ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10): 919-923
http://www.chinaphar.com

Tripterygium wilfordii saponins and interleukin-10 prevent induction of experimental autoimmune thyroiditis by dendritic cells[1]

WANG Sheng-Jun[2], XU Hua-Xi, LIU Gong-Zhi
(*Department of Immunology, Zhenjiang Medical College, Zhenjiang 212001, China*)

KEY WORDS *Tripterygium wilfordii*; saponins; interleukin-10; dendritic cells; autoimmunity; thyroiditis; nitric oxide; tumor necrosis factor

ABSTRACT

AIM: To study the roles of *Tripterygium wilfordii* saponins (T_{II}) and interleukin-10 (IL-10) on dendritic cells (DC)-induced experimental autoimmune thyroiditis (EAT). **METHODS**: We used mice as autoimmune thyroiditis model animals and divided them into 4 groups, namely DC group, T_{II} group, IL-10 group, and control group. The level of thyroglobulin (Tg) antibody was assayed by ELISA. TNF_{β} production in the cultured supernatants and nitric oxide (NO) in the serum were measured by biological activation assay and Griess reaction, respectively. Tg-stimulated proliferation of lymphocytes was detected with MTT incorporation assay. The histopathological analysis of thyroid was carried out. **RESULTS**: Tg-pulsed DC were able to induce EAT with increase in the concentration of TgAb in serum and lymphocytes infiltration in thyroid. After treatment with T_{II} or IL-10, DC could not induce EAT with lower levels of TgAb and no lymphocyte infiltration. The concentration of NO in serum, TNF_{β} activation, and the proliferation of lymphocytes in response to thyroglobulin in T_{II} or IL-10 group were lower than those in DC group. **CONCLUSION**: T_{II} and IL-10 are able to strongly inhibit the ability of DC to induce experimental autoimmune thyroiditis.

INTRODUCTION

Dendritic cells (DC) are the most potent antigen presenting cells (APC) and are able to induce primary immune responses both *in vitro* and *in vivo*[1]. DC possess an exceptional capability to capture, process and present self-antigen, and induce autoimmune disease as previously described[2,3]. *Tripterygium wilfordii* saponins (T_{II}) are effective immunosuppressive principles of a Chinese traditional drug used to treat autoimmune disease and anti-transplantation rejection. Interleukin-10 (IL-10), originally described as a molecule that inhibits cytokine production by Th2 cell, has been also shown to inhibit activation and proliferation of T cell clones. These inhibitory effects were indirect and mediated through inhibition of the function of APC[4,5]. Therefore, we investigated the effects of T_{II} and IL-10 on DC inducing the experimental autoimmune thyroiditis (EAT) to provide more evidence for their clinical application.

MATERIALS AND METHODS

Animals and cell line Female CBA mice (Grade II, Certificate № 01-1009 conferred by Beijing Medical Experimental Animal Management Committee), 8-wk old, were purchased from Animal Institute of Chinese Academy of Medicine and divided randomly into 4 groups of 6 each, namely DC group, T_{II} group, IL-10 group, and control group. WEHI 164.13 cells were kindly provided by Beijing Medical University.

Medium and reagents RPMI-1640 (Gibco) was supplemented with 1×10^5 U·L^{-1} penicillin G, 100 mg·L^{-1} streptomycin, and 10 % FCS (56 ℃ for 30 min). T_{II} and IL-10 were purchased from Taizhou Pharmaceutical Factory and Promega Co, respectively. Nitric oxide (NO) assay kit was purchased from Academy of Military Medical Sciences (Beijing, China). DC mAb (33D1) was a generous gift from Prof Steinman RM (Rockefeller University, USA). All reagents were diluted with medium before use.

Preparation of thyroglobulin (Tg) Thyroids obtained from mice were stored immediately at − 70 ℃ until used. The tissues were thawed and finely chopped,

[1] Project supported by the National Natural Science Foundation of China, № 39370364, and Scientific Research Foundation of Sceince and Technology Committee of Jiangsu.
[2] Correspondence to WANG Sheng-Jun.
Phn 86-511-502-5834, ext 2142. E-mail shengjwang@263.net
Received 2000-02-02 Accepted 2000-07-13

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

and the Tg was extracted in 0.01 mol·L^{-1} phosphate-buffered saline (PBS), PH 7.4, at 4 ℃ for 3 h. The supernatant was chromatographed on sephadex G200 column in 0.01 mol·L^{-1} PBS (PH 7.4). Tg peaks were pooled, the protein concentration was determined at wavelength of 280 nm, and aliquots were stored at −70 ℃.

