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Original Article

Detection of IL-2, IFN-γ, and serum-specific antibodies in the serum of children with atopic dermatitis

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Abstract: Objective: To investigate the expression and significance of serum interleukin-2 (IL-2), interferon-γ (IFN-γ), and serum-specific antibodies in children with atopic dermatitis (AD). Methods: A total of 80 children with AD were selected as Group AD, and 80 infants and young children receiving physical examinations at the same period were selected as the control group. Enzyme-linked immunosorbent assay (ELISA) was used to detect immunoglobulin G (IgG) and immunoglobulin E (IgE) antibodies to allergens as well as the levels of IL-2 and IFN-γ in the serum. The levels of IgG antibodies, IgE antibodies, IL-2, and IFN-γ were compared between the two groups. Results: The concentration of IFN-γ in the peripheral blood of the children with AD was lower than that of normal population. The difference was statistically significant (t=9.89, P=0.020). The concentration of IL-2 in the peripheral blood of the children with AD was higher than that of normal population. The difference was statistically significant (t=21.69, P<0.001). Children with AD showed relatively high positive rates of IgG antibodies to allergens: milk (43.8%), eggs (37.5%) and soybeans (10.0%), and relatively high positive rates of IgE antibodies to the milk (38.8%), eggs (41.3%) and shrimp (21.3%). The positive rates of IgE antibodies to the fish and the shrimp were obviously higher than those of IgG antibodies for children with AD. The differences were statistically significant (both P<0.05). The children with AD had significantly higher positive rates of IgG antibodies to the milk and the eggs than the control group (both P<0.05). They had remarkably higher positive rates of IgE antibodies to the milk, eggs, shrimp, wheat and pork than the control group (all P<0.05). There was a positive correlation between IgG and IgE (r=0.38, P=0.030), a negative correlation between IFN-γ and IL-2 (r=-0.01, P=0.040), a negative correlation between IFN-γ and IgE (r=-0.47, P=0.020) and a positive correlation between IL-2 and IgE (r=0.53, P=0.020). Conclusion: Food allergens and inhaled allergens are important causes of AD in children. It is rapid and convenient to detect IgE and IgG antibodies and the allergens of Group AD can be detected effectively. The changes in the levels of serum IL-2 and IFN-γ play a certain role in the pathogenic process of patients with AD. Early and effective environmental control is very important for treating AD in children and preventing the occurrence of allergic diseases in the respiratory tract.

Keywords: Interleukin-2, interferon-γ, atopic dermatitis, IgG, IgE

Introduction

Atopic dermatitis (AD) is a genetically-related skin disease that is commonly seen in the clinic. It is characterized by pruritus, eczema, etc. In recent years, its incidence in children has been increasing. However, its pathogenesis is still unclear at present [1]. Exogenous allergens of AD in children mainly include food allergens and inhaled allergens. The mechanism of AD is that allergic reactions occur after the body contacts with the allergens. The main antibodies for allergic reactions are immunoglobulin E (IgE) and immunoglobulin G (IgG). IgE mediates immediate hypersensitivity reactions, while IgG is a food delayed response and a food intolerance index. IgE and IgG can be used as indicators to evaluate allergic diseases. Most of the diseases are considered to be related with the imbalance of subsets of helper cells Th1/Th2, which is mainly manifested as a decline in the function of Th1 cells and an enhancement in the function of Th2 cells. Interleukin-2 (IL-2) and interferon-γ (IFN-γ) also play a crucial role in regulation of immune function [2, 3]. In this study, we used ELISA to detect the levels of IL-2 and IFN-γ in the peripheral blood of children with AD. Meanwhile, we investigated the signi-

ficance of IgE and IgG in the detection of allergens for AD in infants and young children. These results provide experimental evidence for the diagnosis and treatment of AD.

Materials and methods

General data

A total of 80 infants and young children with AD treated in the Dermatology Clinic of our hospital from June 2015 to June 2017 were selected as the objects of the study.

