Effects of the polysaccharide nucleic acid fraction of bacillus Calmette-Guérin on the production of interleukin-2 and interleukin-10 in the peripheral blood lymphocytes of patients with chronic idiopathic urticaria

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Abstract. Urticaria is one of the most frequent dermatoses and its prevalence in the general population is estimated to be ~20%, whereas a substantial percentage of the cases may be classified as chronic idiopathic urticaria (CIU). The inflammatory response presenting with spontaneous wheals exhibits pro-inflammatory characteristics, involving a prominent role for lymphocytes with a mixed Th1/Th2 response in which interleukin (IL)-2 and IL-10 are prominently secreted by Th1 and Th2 cells, respectively. In CIU patients, it was demonstrated that IL-10 production was elevated and IL-2 reduced compared to controls. Therefore, inhibition of IL-10 and promotion of IL-2 production by the lymphocytes, indicating Th2 inhibition and Th1 promotion, may facilitate the treatment of CIU. Whether the polysaccharide nucleic acid fraction of bacillus Calmette-Guérin (BCG-PSN), which possesses multiple immunomodulatory properties, has that potential, remains to be elucidated. In this study, BCG-PSN was used on lymphocytes isolated from CIU patients, with healthy donors used as controls. Immunocytochemistry and ELISA were used to detect IL-2 and IL-10 production. It was demonstrated that the IL-2 production by the lymphocytes in the CIU group was significantly lower compared to that in the healthy control group and it increased sequentially with the increase of the concentration of BCG-PSN used. By contrast, the IL-10 production by the lymphocytes in the CIU group was significantly higher compared to that in the healthy control group and decreased sequentially with the increase of the concentration of BCG-PSN used. Thus, it may be concluded that the BCG-PSN has the potential to promote IL-2 and inhibit IL-10 production in the lymphocytes of CIU patients, facilitating the treatment of CIU.

Introduction

Urticaria is a skin disorder presenting with localized edema due to vasodilatation and increased permeability of the small blood vessels in the mucosae and skin. The chronic form is defined as symptoms lasting for a minimum of 6 weeks. Chronic urticaria (CU) is characterized by recurrent, transitory, itchy wheals, which occur daily or almost daily (1). Several conditions are associated with allergy and autoimmune disorders, as well as urticaria, in which the clinical symptoms may be attributed to the release of histamine and other vasoactive mediators induced by the binding of a specific allergen to the IgE antibodies conjugated to the mast cell surface receptors (2,3). However, in a substantial percentage of cases, the allergic trigger cannot be determined and the urticaria is classified as idiopathic (CIU), whereas ~35-40% of cases appear to be of autoimmune origin (4,5). The inflammatory response presenting with spontaneous wheals exhibits pro-inflammatory characteristics, involving a prominent role for lymphocytes with a mixed Th1/Th2 response (6). It was demonstrated that IL-10 production was elevated and IL-2 reduced in CIU patients compared to controls (7).

Certain natural products, such as the polysaccharide nucleic acid fraction of bacillus Calmette-Guérin (BCG-PSN), may possess immunomodulatory properties (8-10). BCG-PSN may increase CD4+ T cells and induce Th1-type immunity (11) and, by contrast, restrain Th2-type immune response, switching the balance of Th1/Th2 towards the Th1 side (12-15). A recent study by Luo et al (16) demonstrated that BCG priming and...
boosting twice with the AMM vaccine induced a potent antigen-specific interferon (IFN)-γ and IL-2 production.

Th1 and Th2 are involved in the pathogenesis of CIU, whereas IL-2 and IL-10 are prominent cytokines secreted by Th1 and Th2 cells, respectively. This study was designed to investigate whether the effects of BCG-PSN on the Th1/Th2 balance, determined by its effects on IL-2 and IL-10 production by the lymphocytes of CIU patients, may facilitate the management of CIU.

**Materials and methods**

** Subjects.** This study included 30 CIU patients (15 males and 15 females), aged 18-60 years (average, 38.13±12 years), recruited in The Affiliated Hospital of Chengde Medical College (Chengde, China). All the patients met the following selection criteria: age 18-65 years, sharply defined skin wheals persisting for ≥6 weeks, small (<1 cm) to large (>8 cm), erythematous or white wheals with an erythematous rim,
round, oval, acriform, annular or serpiginous, due to confluence and resolution in one area and progression in another, without definite provocation, pruritic and transient. Patients during pregnancy or lactation, with a history of autoimmune disease or administration of antihistamine drugs, glucocorticosteroids or immunomodulating drugs within the 4 weeks preceding enrollment, were excluded. The 30 controls were healthy donors without any history of allergies.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of our hospital. All the participants provided written informed consent prior to enrollment.