Isolation of DC from spleen The procedure used for isolation of DC from spleen was described previously[6]. In brief, the cells from spleen were prepared in a general method and made into suspension with RPMI-1640. Splenocytes were layered over Ficoll-Hypaque (ρ = 1.080) and centrifuged at 800 × g for 20 min, the mononuclear cells were harvested, washed twice, and allowed to adhere to 24-well tissue plates (5 × 10^6 cells/well). After 2 h at 37 ℃ in 5 % CO_2 the nonadherent cells were removed and adherent cells (mostly DC and a few contaminating Mφ) were retrieved by gentle scraping and subjected to a second round of adherence at 37 ℃ in 5 % CO_2 for 18 h to deplete the contaminating Mφ. Non-adherent cells were pooled as splenic DC and the purity was more than 80 %. Viability of cells exceeded 90 % as determined by trypan blue exclusion.

Injection DC were added in 24-well tissue plates (1 × 10^6 cells/well) and treated with T_{II} (20 mg·L^{-1}) or IL-10 (0.2 mg·L^{-1}) at 37 ℃ for 24 h. Treated or non-treated DC were incubated with Tg (100 mg·L^{-1}) at 37 ℃ in 5 % CO_2 for 18 h, washed with PBS, then were injected into recipient mice once daily for 7 consecutive days. All injections were intravenous in 0.1 mL medium at a concentration of 1 × 10^9 cells/L.

Detection of thyroglobulin antibody (TgAb) in serum Indirect enzyme-linked immunosorbent assay (ELISA) was used.

TNF$_\beta$ activation assay Splenic mononuclear cells were added into 24-well tissue plates at a concentration of 1 × 10^{10}/L medium, together with ConA (Sigma) at a concentration of 5 mg·L^{-1}. Culture supernatants were removed after 48 h and assayed for the activation of TNF$_\beta$.

The MTT tetrazolium cytotoxicity assay was used to measure TNF$_\beta$ activation[7]. WEHI164.13 cells were seeded in 96-well culture microplates (Nunclon) at a concentration of 2 × 10^4 cells/well in 0.1 mL medium. The culture supernatant was added to the target cells. After 48-h incubation at 37 ℃, 10 μL MTT at a concentration of 5 g·L^{-1} in PBS was added and further incubated at 37 ℃ for 4 h. After aspirating 0.1 mL of the supernatant from the wells, 0.1 mL isopropanol with 0.04 mol·L^{-1} HCl was added to all wells. After dissolving the dark blue formazan crystals, the plates were read at wavelength of 570 nm. Individual data points were set up in duplicate. TNFβ activation was expressed with % dead cells.

$$\%\ \text{dead cells} = 100 - \frac{\text{optical density in detected wells}}{\text{optical density in control wells}} \times 100$$

Assay of NO NO concentration was detected by NO assay kit. First, 0.1 mL serum was mixed with 0.1 mL nitrate reductases and 0.1 mL PBS at 37 ℃. After 1 h, 1 mL of Griess reagent was added at room temperature for 10 min and the absorbance (A) was determined at 530 nm. NO level was represented as nitrite level with Griess reaction[8], and was determined with reference to a standard curve of sodium nitrite.

Tg-stimulated lymphocyte proliferation Splenic mononuclear cells (1 × 10^{10} cells/L) were incubated with Tg (20 mg·L^{-1}) for 72 h in 96-well culture microplates. For the last 6 h at the end of the culture, 10 μL MTT at a concentration of 5 g·L^{-1} was put into the wells. Then, acid-isopropanol (0.1 mL of HCl 0.04 mol·L^{-1} in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals and kept for a few minutes at room temperature to ensure that all crystals were dissolved. The amount of reaction production was assessed on an ELISA plate reader at 570 nm. Individual data points were set up in duplicate.

Histopathological analysis Thyroid tissues were freshly removed from mice and fixed in formal saline. The entire thyroid gland was sectioned semiserially for histologic examination of hematoxylin-eosin-stained sections.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analysis of variance was used to analyze significance.