Inclusion criteria: Patients meeting the diagnostic criteria for AD; patients without the history of infectious diseases; patients without heart, liver or kidney diseases; patients free from the use of immunomodulatory drugs; patients with informed consent obtained from their guardians; patients with complete clinical data.

Exclusion criteria: Patients with infectious diseases; patients with the history of using immunosuppressive agents due to illness; patients whose clinical data were incomplete.

The patients included 57 males and 23 females aged 1-4 years old with an average of 2.24± 0.36 years. The course of the illness was 1-4 years with an average of 2.34±1.7 years. Meanwhile, 80 infants and young children receiving physical examinations in the same period were selected as the control group, including 52 males and 28 females aged 1-3 years old. The differences in gender, age, etc. of the two groups of children had no statistical significances (all $P > 0.05$). They were comparable.

Detection of serum concentrations of IL-2 and IFN-γ

A total of 5 mL of peripheral blood was taken from the forearm of the participants, and centrifuged at 3,000 r/min for 15 min. Supernatant was taken, and stored at -80°C for future testing. A total of 50 μL of enzyme-labeling solution and 100 μL of standard substance or sample were added to corresponding sites in the enzyme-labeling plate, respectively. The plate was placed at 18-25°C for reaction for 90 min. Then they were washed 5 times via a plate-washing machine. A total of 50 μL each of base solution A and base solution B were added to each well in the enzyme-labeling plate at an interval of 20 s between each time of washing. They were then incubated at 18-25°C away from light for a reaction time of 15 min. Finally, 50 μL of stop solution was added to each well in the enzyme-labeling plate to terminate the reactions. The value of optical density (OD) was measured, and corresponding concentrations of IL-2 and IFN-γ were obtained according to the OD value.

Detection of serum IgE and IgG

The conditions of IgG antibodies to 7 food allergens (eggs, milk, fish, shrimp and crab, beef and mutton, peanuts and soy, mango as well as wheat) and 7 inhaled allergens (dust mites, artemisia, dander of cats and dogs, cockroaches, branch spore of penicillium notatum, cypress sycamore triangular leaves as well as humulus japonicus) and IgE antibodies to 14 food allergens (beef, milk, eggs, chicken, pork, shrimp, crab, cod, rice, corn, wheat, tomatoes, soybeans, and mushrooms) were detected by ELISA. If the serum sample contained IgE antibodies to one or more allergens, half an hour after the substrate (indicator P) was added, the corresponding reaction sites in the tube would turn purple, and it was judged as positive. The concentration of IgG was calculated based on the OD value of IgG antibodies measured with an enzyme-labeling instrument. IgG ≥ 0.02 pg/mL and IgE > 0.35 pg/mL suggested that the result was positive. Positive rate = number of positive cases/number of total cases *100%.

Statistical methods

SPSS 11.0 was used in this study for data processing. Positive rates of IgE and IgG were expressed as %, and X^2 test was adopted for the comparison between two groups. The concentrations of IL-2 and IFN-γ are expressed as ($\bar{x} \pm sd$), and t test was adopted for the comparison between two groups. $P<0.05$ indicated that the difference was statistically significant.

Results

Detection results of serum IFN-γ and IL-2

The concentration of IFN-γ in the peripheral blood of the children with AD was lower than that of the normal population. The difference was statistically significant ($t=9.89$, $P=0.020$).

Table 1. Detection results of serum IFN-γ and IL-2 ($\bar{x}$ ± sd)

Group	IFN-γ (pg/mL)	IL-2 (pg/mL)
AD group (n=80)	22.38±7.82	1,150.72±391.80
Control group (n=80)	28.13±5.77	817.52±187.62
t	9.89	21.69
P	0.020	0.000

Note: IFN-γ, interferon-γ; IL-2, interleukin-2; AD, atopic dermatitis.

