ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Apr; 20 (4): 353 - 357
http://www.simm.ac.cn; www.chinainfo.gov.cn/periodical

IL-10 and trichosanthin inhibited surface molecule expression of antigen processing cells and T-cell proliferation[1]

FAN Zu-Sen[2], MA Bao-Li
(*Shanghai Institute of Immunology, Shanghai Second Medical University, Shanghai 200025, China*)

KEY WORDS interleukin-10; trichosanthin; antigen-presenting cells; T-lymphocytes; cell division; surface antigens; messenger RNA

ABSTRACT

AIM: To study immunoinhibitory effects and preliminary mechanism of IL-10 and trichosanthin. **METHODS:** Surface molecule expression on antigen processing cells (APC) was stained with fluorescence and analyzed by FACScan. B7-1 mRNA expression was detected with nested RT-PCR. **RESULTS:** IL-10 2 mg·L^{-1} and trichosanthin 10 mg·L^{-1} inhibited B7-1 molecule expression. By contrast, they had not the same effects on ICAM-1. IL-10 and trichosanthin down-regulated LFA-1 expression, but had no regulatory effect on CD40. IL-10 and trichosanthin dramatically inhibited T-cell proliferation and IL-2 production. B7-1 mRNA expression was undetectable in APC treated with IL-10 and trichosanthin. **CONCLUSION:** IL-10 and trichosanthin inhibit surface molecule expression on APC. They exert multiple immunoinhibitory effects.

INTRODUCTION

Antigen processing cells (APC) intake extracellular antigens, process them, and form immune peptides. The immune peptides combined with major histocompatibility complex (MHC) class Ⅱ molecule are presented to T-cells by APC. Many surface molecules on APC interacting with counter-receptors on T-cells provide cellular adhesion and costimulation, which is required for T-cell activation. APC can express many surface molecules, including MHC class Ⅰ and Ⅱ molecules, adhesive and costimulatory molecules, etc[1,2]. These surface molecules on APC play an important role in immunoresponse. IL-10 is an immunosuppressor cytokine produced by a variety of cells. It exerts a crucial role in immunoregulation via controlling the balance between T_h1 and T_h2 cells. IL-10 inhibited antigen presentation by down-regulation B7 expression on APC[3]. Trichosanthin is an effector drug extracted from the root tuber of *Trichosanthes kirilowii* Maxim, which has been used to treat chorio carcinomas and as an abortifacient. Trichosanthin had an antiviral activity and inhibited HIV replication, and improved the symptoms of AIDS[4,5].

This study was to investigate the immunoinhibitory effects of IL-10 and trichosanthin and their preliminary mechanisms, which will provide theoretical evidence for their clinical applications.

MATERIALS AND METHODS

Materials Peripheral blood of healthy volunteers was provided by Shanghai Blood Center; CTLL-2 cell line and EBV-transformed B cell line were stored by our laboratory.

Reagents Tetanus toxoid (TT) was provided by Prof YE Min in Shanghai Institute of Cell Biology, Chinese Academy of Sciences; B7-1 mAb (BB1), ICAM-1mAb (H616), LFA-1mAb (CD11 : $Ts_{1/22}$), and CD40mAb (G28-5) were kindly provided by Dr CA Clark (USA); IL-10 (Genzyme), trichosanthin was provided by Dr HONG Jian; FITC-conjugated

[1] Project supported by Shanghai Municipal Commission of Science and Technology, № 94JC14005.
[2] Correspondence to Dr FAN Zu-Sen, now in *Group 406, State Key Laboratory of Molecular Biology, Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai 200031, China*.
Phn 86-21-6437-4430, ext 252. Fax 86-21-6433-8357.
E-mail aosz@server.shcnc.ac.cn
Received 1998-07-13 Accepted 1998-10-27

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Apr; 20 (4)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

goat-anti-mouse Ig (HuaMei Co); AET (Sigma); PWM, Trizol kit, RT-PCR kit (Gibco).