RESULTS

Transfer of autoimmunity using Tg-pulsed DC The autoimmune thyroiditis is characterized by both antibody production to Tg and infiltration of the thyroid by lymphocytes. Tg-pulsed DC caused lymphocyte infiltrates in many recipient mice. Five of 6 mice had lymphocyte infiltration with destruction of thyroid follicular epithelial cells in their thyroids 6 weeks later (Fig 1A). In the experiment described in Fig 2 there were high

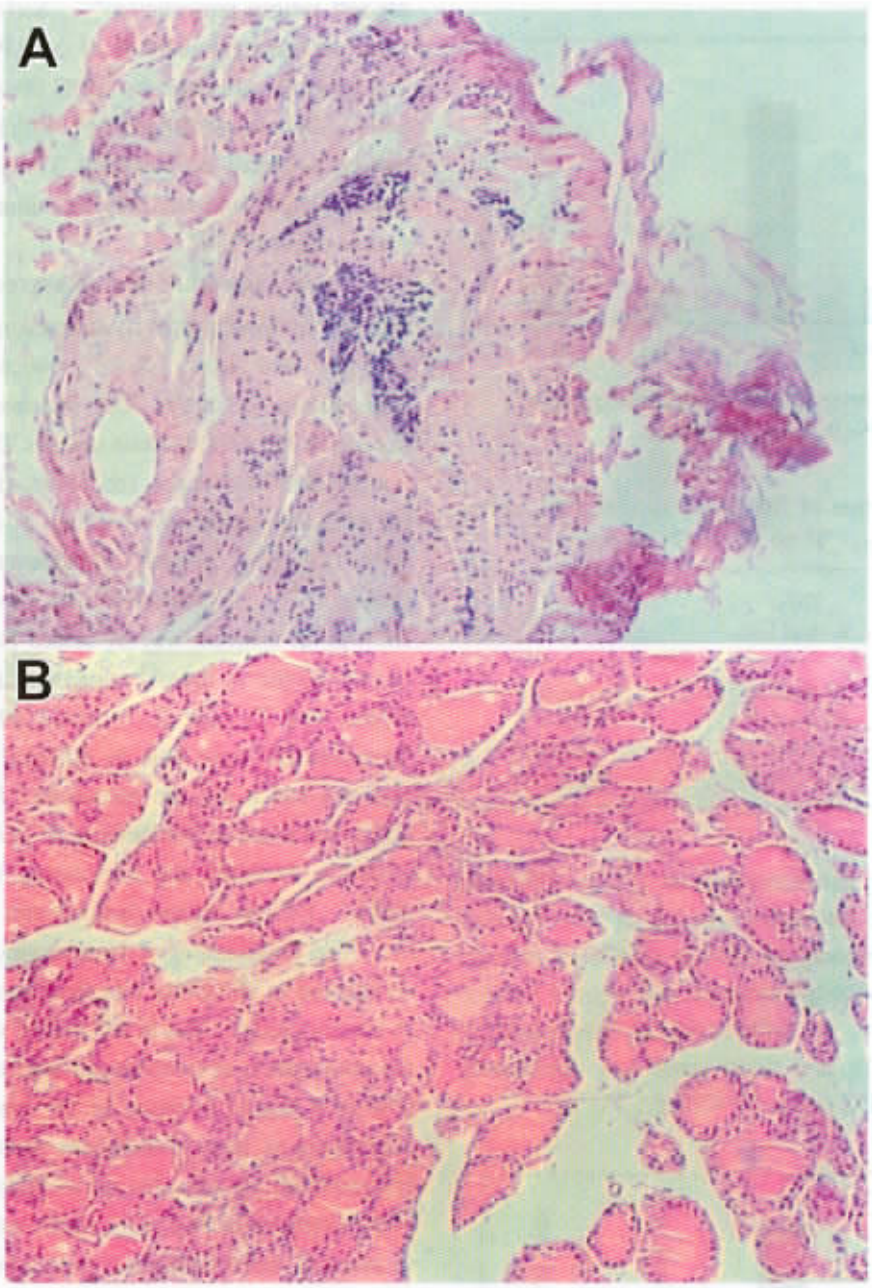

Fig 1. A) Infiltration of lymphocytes with destruction of thyroid follicular epithelial cells (HE stain, ×100). B) Normal thyroid tissue (HE stain, ×100).

levels of antibody to Tg in mice receiving an injection of Tg-pulsed DC.

Inhibition by exposure of DC to T_{II} and IL-10 in induction of EAT When DC were treated *in vitro* with T_{II} or IL-10 before transfer, recipient mice showed no lymphocyte infiltration in their thyroid tissue (Fig 1b). The levels of TgAb in T_{II} and IL-10 group were lower than those in DC group ($P < 0.05$, Fig 2). Thus, after being treated with T_{II} or IL-10, DC could not induce experimental autoimmune thyroiditis.

