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Research Paper

Effect of IL-10 gene transmission on the PTg-stimulated splenocytes proliferation and Th1 cytokines production from experimental autoimminue thyroiditis rats☆

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Abstract

Objective: To investigate the effects of gene therapy with IL-10 on PTg-induced proliferation of splenocytes and Th1 cytokine production from PTg-stimulated splenocytes. **Methods**: EAT rats were divided into four groups: group A (PBS+PLL), group B (pORF+PLL), group C (pORFmIL10+PLL), and group D (pORFmIL10+ MEM). The substances mixed with lipofectamine were injected into the thyroid tissues of rats on the 18th dday after immunization. The rats were sacrificed at the 8th week. *In vitro* proliferative responses to ConA and different concentration of PTg were measured by culturing 4×105 splenocytes pulsed with 18.5KBq of [^{3}H] thymidine for the final 12h and then harvested for liquid scintillation counting. In vitro splenocytes were cultured with PTg (25 mg/L). Th1 cytokine IFN-γ, TNF-αand IL-2 were detected by ELISA. **Results**: The proliferative response to PTg was suppressed in group C, compared with that of group A and B ($P < 0.05$). The levels of IFN-γ, TNF-αand IL-2 in the supernatant of PTg-stimulated splenocytes were 3548.25 ± 779.47 pg/ml, 27.66±10.50 pg/ml and 3617.73± 609.15 pg/ml, respectively, which were much lower in group C than those in group A and B($P < 0.01$, $P < 0.05$, $P < 0.001$, respectively). **Conclusion**: IL-10 gene transmission in thyroid tissues could inhibit PTg specific proliferation of splenocytes from EAT rats and the secretion of Th1 cytokines from PTg-stimulated splenocytes.

Keywords: interleukin-10, autoimmune thyroiditis, lymphocytic proliferation, autoimmunity

INTRODUCTION

Autoimmune lymphocytic thyroiditis (LT) is a common organ special autoimmune disease. It is a common cause of primary hypothyroidism, with some coexiting complications such as dyslipidemia, cardiomyopathy, osteoporosis, and so on. But it is difficult to investigate early stages of thyroiditis in humans, because surgical specimens mostly reflect the autoimmune disease at a late stage. Experimental autoimmune thyroiditis animals were used as human lymphocytic thyroiditis model with some characters such as increased TGAb, pathology changes like human autoimmune thyroiditis in thyroid tissues[1-2].

The representation of experimental autoimmune thyroiditis (EAT) depends on the balance between regulative and effective T cells, including the balance between Th1 and Th2 profiles. Th1 cytokines such as IL-2 and IFN-γ may aggravate the disease[3], however Th2 cytokine IL-10 and IL-4 may exert antiinflammation function and ameliorate thyroidits. IL-10 as an important negative regulative factor can inhibit the transformation of Th0 to Th1, hold back transference of inflammatory cells and reduce production of Th1 cytokines, so to maintain the balance in cellular immunity.

MATERIALS AND METHODS

Reagents and instruments

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pORFmIL10 plasmid(Invivogen company), lipofectamine (Gibco, America), poly-l-lysine(Sigma, America), Opti-MEM Reduced Serum Medium (Gibco, America), porcine thyroglobulin (Sigma, America), complete Fraund's adjuvant (CFA) and incomplete Fraund's adjuvant(IFA) (Sigma, America), NaI (Amresco, America), MAXIPREP GFI-ITM Endo-Free Kit (Q.BIO gene), ConA (CNI, America), IFN-γELISA kit (DIACLONE, French), TNF-αELISA kit (Bender MedSystems, Austria), IL-2 ELISA kit (BIOSOURCE, America). Liquid scintillation counting machine (1219RackBeta LKb Wallac).

Animals

SPF female Wistar rats, weighing 60~80 g, were purchased from Shanghai Laborotory animal center Chinese Academy of Science. License:[SCXK(Hu)(No2002-0010,China)], environment and quality certificate:[SYXK(Su)(No2002-0013,China)]. Experimental LT animals were divided into four groups: group A(PBS+PLL), group B (pORF plasmid+PLL), group C (pORFmIL10 plasmid +PLL), group D (pORFmIL10 plasmid).

EAT animal model

The animals were immunized with subcutaneous injuection of 100 μg PTg emulsified with CFA. Then the immunization was enhanced with injection of 100 μg PTg emulsified with IFA at the 2nd,3rd, 4th week. All rats were fed with 0.05% NaI. Thyroidectomy was carried out at the 8th week.