APC and T-cell isolation SRBC was treated by AET 0.14 mol·L^{-1} at the ratio of (5 – 10) : 1, incubating at 37 ℃ for 20 min. Cells were washed, prepared for 2 % SRBC solution. PBMC freshly isolated by Ficoll-Paque was mixed with 2 % SRBC, incubated at 0 ℃ for 1 h, then seperated APC and T-cells with Ficoll-Paque. The upper layer was APC, the bottom was T-cells and SRBC. T-cells were isolated by lyzing SRBC.

Flow cytometry APC or EBV-transformed B cells were added in 24-well tissue plate (1×10^6/well) and treated respectively with IL-10 (2 mg·L^{-1}), trichosanthin (10 mg·L^{-1}) at 37 ℃ for 24 h. APC were harvested, washed with PBS, and incubated in 5 % FCS RPMI 1640 at 4 ℃ for 1 h with the following murine mAb: B7-1 mAb, ICAM-1 mAb, LFA-1 mAb, CD40 mAb. Cells were washed and stained with FITC-conjugated goat-anti-mouse Ig. Samples were analyzed by FACScan (Becton Dickinson).

Detection of T-cell proliferation and IL-2 activity T-cells mixed with APC treated with TT antigen plus IL-10 (2 mg·L^{-1}) or trichosanthin (10 mg·L^{-1}). Cultured at 37 ℃ for 5 d, cell proliferation was assayed by uptake of [^{3}H]thymidine. For the last 16 h, at the end of the culture, the cells were harvested and [^{3}H]thymidine incorporation was measured. Culture supernatants were collected for IL-2 detection using biological activity method with CTLL-2 cell line.

Analysis of B7-1 mRNA expression with nested RT-PCR APC or EBV-transformed B-cells (5×10^6/well) were treated with IL-10 or trichosanthin at 37 ℃ for 24 h, 48 h, 72 h. Then cells were havested and washed. Total RNA was isolated by Trizol kit. Single-strand cDNA was synthesized using P4 as a primer and PCR was performed as described[6], with the following primers:
P1 133-161 (5′-CCACAAGCTTTGGAGACCCAGGA-ACACCC-3′), P2 59-79 (5′-GTGGAGTCGTACCCT-GAAATC-3′), P3 1249-1281 (3′-GGAAGAATTC-CTCATGATCCCCACGATCCCATG-5′), P4 1358-1379 (3′-AGGAGAGGGATGCCAGCCATCC-5′).

RESULTS

Effects of IL-10 and trichosanthin on B7-1 and ICAM-1 expression of APC IL-10 and trichosanthin inhibited B7-1 expression on APC (Fig 1A). IL-10 and trichosanthin had no evident influence on ICAM-1 molecule (Fig 1B).

Effects of IL-10 and trichosanthin on LFA-1 and CD40 expression of APC IL-10 and trichosanthin had no significant regulatory role in CD40 expression (Fig 1C); By contrast, IL-10 and trichosanthin down-regulated LFA-1 molecules (Fig 1D).

Inhibitory role of IL-10 and trichosanthin in T-cell proliferation and IL-2 production IL-10 and trichosanthin dramatically suppressed TT-induced T-cell proliferation ($P < 0.01$) and IL-2 production ($P < 0.01$) (Tab 1).

Tab 1. Influence of IL-10 and trichosanthin on T-cell proliferation and IL-2 production induced by TT antigen. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **TT control.**

Group	T-cell proliferation/Bq per well	IL-2/kU·L^{-1}
TT control	1498 ± 59	193 ± 21
IL-10	106 ± 9^c	34 ± 5^c
Trichosanthin	77 ± 6^c	37 ± 6^c
T cell control	21 ± 5	24 ± 4

Effect of IL-10 and trichosanthin on B7-1 mRNA expression in APC Freshly isolated APC expressed B7-1 mRNA, PWM and IL-2 enhanced B7-1 mRNA expression. B7-1 mRNA in APC treated by IL-10 and trichosanthin were undetectable. B7-1 mRNA was expressed at peak level for 24 h, and was detectable at 72 h in APC stimulated by PWM and IL-2 (Fig 2).