Table 2. Detection of positive rates of IgG and IgE antibodies to allergens in children with AD (n, %)

Allergen	Positive IgE antibody	Positive IgG antibody	X^2	P
Milk	31 (38.8)	35 (43.8)	0.908	0.218
Egg	33 (41.3)	30 (37.5)	0.334	0.165
Soybean	15 (18.8)	8 (10.0)	0.125	0.873
Fish	12 (15.0)	6 (7.5)	5.215	0.011
Shrimp	17 (21.3)	7 (8.8)	11.084	0.000
Wheat	9 (11.3)	5 (6.3)	2.102	0.118
Pork	8 (10.0)	6 (7.5)	3.458	0.095

Note: IgG, immunoglobulin G; IgE, immunoglobulin E; AD, atopic dermatitis.

The concentration of IL-2 in the peripheral blood of the children with AD was higher than that of the normal population. The difference was statistically significant (t=21.69, P=0.000). See **Table 1**.

Detection of IgG and IgE antibodies to exogenous allergens in children with AD

Children with AD showed relatively high positive rates of IgG antibodies to the milk (43.8%), eggs (37.5%) and soybeans (10.0%), and relatively high positive rates of IgE antibodies to the milk (38.8%), eggs (41.3%) and shrimp (21.3%). Positive rates of IgE antibodies to fish and shrimp were obviously higher than those of IgG antibodies for children with AD. The differences were statistically significant (both P<0.05). See **Table 2**.

Detection of IgG and IgE antibodies in the two groups of children

Children with AD had significantly higher positive rates of IgG antibodies to the milk and the eggs than the control group (both P<0.05). They had remarkably higher positive rates of IgE antibodies to the milk, eggs, shrimp, wheat, and pork than the control group (all P<0.05). See **Tables 3** and **4**.

Pearson-related test

There was a positive correlation between the positive rate of IgG and that of IgE (r=0.38, P=0.030), a negative correlation between the positive rate of IFN-γ and that of IL-2 (r=-0.01, P=0.040), a negative correlation between the positive rate of IFN-γ and that of IgE (r=-0.47, P=0.020) and a positive correlation between the positive rate of IL-2 and that of IgE (r=0.53, P=0.020).

Discussion

AD is a common skin disease in children. The body's first exposure to allergens will produce an immune response. Corresponding body damage will be caused when the body exposes to the same allergens again. The immune cells involved in this process are mostly mast cells and basophils. IgE-mediated immune responses mainly refer to the allergic reactions induced by the variety of media secreted by these cells [4, 5]. Specific dermatitis is sometimes a late-onset anaphylactic reaction, in which mast cells play a major role, and IgG antibodies mainly take an effect in the delayed hypersensitivity reaction induced by mast cells. This effect has gotten increased attention [6]. Allergens are divided into food allergens and inhaled allergens currently. Food allergy is mainly mediated by IgE, and part of it is non-IgE-mediated. IgE mainly mediates immediate hypersensitivity reactions to single food such as eggs, milk and pork. IgG antibodies are positive to various foods. Inhaled allergens are also the major allergens of AD, among which IgE antibodies have high positive rates to the dust, mold, pollen, dust mites, etc. [7].

At present, detection of allergen-specific antibodies has been widely used in patients with AD. Some studies suggested that detection of IgE and IgG antibodies is quick and convenient, which can provide correct guidance for clinical treatment and diet [8]. The results of this study showed that children with AD had relatively high positive rates of IgG antibodies to allergens: milk (43.8%), eggs (37.5%) and soybeans (10.0%), and relatively high positive rates of IgE

Table 3. Positive rate of IgG antibodies in the two groups of children (n, %)

Allergen	AD group	Control group	X^2 value	*P* value
Milk	35 (43.8)	8 (10.0)	28.351	0.000
Egg	30 (37.5)	13 (16.3)	10.752	0.000
Soybean	8 (10.0)	3 (3.8)	1.659	0.762
Fish	6 (7.5)	4 (5.0)	2.026	0.101
Shrimp	7 (8.8)	6 (7.5)	1.469	0.103
Wheat	5 (6.3)	5 (6.3)	3.521	0.156
Pork	6 (7.5)	7 (8.8)	0.827	0.256

Note: IgG, immunoglobulin G; AD, atopic dermatitis.