**Immunocytochemistry.** Human peripheral blood mononuclear lymphocytes were collected from the venous blood of the subjects by Ficoll-Paque gradient centrifugation. The lymphocytes were adjusted to a final concentration of 2x10⁶/ml in RPMI-1640 medium with 10% fetal bovine serum. The lymphocytes in the RPMI-1640 medium (100 µl, 2x10⁶/ml) were added to a 96-well culture plate, in which 10 µl of phytohemagglutinin (2 mg/ml) and differently diluted (0, 5, 10, 20, 40 and 80 µl/ml) of BCG-PSN (0.35 mg BCG polysaccharide with ≥30 µg/l nucleic acid) had been previously added. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂ for 96 h. BCG-PSN was obtained from Zhejiang Wanma Pharmaceutical Co., Ltd., Hangzhou, China.

The culture supernatants were collected for ELISA, whereas the cells were smeared on slides and fixed with cold acetone for 5 min. The endogenous peroxidase activity was inactivated with 3% H₂O₂ for 20 min and blocking was performed for 30 min in 10% calf serum. Cells were incubated overnight at 4°C with the primary antibody against IL-2 (Santa Cruz Biotechnology Inc., Dallas, TX, USA) or IL-10 (Santa Cruz Biotechnology Inc.), rinsed and incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody for 30 min at 37°C, followed by visualization with diaminobenzidine and counterstaining with hematoxylin prior to mounting. Replacement of the primary antibody with phosphate-buffered saline was used as negative control. The slides were observed under a light microscope. The expression of IL-2 and IL-10 was identified in the cytoplasm and cell membrane as brown colour. The number of positive cells in five separate fields was calculated with a balanced cell number on every slide.

**ELISA.** The ELISA was performed using the ELISA kit (NeoBioscience, Shenzhen, China) according to the manufacturer's instructions. In brief, the 96-well flat-bottom plate was precoated with anti-human IL-2 or IL-10 antibody. The diluted standards and samples were added to each coated well (100 µl/well) and incubated at 36°C for 90 min. After 5 washes, biotin-conjugated anti-human IL-2 or IL-10 antibody was added to each well and incubated at 36°C for 60 min. After an additional 5 washes, 100 µl of HRP-conjugated avidin was added to each well and incubated at 36°C in the dark for 30 min. The plate was washed 5 times and 100 µl of the tetramethylbenzidine solution was added to the wells, followed by incubation at 36°C in the dark for 15 min. The reaction was stopped with 100 µl of 2 M sulphuric acid. The optical density was measured at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). The concentrations of IL-2 and IL-10 (pg/ml) were determined with a standard curve derived from a known amount of the relevant cytokines. The minimum detection level was 8 pg/ml for IL-2 and 1 pg/ml for IL-10.

**Statistical analysis.** The differences between the experimental and control groups were compared with the Pearson's Chi-square test for categorical variables or one-way ANOVA followed by the Dunnett's t-test for continuous variables. The results are presented as percentages for categorical variables, or means (± SD) for continuous variables. P<0.05 indicated a statistically significant difference.

**Results**

**Effects of BCG-PSN on IL-2 production by the lymphocytes of CIU patients.** IL-2 is a prominent cytokine secreted by Th1 cells; therefore, it may reflect Th1 function. It was demonstrated by immunocytochemistry that the highest percentage of IL-2-positive lymphocytes was observed in the healthy control group, the lowest in the untreated CIU group (0 µg/ml BCG-PSN) and there was a sequential increase with the elevation of the BCG-PSN concentration (Fig. 1).

It was also demonstrated by ELISA that the levels of IL-2 in the culture supernatant of the lymphocytes were the highest in the healthy control group, the lowest in the untreated CIU group (0 µg/ml BCG-PSN) and there was a sequential increase with the elevation of the BCG-PSN concentration (Fig. 2).

**Effects of BCG-PSN on IL-10 production by the lymphocytes of CIU patients.** IL-10 is a prominent cytokine secreted by Th2 cells; therefore, it may reflect Th2 function. It was demonstrated by immunocytochemistry that the lowest percentage of IL-10-positive lymphocytes was observed in the healthy control group, the highest in the untreated CIU...
group (0 µg/ml BCG-PSN) and decreased sequentially with the increase of the BCG-PSN concentration (Fig. 3). It was also demonstrated by ELISA that the levels of IL-10 in the culture supernatant of the lymphocytes were the lowest in the healthy control group, the highest in the untreated CIU group (0 µg/ml BCG-PSN) and there was a sequential decrease with the increase of the BCG-PSN concentration (Fig. 4).

**Discussion**

CU causes patients significant physical and mental discomfort. CU has been defined as a daily or almost daily occurrence of wheals and/or angioedema, occurring over a period of ≥6 weeks (17-19). Furthermore, the direct cost of CU, in terms of healthcare visits, investigation and treatment, is high (20). Several aetiological factors have been associated with the onset
In this study, it was demonstrated that the secretion of IL-2 was increased while that of IL-10 was decreased in the peripheral blood lymphocytes of patients with CIU following treatment with BCG-PSN at different concentrations in vitro for 96 h. In addition, with the increase of drug concentration, the levels of IL-2 and IL-10 were more efficiently restored, suggesting that BCG-PSN may restore the Th1/Th2 imbalance in patients with CIU.

References