Plasmid preparation

The transmission, reproduction, purification were carried out according to the MAXIPREP GFII™ Endo-Free Kit protocol. Plasmid or PBS (10 μl) were mixed with PLL2.5 μg/10 ul opti-MEM at room temperature for 30 minutes, then added 30 μg lipofectamine/15ul for 15 minutes. Plasmid were injected into the thyroid tissues on the 18th day.

In vitro proliferative responses of splenocytes to PTg

Splenocytes were isolated from rats at the 8th week, in vitro proliferation were measured by culturing 4×10^9/ L splenocytes incubated with ConA and different concentration PTg in 100μl for 72 h. Cells were pulsed with 18.5KBq of[^{3}H]-TdR for the final 12 h, then harvested for liquid scintillation counting.

In vitro cytokine responses of splenocytes to PTg

Splenocytes were isolated from rats at the 8th week, in vitro cytokine responses were measured by culturing 4×10^9/L spleen cells incubated with 25 mg/L PTg for 72 h. Supernatant from parallel cultures harvested at 72 h, IL-2,TNF-α and IFN-γ were detected by ELISA.

Statistics

Data were expressed as mean ±SD. Differences were considered statistically significant as analyzed by ANOVA test.

RESULTS

Pathologic Thyroids were moved for histologic analysis at the 8th week after primary immunization. Normal thyroid had integrity follicles engorged with colloid substance(***Fig. 1***). Dramatic lymphocytic infiltration was observed in thyroids of experimental autoimmune thyroiditis rats (***Fig. 2***).

Effect of IL-10 on proliferative responses of splenocytes to PTg

When splenocytes were incubated with PTg, the degree of proliferative response to PTg was more suppressed in group C than that in group A and B($P < 0.05$). There was no different responses to ConA between each group. (***Table 1***).

Effect of I L-10 on cytokine production of splenocytes to PTg

The levels of IFN-γ,TNF-αand IL-2 in the supernatant of PTg-stimulated splenocytes were 3548.25 ± 779.47 pg/ml, 27.66 ± 10.50 pg/ml and 3617.73 ± 609.15 pg/ml, respectively, which were much lower

Table 1 **Proliferative responses to PTg-stimulation (SI) in splenocytes co-incubated with ConA and different concentration PTg for 72 h.**

Groups	PTg 25 mg/L	PTg 50 mg/L	PTg 200 mg/L	ConA 5 mg/L
A	68.50 ± 16.41	79.48 ± 14.42	193.11 ± 39.90	70.78 ± 20.40
B	59.23 ± 10.57	77.95 ± 18.13	173.18 ± 53.37	62.65 ± 15.48
C	29.74 ± 3.38*	38.37 ± 4.71*	50.17 ± 7.59*	59.25 ± 8.48
D	50.54 ± 11.82	72.32 ± 22.10	128.10 ± 47.14	57.73 ± 9.61

Compared with group A and B, *$P < 0.05$

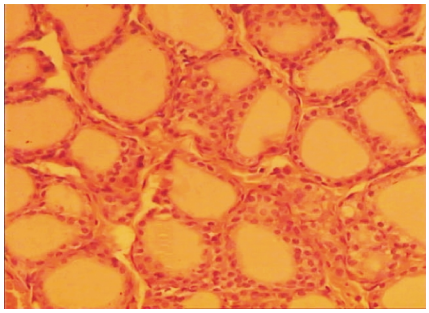

Fig. 1 Normal thyroid (HE × 200)

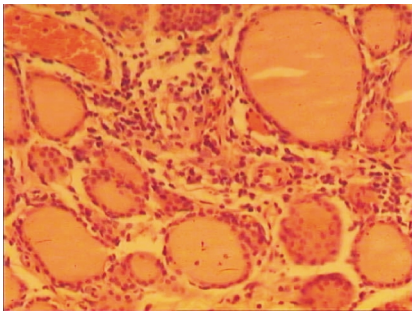

Fig. 2 Experimental autoimmune thyroiditis (HE × 200)

in group C than those in group A and B ($P < 0.01$, $P < 0.05$, $P < 0.001$, respectively) (***Fig. 3***,***Fig. 4***, ***Fig. 5***).

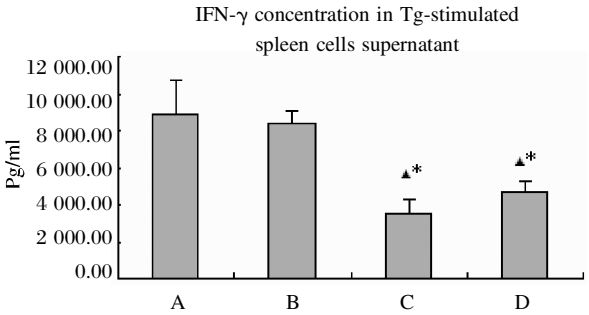

Fig. 3 IFN-γ production by PTg-activated splenocytes from rats, comparison with group A and B, $^{▲}P < 0.01$; $^{*}P < 0.05$.