The concentration of NO in serum and TNF_β activation in different groups DC were able to increase the concentration of NO in serum and activate TNF_β. But when DC were treated with T_{II} or IL-10, the concentration of NO in serum and TNF_β activation

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

markedly decreased ($P<0.05$, Tab 1).

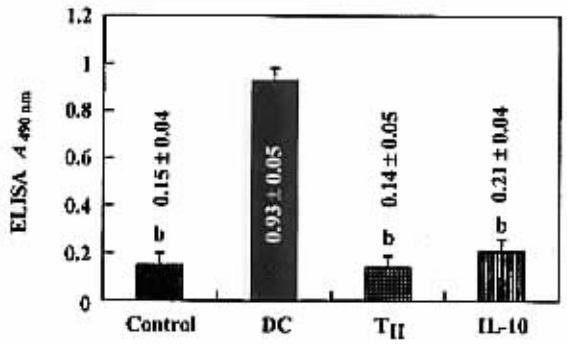

Fig 2. Levels of serum TgAb in different groups. $n=6$. $\bar{x}\pm s$. $^bP<0.05$ *vs* DC group.

Tab 1. Concentration of NO in serum and TNF_β activation. $n=6$. $\bar{x}\pm s$. $^bP<0.05$ *vs* DC group.

Group	TNF_β/ % dead cells	NO/μmol·L^{-1}
Control	20.6±2.6	6.4±2.1
DC	37.3±4.2	15.3±4.1
T_{II}	22.3±3.3^b	7.8±3.5^b
IL-10	23.7±4.8^b	6.2±2.9^b

Lymphocyte proliferation to Tg in different groups It was shown earlier that the *in vitro* lymphocyte proliferative response to Tg was studied in relationship to *in vivo* antibody formation and thyroid infiltration. In this study, the proliferation of lymphocytes to Tg stimulation in T_{II} and IL-10 group was significantly lower than that in DC group ($P<0.05$, Fig 3).

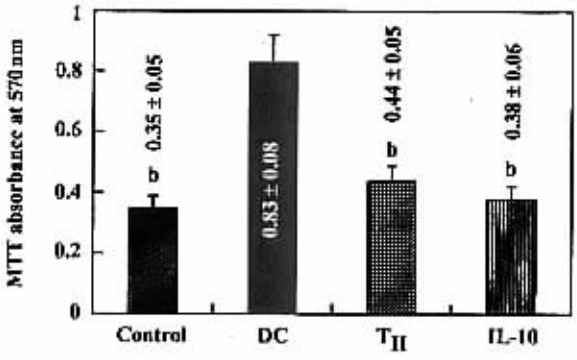

Fig 3. *In vitro* lymphocyte proliferation response to Tg. $n=6$. $\bar{x}\pm s$. $^bP<0.05$ *vs* DC group.

DISCUSSION

In the case of experimental autoimmune thyroiditis, T cell-mediated autoimmunity is the result of inappropriate self-antigen presentation[9]. Self-antigen that has entered the endocytic pathway of the APC is processed there and generally presented by MHC class Ⅱ molecules to T-cells. DC are specialized APC which are able to present antigen to naive or quiescent T-cells and consequently play a central role not only in initiation but also in the maintenance of EAT.

T_{II} is a powerful immunosuppressor that inhibits the function of lymphocytes by suppressing nuclear factor κB (NF-κB) activation. IL-10 produced by Th2 cells has been shown to inhibit Th1 responses. The cytokine skews the Th1/Th2 balance towards Th2 by down-regulation of MHC molecules and of co-stimulation molecules on APC and selectively blocking IL-12 synthesis by the APC that play a role of adjuvant of immune response[10,11]. In this study, we showed that Tg-pulsed DC was an efficient way to induce EAT with infiltration of lymphocyte in thyroid tissue and increase of TgAb in serum, and T_{II} and IL-10 could inhibit this pathway. Tg-pulsed DC were able to efficiently stimulate B-lymphocytes which are the precursors of antibody-secreting cells, and result in the increase of TgAb. T_{II} and IL-10 evidently inhibited the stimulatory role by decreasing TgAb. DC can activate lymphocytes *in vivo* and *in vitro*. The proliferation of lymphocytes in response to Tg stimulation in DC group was significantly higher than that in control group. But in DC treated with T_{II} and IL-10, the proliferation of lymphocytes was markedly decreased. TNF is a central cytokine in the immunoregulation and contributes to many pathologies such as inflammation. NO produced by inducible nitric-oxide synthase has been also recognized as one of the important effector molecules in immune and inflammatory responses. It up-regulates the inflammatory reaction through stimulation of both T- and B-cells, resulting in antibody production and tissue injury as previously described[2,12]. In this study, our results also showed that the concentration of NO and TNF_β activation in DC-induced EAT mice significantly increased. But T_{II} and IL-10 inhibited the function of DC, the concentration of NO and TNF_β activation in T_{II} and IL-10 group were markedly lower than those in DC group.

