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Allergen-specific B cell subset responses in cow's milk allergy of late eczematous reactions in atopic dermatitis

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ABSTRACT

B cells have regulatory functions in immune responses. Antigen-specific responses of B cell subsets by allergen stimulation ex vivo were examined in milk allergy of late eczematous reactions. Eight milk allergy subjects and 13 milk tolerant subjects were selected by DBPCFC. PBMCs were stimulated by casein ex vivo and stained for B cell subsets using monoclonal antibodies. CD19+ B cells unchanged from 8.7 ± 3.8% to 8.0 ± 5.1% ($p = 0.504$, $n = 8$) in the milk allergy group and decreased in the milk tolerant group from 8.5 ± 3.2% to 5.0 ± 1.6% ($p = 0.001$, $n = 13$). The fraction of apoptotic B cells in B cells significantly decreased 4.4 ± 3.1% to 1.3 ± 0.4% ($p = 0.027$, $n = 4$) in the allergy group and insignificantly increased from 2.8 ± 0.6% to 5.4 ± 2.6% ($p = 0.059$, $n = 6$) in the milk tolerant group. CD5+ regulatory B1 cell% in B cells decreased in milk allergy subjects from 36.2 ± 5.0% to 31.0 ± 5.7% ($p = 0.010$) and unchanged in milk tolerant subjects from 41.6 ± 10.2% to 43.8 ± 10.0% ($p = 0.413$). IL-10 producing CD19+CD5+ regulatory B cell% in CD19+CD5+ regulatory B cells significantly decreased from 24.9 ± 6.5% to 13.8 ± 5.6% ($p = 0.002$, $n = 5$) by casein stimulation in milk allergy group and unchanged from 44.8 ± 11.3% to 43.9 ± 10.0% ($p = 0.297$, $n = 5$) in the milk tolerant group. B cell subset responses to IL-4 and IL-5 were also similar in both groups. B cell subset changes seemed to have diagnostic value. Exact immunologic roles of regulatory CD5+ B1 cells need further investigation.

1. Introduction

Food allergies have increased significantly in the past decade and should be classified into IgE, non-IgE, or mixed response categories [1]. Far less is understood regarding non-IgE-mediated food allergies compared to those that are IgE-mediated, and the clinical relevance is likely underestimated in most cases [2,3]. Clinically, non-IgE-mediated food allergies are not well defined due to the lack of easily accessible diagnostic measures.

Food allergy has been known to be modulated by relative imbalances in the Th1/Th2 paradigm [4]. In vitro production of IL-4 in peripheral blood mononuclear cells (PBMC) was enhanced in children with isolated immediate IgE-mediated food hypersensitivity [5]. Serum IL-5 levels were increased in cow milk allergy [6].

Recently negative regulatory mechanisms have been suggested including CD19+CD5+ regulatory B cells [7] in addition to CD4+Foxp3+ regulatory T cells [8] for immune tolerance. CD5 is a pan T cell marker also expressed at various developmental and activation states on human B cells and is a well-established negative regulator of TCR and BCR signaling [9]. CD5 plays a key role in B and T cell selection as well as generation and maintenance of tolerance [10]. A small subset of mouse and human B cells which express CD5 produces much of the serum immunoglobulin, including many common autoreactive antibodies, and accounts for most cases of B cell chronic lymphocytic leukemia [11]. These CD5+ B cells are known to have negative regulatory functions in immune responses [7].

Although, there are many reports concerning the responses of isolated cells to allergen, the changes and resultant responses of PBMCs in which all immunocompetent cells and all possible cytokines may involve the immune responses by ex vivo allergen stimulation may be different. However, PBMCs are the most similar to in vivo conditions and the change of PBMCs may be regarded as important for understanding of integrated in vivo

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responses by allergen stimulation in allergy and tolerance. Also, this understanding is important for diagnostic purposes and for the evaluation of therapeutic effects of tolerance induction. For these reasons, PBMCs were stimulated ex vivo with casein in milk allergy patients and milk tolerant subjects, and the changes of B cell subsets including CD19+CD5+ regulatory B1 cell were compared.

2. Materials and methods

2.1. Subjects and inclusion criteria

Subjects who visited the Health Guidance Division at the Seodaemun-Gu Health Center for the 'Atopy Safe School Project' and the Department of Allergy and Clinical Immunology at the Seoul Allergy Clinic between March and July 2009 and were suffering from a repeated history of late eczematous reactions or exacerbations of AD, were screened for the current study among the subjects between 8 and 12 years of age. They received blood tests and skin prick tests as described below and fulfilled the criteria of Hanifin and Rajka [12]. Signed consent forms were obtained from either the patient or the parent. The study was approved by the Ethical Committee of the Seoul Allergy and Immunology Association, Seoul, Korea.