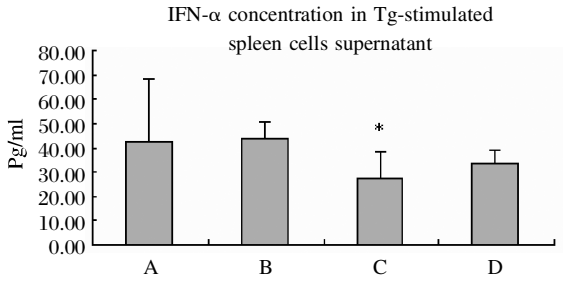

Fig. 4 TNF-α production by PTg-activated splenocytes from rats, comparison with group A and B, $^{*}P < 0.05$.

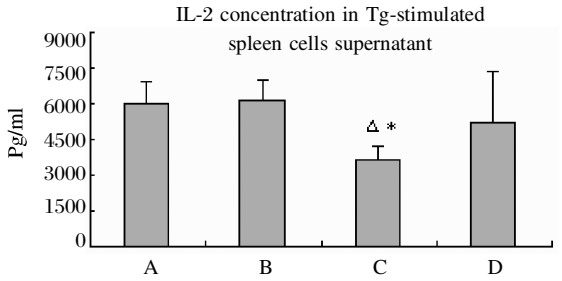

Fig. 5 IL-2 production by PTg-activated splenocytes from rats, comparison with group A and B, $^{△}P < 0.001$; comparison with group D, $^{*}P < 0.05$.

DISCUSSION

Hashimoto's thyroiditis is categorized as a T cell-mediated organ special autoimmune disorder. Experimental autoimmune thyroiditis produced in rats by immunization with thyroglobulin plus Freund's adjuvant represents a valuable model for studying the pathogenesis of Hashimoto's thyroiditis, which is characterized both by thyroid infiltration with mononuclear cells and by elevated circulating thyroglobulin antibodies. The immune response is specific for thyroglobulin, both in terms of antibody response and T-cell proliferation. The animal model provides an opportunity for investigating in detail the mechanisms by which an environmental agent can trigger a pathogenic autoimmune response. Cytokines play a crucial role in immunoregulation and pathology of experimental autoimmune thyroiditis[4]. EAT is a Th1 cell dependent abnormal immune condition[5].

As anti-inflammatory cytokine, IL-10 belongs to 4α-helix cytokine family. It may exert a curative effect in some disease such as type 1 diabetes, experimental allergic encephalomyelisis, inflammatory bowel disease. It also exerts key regulating function on development of tissue specific autoimmune injury such as lymphocyte thyroiditis. The mechanism of its modulation function in autoimmune thyroiditis includes: ① Inhibiting proinflammation factors such as: IFN-γ, TNF-α, IL-1, IL-2, IL-12 and chemotatic factors produced by T cell and macrophages; ② Inhibiting the expression of MHC-II molecules and some costimulating factors B7 on antigen presenting cells; ③ Inhibiting the function of macrophages and NK cells; ④Inhibiting differentiation of Th0 to Th1 and transference of inflammation cells.

Systemic and local administration of IL-10 has a curative effect on EAT [6]. Some studies have proved that IL-10 may alleviate infiltration of lymphocytes in thyroid tissues and decrease serum autoantibodies[7-8].

To avoid side effects of high dose and frequent use of cytokine, we devised an method of gene therapy to deliver pORFmIL10 plasmid DNA encoding IL-10 into the inflamed thyroid to investigate the immunorepressive function of the Th2 cytokine. Our data showed that IL-10 decreased rat serum autoantibodies TmAb, TgAb. These results were paralleled by significantly decreased proliferative response of splenocytes to PTg and reduced production of Th1 cytokines from splenocyte response to PTg *in vitro*.

The results showed that Tg-responsive immune disorder had a pathogenic role in the development of experimental autoimmune thyroid disease. In vitro studies strengthened our demonstration that IL-10 might suppress the specific immune response to LT-induced antigen. Local IL-10 gene therapy with lipofectamine-PLL complexes induced faster and more long-lasting expression of IL-10 than lipofectamine alone on rat thyroid follicular cells, and might be a curative treatment of EAT in rats.

The low amount of cytokines produced locally would probably minimize the side effects after high dose systemic injection. Non-viral vector gene transmission was a safe and effective method avoiding the risk of gene mutation[9]. In conclusion, local IL-10 gene therapy using non-viral vectors is a novel and promising approach for the treatment of thyroid autoimmune disorders that could be applied in the future.

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