40 蛋白表达和 mRNA 转录的影响。 **方法:** 采用 GM-CSF、IL-4 和 TNF $\alpha$  体外培养体系获得人外周血树突细胞(DC), HLA-DR 和 CD80 抗原的表达经免疫荧光染色后采用流式细胞仪进行分析, 用酶联免疫吸附测定和逆转录聚合酶链反应分别检测 IL-12 p40 蛋白水平和 mRNA 的转录。 **结果:** 雷公藤多甙 5-20 mg/L 显著下调 HLA-DR 和 CD80 抗原的表达, IL-10 50-200  $\mu$ g/L 能抑制 HLA-DR 和 CD80 抗原的表达, 并都具有剂量依赖关系; 雷公藤多甙 5-20 mg/L 和 IL-10 50-200  $\mu$ g/L 均能抑制 DC 分泌 IL-12 p40 蛋白, 同时经雷公藤多甙 20 mg/L 和 IL-10 200  $\mu$ g/L 处理的 DC 中 IL-12 p40 mRNA 的表达受到显著抑制。 **结论:** 雷公藤多甙和 IL-10 能通过抑制 DC 表面分子的表达和 IL-12 的合成而发挥免疫抑制作用。

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