DISCUSSION

APC, as an immune competent cell, plays an important role in immune response. APC uptake antigens, process them and present them to the surface of APC via binding with MHC class Ⅱ molecules. APC can express a variety of surface molecules, including MHC Ⅰ, Ⅱ, adhesive and costimulatory molecules, etc. T-cell activation requires two signals,

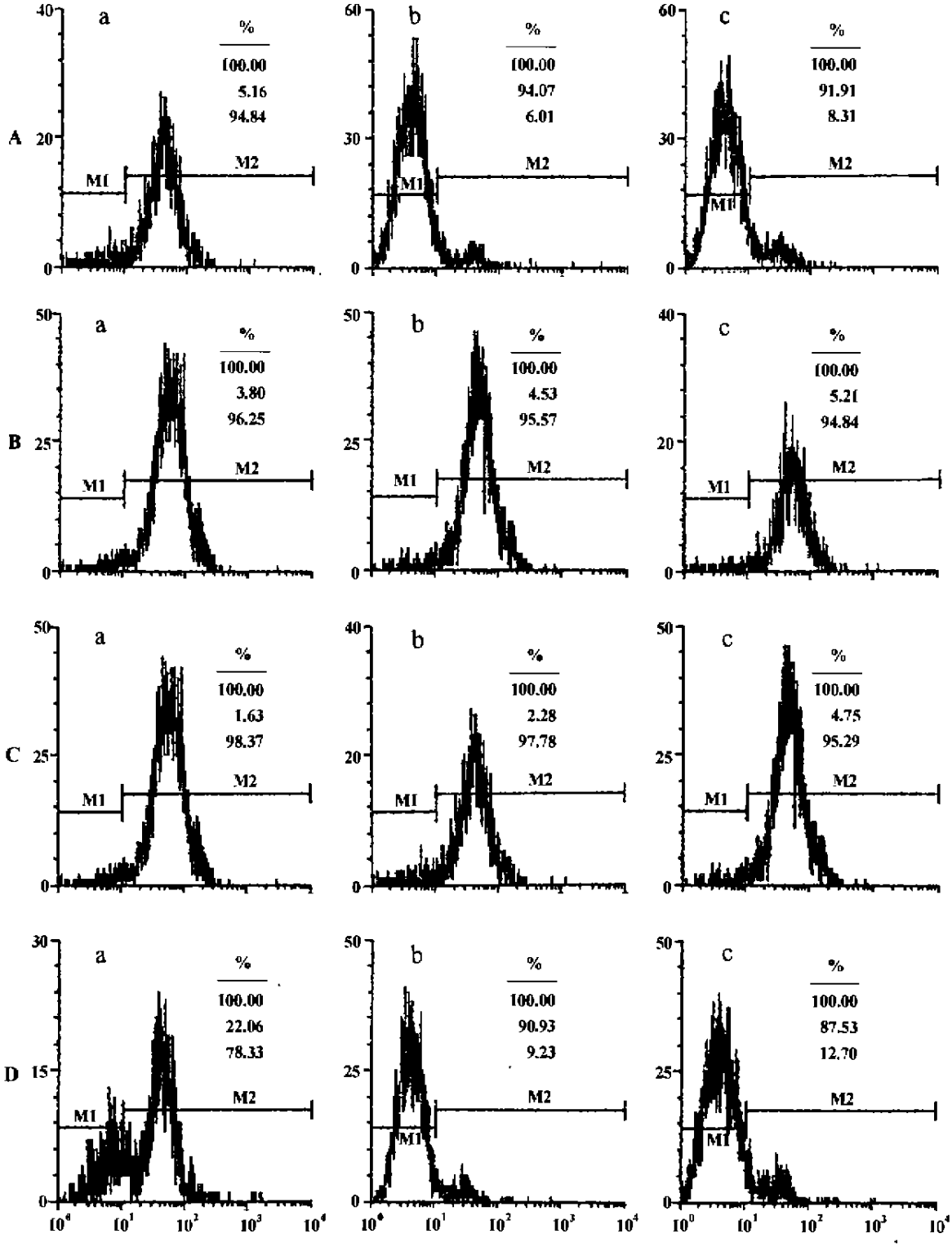

Fig 1. Effects of IL-10 and trichosanthin on B7-1 (A), ICAM-1 (B), CD40 (C), and LFA-1 (D) expression in EBV-transformed B-cells. X axis represents fluorescence. Y axis represents relative cell number. (a) EBV-transformed B cells; (b) IL-10; (c) trichosanthin.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 1999 Apr; 20 (4)
E-mail aps@server.shcnc ac.cn Phn/Fax 86-21-6474-2629