Generally, these results suggest that T_{II} and IL-10 strongly suppress the function of DC, and therefore prevent induction of adoptively transferred EAT.

REFERENCE

1 Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245 - 52.
2 Wang SJ, Xu HX, Jiang SF, Liu GZ, Liu LT. The effects of dendritic cell in murine experimental autoimmune thyroiditis. Chin J Immunol 1999; 15: 294 - 8.
3 Dittel BN, Visintin I, Merchant RM, Janeway CA Jr. Presentation of the self antigen myelin basic protein by dendritic cells leads to experimental autoimmune encephalomyelitis. J Immunol 1999; 163: 32 - 9.
4 Fiorentino DF, Bond MW, Mosmann TR. Two type of mouse T helper cell. Ⅳ. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989; 170: 2081 - 96.
5 Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, *et al*. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol 1991; 146: 3444 - 51.
6 Xu HX, Liu GZ. Isolation and identification of mouse spleen dendritic cells. Shanghai J Immunol 1991; 11: 129 - 32.
7 Espevik T, Nissen-Meyer J. A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis from human monocytes. J Immunol Methods 1986; 95: 99 - 105.
8 Kiechel FL, Malinski T. Nitric oxide: biochemistry, pathophysiology, and detection. Am J Clin Pathol 1993; 100: 567 - 75.
9 Brown TR, Zhao G, Palmer KC, Sundick RS. Thyroid injury, autoantigen availability, and the initation of autoimmune thyroiditis. Autoimmunity 1998; 27: 1 - 12.
10 Chang CH, Furue M, Tamaki K. B7-1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is down-regulated by interferon-γ or by interleukin-10. Eur J Immunol 1995; 25: 394 - 8.
11 Qin Z, Noffz G, Mohaupt M, Blankenstein T. Interleukin-10 prevents dendritic cell accumulation and vaccination with granulocyte-macrophage colony-stimulating factor gene-modified tumor cells. J Immunol 1997; 159: 770 - 6.
12 Colin IM, Kopp P, Zbaren J, Haberli A, Grizzle WE, Jameson JL. Expression of nitric oxide synthase Ⅲ in human thyroid follicular cells: evidence for increased expression in hyperthyroidism. Eur J Endocrinol 1997; 136: 649 - 55.

雷公藤皂苷和白介素-10抑制树突状细胞诱导实验性自身免疫性甲状腺炎[1]

王胜军[2], 许化溪, 刘恭植
(镇江医学院免疫学研究室, 镇江 212001, 中国)

关键词 雷公藤; 皂苷类; 白介素-10; 树突状细胞; 自身免疫; 甲状腺炎; 一氧化氮; 肿瘤坏死因子

目的: 研究雷公藤皂苷和白介素-10 (IL-10)在树突状细胞(DC)诱导实验性自身免疫性甲状腺炎(EAT)中的作用. **方法**: ELISA法检测甲状腺球蛋白抗体水平, TNF_β和NO分别采用生物学方法及Griess法测定, 淋巴细胞增殖试验采用MTT掺入法. **结果**: DC能诱导EAT的发生, 表现为甲状腺内出现淋巴细胞浸润并伴有血清中TgAb明显升高; 但经雷公藤皂苷和IL-10处理后, DC不能诱导EAT的发生, 表现为甲状腺无病理改变, 血清中TgAb明显低下. 同时, 雷公藤皂苷组和IL-10组小鼠TNF_β活性、NO浓度和淋巴细胞增殖能力显著低于树突状细胞组($P<0.05$). **结论**: 雷公藤皂苷和IL-10能显著抑制DC诱导EAT的发生.

(责任编辑 吕 静)