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ORIGINAL ARTICLE

Differential Th1/Th2 balance in peripheral blood lymphocytes from patients suffering from flea bite-induced papular urticaria

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KEYWORDS
Papular urticaria;
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Intracellular cytokine;
Skin allergy;
Ectoparasite

Abstract
Background: The Th1/Th2 balance has not been characterized in patients suffering from flea bite-induced papular urticaria (FBPU). Our aim was to improve understanding of the immunopathogenesis of CD4+ and CD8+ T-cells in humans suffering from flea bite-induced papular urticaria.
Methods: Peripheral blood mononuclear cells were obtained from 18 pediatric patients and 10 age-matched healthy controls. Cellular phenotypes, intracellular production of interferon gamma (IFNγ) and interleukin-4 (IL-4) in T-cells stimulated with polyclonal stimuli was determined by flow cytometry following short-term *in vitro* stimulation.
Results: The results revealed lower frequencies of IFNγ-secreting ($p = 0.02$) and higher frequencies of IL-4-secreting ($p = 0.03$) CD4+ T-cells in patient lymphocyte cultures compared to healthy control cultures in the presence of polyclonal stimuli. This is the first description of differential cytokine patterns in papular urticaria patients.
Conclusion: Patients suffering from papular urticaria have an atopic status compared to healthy children.

Introduction

Allergic diseases are characterized by a complex immune response that is predominantly related to the secretion of Th2-associated cytokines. These cytokines are responsible for inducing and maintaining allergic inflammation[1,2].

Papular urticaria has been characterized as a chronic allergic disease, manifesting itself as hypersensitive reactions on the skin caused by exposure to ectoparasites, such as fleas[3]. Previous studies concerning the immunological mechanisms involved in this disease have shown large eosinophil infiltrates in biopsies from patients' cutaneous lesions[4]. Moreover, it has been shown that simultaneous exposure of patients' dendritic cells to flea antigens and lipopolysaccharide induces increased expression of the CD86 co-stimulating molecule that is associated with the induction of a Th2 re-

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sponse[5]. Humoral immunity studies have revealed greater protein recognition in flea extract by IgG1 and IgG3 from healthy individuals and by IgE from patients with papular urticaria (unpublished data).

The presence of eosinophils in skin lesions and differential antigenic recognition by some antibody isotypes led to the hypothesis that patients suffering from flea bite-induced papular urticaria (FBPU) would have a Th2-dominant response, as has been described for other allergic diseases. The objective of the current work was to identify cytokines secreted by CD4+ and CD8+ lymphocytes (IFNγ and IL-4) as indicators of Th1 and Th2 responses, respectively, after stimulation with a polyclonal stimuli.

Materials and Methods

Study population

The sample included 18 patients, aged one to 13, who had been clinically diagnosed as suffering from FBPU. These patients were attended by the Pediatric Dermatology and Allergy Department of the Fundación Santa Fe de Bogotá, in Bogotá, Colombia. Patients excluded from consideration for participation in the study included those having secondary infected lesions or suffering from immuno-suppression caused by systemic disease, as well as those who had been treated with immuno-suppressive medication; those who had received antihistamines 5 days before the consultation, and/or who had been treated with flea extract. Ten healthy children admitted to the same institution for elective surgery and sharing the same age group and socioeconomic characteristics as the FBPU patients were included as controls. The investigation was approved by the ethics committees at both the Fundación Santa Fe de Bogotá and the Universidad Javeriana.

Disease diagnosis

FBPU was diagnosed according to clinical characteristics. Patients usually had lesions appearing as grouped papules, a symptom commonly associated with pruritus. Papules were often excoriated or crusted, appearing intermittently in a chronic course and leaving hypo- or hyper-pigmented macules behind. These were most often located in areas where clothing fits snugly such as the socks and the waist-band. In some patients, exposed areas of the extremities were also affected.

PBMC isolation and lymphocyte stimulation

Peripheral blood mononuclear cells (PBMC) were prepared from fresh heparinised blood using a Ficoll-hypaque density gradient (Sigma, St. Louis, MO). The cells were adjusted to $2x10^6$ cells in a final 2 ml volume of RPMI 1640 medium containing antibiotics, non-essential amino acids, sodium pyruvate, and 10% FCS. PBMC were incubated with 1 μg/ml of anti-CD28 and anti-CD49d. A positive control of 3.7 μg/ml of staphylococcal enterotoxin B (SEB) was used[6]. A negative control (no stimulus) was included in every experiment. The cultures were incubated for 9 h at 37 °C in 5% CO_2, followed by an additional 3 h incubation in the presence of 10 μg/ml Brefeldin A (Sigma). Surface staining was performed with anti-CD3-FITC, anti-CD4-PerCP or anti-CD8-PerCP, anti-CD69-APC (BD Pharmingen); after fixation and permeabilisation, the cells were then stained with IFNγ-PE or IL-4-PE (BD Pharmigen). Data were acquired and analysed using a FACSCalibur flow cytometer (BD Immunocytometry Systems) and Cell Quest software.

Quantifying cytokine levels in plasma

Cytokines were quantified in plasma using a Cytometric Bead Array kit (BD Biosciences), following the manufacturer's instructions. Beads displaying different PerCP fluorescence intensities were used for the quantification; they were coated with PE-conjugated capture antibodies that were specific for IFNγ and IL-4. Concentration was calculated using different cytokine patterns at known concentrations. Data were acquired using a FACSCalibur flow cytometer (BD Immunocytometry Systems), and Cell Quest software was used for analysis.

Statistical analysis

SPS statistical software was used for analysing the data. All categorical variables were described using frequencies, while continuous variables were reported as means or medians with their corresponding 95% confidence intervals and standard deviations. The U Mann-Whitney test was used for making comparisons; the significance level was set at < 0.05.

Results

PBMC were obtained and cultured in the presence of polyclonal stimuli to characterize the Th1/Th2 cytokine balance in lymphocytes from healthy controls and paediatric patients suffering from FBPU.

The patients were 50/50% with respect to sex, the age average was 3.5 years (SEM 0.7 years). Twenty-eight percent of the patients in this study reported a personal history of atopy (asthma, allergic rhinitis, or atopic dermatitis) while 78% of them reported a family history of atopy. Seventy percent of healthy children were males and the age average was 3.4 years (SEM 0.6 years); none reported a personal history of atopy, and 11% reported a family history of atopy.

Plasma levels of IFNγ and IL-4 were quantified by flow cytometry to establish differences in plasmatic cytokine levels between the patients and controls. The results (32.7 pg/ml, SEM 3.3 pg/ml in healthy children and 29.5 pg/ml, SEM 2.5 pg/ml in patients for IFNγ and 45.6 pg/ml, SEM 3.6 pg/ml in healthy children and 55.8 pg/ml, SEM 2.7 pg/ml for IL-4) revealed no quantitatively significant differences between patients and healthy controls for any of the cytokines evaluated.

Expression levels of cellular activation markers were examined in CD3+/CD4+ and CD3+/CD8+ T-cells to assess the activation status of the T-cells after various culture conditions. When cells were unstimulated or activated with SEB as a polyclonal stimulus, CD69 expression levels were similar in both patients and controls (24.2% in healthy children and 29.3% in patients).