Elimination diet was performed as a first step and subsequent double-blind placebo-controlled food challenge (DBPCFC) tests were conducted for milk as described below. Eight positive milk allergy subjects (mean age = 8.4 ± 1.0 yr, M:F = 3:5) were selected according to the diagnostic criteria for milk allergy of late eczematous reactions (a milk allergy group) and 13 subjects (mean age = 9.5 ± 1.4 yr, M:F = 4:9) who did not react to milk were involved as a milk tolerant group (Table 1).

Diagnostic criteria for food allergy of late eczematous reactions were based on the following criteria: (1) late eczematous reactions including exacerbation of AD by DBPCFC, (2) exacerbation onset 4 h after the food challenge, and (3) negative skin prick test and undetectable food-specific IgE level for milk [13].

Table 1
Milk allergy group and milk tolerant group.

	Positive	Negative
n	8	13
M:F	3:5	4:9
Mean age	8.4 ± 1.0	9.5 ± 1.4
Mean WBC counts (counts × 1000/μl)	7.6 ± 2.0	7.6 ± 2.7
Mean eosinophil fractions	7.4 ± 3.1	7.7 ± 4.3
Mean serum total IgE (IU/L)	971 ± 1063	900 ± 1188
Mean blood lymphocyte % in WBC (%)	47.5 ± 10.5	40.9 ± 6.3
Mean milk-specific IgE (kU A/L)	0.04 ± 0.02	0.02 ± 0.02
Mean casein-specific IgE	0.02 ± 0.02	0.02 ± 0.02

2.2. Blood tests and skin prick tests

Subjects who were allergic to milk received blood tests and skin prick tests. Blood tests were performed including a CBC with differential count for the eosinophil fraction and serum total IgE levels. Serum food-specific IgE levels were also measured using the ImmunoCAP System (Phadia AB, Uppsala, Sweden). Serum food-specific IgE levels less than 0.35 kU A/L were classified as undetectable food-specific IgE levels.

Skin prick tests were conducted on the patient's left forearm using commercial allergen extracts (Bencard, Brentford, England). Histamine hydrochloride (1 mg/ml) (Bencard) was used as a positive control. Physiological saline and glycerol were used as negative controls. Reactions were read after 15 min and classified as negative (0: no reaction, +: reaction greater than the control reaction but smaller than half that caused by histamine) or positive (++: half the level, +++: equal to, and ++++: twice the level caused

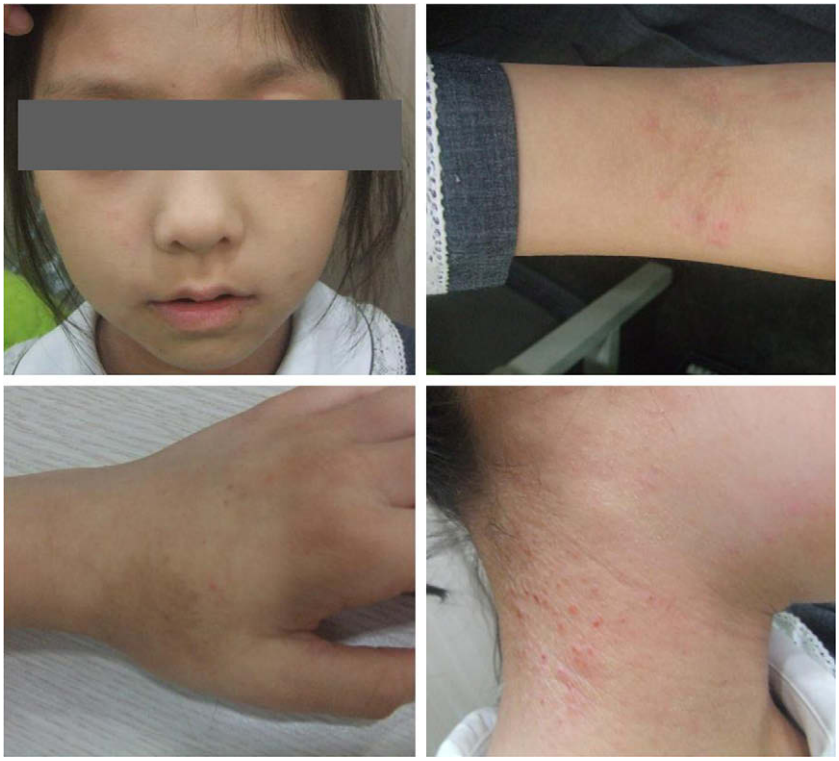

Fig. 1. Skin manifestations of milk allergy in atopic dermatitis. Patients showed erythematous popular eruption with severe itching.