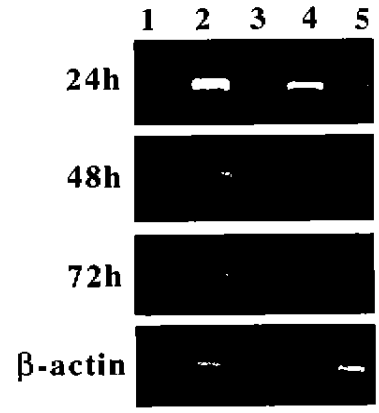

Fig 2. Effects of IL-10 and trichosanthin on B7-1 mRNA expression dynamics in EBV-transformed B-cells. 1) IL-10, 2) PWM, 3) trichosanthin, 4) TT control, 5) EBV-transformed B-cells control.

antigenic and costimulatory signals. Costimulatory signals were provided by APC. Therefore, APC exert a crucial role in T-cell activation. Elevated expression of some surface molecules on APC were closely related to the pathogenesis of some autoimmune diseases such as rheumatoid arthritis[7,8]. Antigenic stimulation can lead to a state of unresponsiveness without appropriate costimulatory signals provided by APC[9]. IL-10 is a potent inhibitory cytokine, it can interfere with antigen presentation by inhibiting expression of some molecules. IL-10 showed a satisfactory therapeutic effect on some animal models of autoimmune diseases as a potent inhibitor[10]. There was evidence that trichosanthin inhibited murine and human lymphocyte proliferation[11]. This study was to elucidate the role of IL-10 and trichosanthin in surface molecule expression on APC and to understand their immunoinhibitory mechanisms.

ICAM-1 and LFA-1 can express on APC and T-cells, which exert both adhesive and costimulatory role during interaction between APC and T-cells[12]. IL-10 and trichosanthin had no evident effect on ICAM-1 expression, but they inhibited LFA-1 molecules. CD40 expresses on APC and belongs to the superfamily of TNF-R. Its receptor is CD40L. CD40/CD40L costimulation participates in B-cell activation and antibody secretion. Blockade of CD40/CD40L pathway can lead to B-cell anergy[2]. IL-10 and trichosanthin had no regulatory effect on CD40, which suggested that IL-10 and trichosanthin may not interfere with CD40/CD40L costimulation.

IL-10 and trichosanthin significantly inhibited B7-1 molecule expression on APC. B7-1 mRNA in APC treated with IL-10 and trichosanthin were undetectable. It indicated that IL-10 and trichosanthin could interfere with B7/CD28 costimulation. Their inhibitory effects derived from gene transcription. B7/CD28 costimulation is a major costimulatory pathway in interaction between APC and T-cell. Blocking B7/CD28 costimulation can lead to T-cell anergy. Its function *in vivo* was confirmed by CD28 gene knock-out mice[13]. Blockade of B7/CD28 *in vivo* can promote symptoms of some autoimmune diseases and prevent transplantation rejection[14,15]. In conclusion, IL-10 and trichosanthin significantly suppressed B7-1 expression, T-cell proliferation, and IL-2 production as well. They had multiple inhibitory effects.

REFERENCES

1 Kooten CV, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells.
Curr Opin Immunol 1997; 9: 330 - 7.

2 VanGool SW, Vandenberghe P, Boer M, Ceuppens JL. CD80, CD86 and CD40 provide accessory signals in a multiple-step T cell activation model.
Immunol Rev 1996; 153: 47 - 82.

3 Ozawa H, Aiba S, Nakagawa S, Tagami B. Interferon-γ and IL-10 inhibit antigen presentation by Langerhans cells for T helper type Ⅰ cells by suppressing their CD80 expression.
Eur J Immunol 1996; 26: 648 - 52.

4 Wang Y, Qian RQ, Gu ZW, Huang HL.
Scientific evalution of Tian Hua Fen-history, chemistry and application.
Pure Appl Chem 1986; 58: 789 - 93.

5 McGrath MS, Hwang KM, Caldwell SE, Gaston I, Luk KC, Wu P, *et al*.
GLQ223, an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage.
Proc Natl Acad Sci USA 1989; 86: 2844 - 8.

6 Wang SY, Huang OH, Chen Z, Ma BL.
Nested RT-PCR method to clone human B7-1 cDNA.
Shanghai J Immunol 1995; 15: 240 - 1.

7 Winhagen A, Newcombe J, Dangond F, Strand C, Woodroofe MN, Cuzner ML, *et al*.
Expression of costimulatory molecules B7-1 (CD80), B7-2 (CD86) and IL-12 cytokine in multiple sclerosis lesions.
J Exp Med 1995; 182: 1985 - 96.

8 Ranhein EA, Kipps TJ. Elevated expression of CD80 and other accessory molecules on synovial fluid mononuclear cell subsets in rheumatoid arthritis.
Arthritis Rheum 1994; 37: 1637 – 43.
9 Schwartz RH.
A cell culture model for T lymphocyte clonal anergy.
Science 1990; 248: 1349 – 56.
10 Fan ZS, Cao RH, Sun WS, Zhang QY, Song J, Ma DL.
The study of IL-10 on treating rat adjuvant arthritis and mechanisms.
Acta Acad Med Shandong 1996; 34: 194 – 7.
11 Ye M, Chi YY, Shen RY, Wang MN. The study of *in vitro* and in *vivo* response to trichosanthin in mouse.
Acta Biol Exp Sin 1986; 19: 81 – 6.
12 Wingren AG, Parra E, Varga M, Kalland T, Sjogren HO, Hedlund G. T cell activation pathways: B7, LFA-3 and ICAM-1 shape unique T cell profile.
Crit Rev Immunol 1995; 15: 235 – 53.
13 Shahinian A, Preffer K, Lee KP, Kundig TM, Kishihara K, Wakeham A, *et al*.
Differential T cell costimulatory requirements on CD28-deficient mice.
Science 1993; 261: 609 – 12.
14 Tivol EA, Schweitzer AN, Sharpe AH.
Costimulation and autoimmunity.
Curr Opin Immunol 1996; 8: 822 – 30.
15 Bolling SF, Lin H, Wei RQ.
Preventing allograft rejection with CTLA4Ig: effect of donor-specific transfusion route or timing.
J Heart Lung Transplant 1996; 15: 928 – 35.

353 ~ 357

白细胞介素-10和天花粉蛋白抑制抗原提呈细胞表面分子表达及T-细胞增殖

R979.5

范祖森，马宝骊 （上海第二医科大学，
上海市免疫学研究所，上海 200025，中国）

关键词 白细胞介素10；天花粉蛋白；
抗原提呈细胞；T-淋巴细胞；细胞分裂；
表面抗原；信使 RNA

目的：探明 IL-10 和天花粉蛋白的免疫抑制作用，为其临床应用提供理论依据. **方法**：APC 表面分子经荧光染色用 FACS 分析结果，用^{3}H-TdR 掺入法测定 T-细胞增殖，巢式 RT-PCR 检测 B7-1 mRNA 的表达. **结果**：IL-10 和天花粉蛋白均能显著抑制 B7-1 分子的表达，两者对 ICAM-1 的表达未显示抑制作用. IL-10 和天花粉蛋白对 CD40 的表达未见影响，但皆抑制 LFA-1 的表达. IL-10 和天花粉蛋白处理过的 APC 未检测到 B7-1 mRNA 的表达. IL-10 和天花粉蛋白都能抑制 T-细胞的增殖（$P < 0.01$）和 IL-2 的产生（$P < 0.01$）. **结论**：IL-10 和天花粉蛋白抑制 APC 多种表面分子表达并抑制 T-细胞增殖.

（责任编辑 周向华）