Table 4. Positive rate of IgE antibodies in the two groups of children (n, %)

Allergen	AD group	Control group	X^2 value	*P* value
Milk	31 (38.8)	8 (10.0)	31.028	0.000
Eggs	33 (41.3)	8 (10.0)	33.259	0.000
Soybeans	15 (18.8)	5 (6.3)	0.759	0.223
Fish	12 (15.0)	4 (5.0)	3.568	0.257
Shrimp	17 (21.3)	6 (7.5)	7.479	0.000
Wheat	9 (11.3)	3 (3.8)	11.461	0.000
Pork	8 (10.0)	3 (3.8)	10.056	0.000

Note: IgE, immunoglobulin E; AD, atopic dermatitis.

antibodies to the milk (38.8%), eggs (41.3%) and shrimp (21.3%). The positive rates of IgE antibodies to the fish and the shrimp were obviously higher than those of IgG antibodies for children with AD (both $P<0.05$). Children with AD had significantly higher positive rates of IgG antibodies to the milk and the eggs than the control group (both $P<0.05$). They had remarkably higher positive rates of IgE antibodies to the milk, eggs, shrimp, wheat, and pork than the control group (all $P<0.05$). These results suggested that both IgG and IgE antibodies were associated with AD, which is consistent with the results obtained in other studies [9-11]. Therefore, combined detection of IgE and IgG antibodies is an effective way to find allergens, but its exact mechanism still needs to be studied further.

With greater understanding of both immunology and molecular biology, more and more evidence shows that immune dysfunction plays an important role in the pathogenesis of AD. Numerous studies have confirmed that Th2-predominant Th1/Th2 imbalance is the intrinsic mechanism for the onset of AD [12]. The clinical manifestations are the increase of Th2-type cytokines, such as IL-2, and the decrease of Th1-type cytokines, such as IFN-γ. How to effectively prevent the synthesis of leukotrienes, and how to regulate the balance of Th-1/Th-2 are new ideas for the treatment of AD.

Skin lesions of AD are manifested as double-phase (acute phase and chronic phase) lesions. The immune pathogenesis generally goes through three processes: activation of Th2 cells, generation of IgE, and activation of Th1 cells. The cytokines produced by Th2 cells include IL-4, IL-5, IL-2 and IL-10. They play a positive regulatory role on IgE. Cytokines such as IFN-γ produced by Th1 cells have an inhibition on IgE, and can stimulate the production of IgG antibodies. IL-2 plays an important role in maintaining the balance between Th1 and Th2. Meanwhile, the intradermal injection of IL-2 caused skin pruritus in AD patients and normal people in foreign countries [13]. Thus it was thought that IL-2 may play an important role in the occurrence and the development of AD. Although changes of IL-2 levels in patients with AD have been reported differently, the role of IL-2, one of the major factors secreted by Th2 cells will attract more and more attention as the theory that the activation of Th2 to Th1 in a sequence is the cause of AD is proposed. However, the specific mechanism of the involvement of IL-2 in the onset of AD remains unclear and needs to be further confirmed. IFN-γ is mainly secreted by Th1 cells. It mediates cellular immunity, differentiation, and activation of cytotoxic T lymphocyte as well as activation of macrophages, and suppression of B cells, which plays an important role in the clearance of intracellular microbial infections [14]. The interaction between IL-2 and IFN-γ is the key to maintaining the balance between Th1 and Th2. Th2 induces the recruitment and activation of eosinophils as well as the transformation of B-cell subtypes by secreting IL-4, IL-5 and IL-13 [15]. However, INF-γ can regulate Th2, Th17, NKT, B cells, and other macrophages, neutrophils, eosinophils and other inflammatory cells, and inhibit allergic inflammation as well as the release of IgE [16]. Th1 and Th2 are balanced in normal population. Patients with AD have immune dysfunction, so Th1 and Th2 are imbalanced. The results of this study

suggest that compared with the normal population, patients with AD have elevated IL-2 and decreased INF-γ. It is speculated that the suppressive cytokine IL-2 in the patients with AD is increased, and INF-γ is decreased, which fails to block the synthesis and release of Th2-type cytokines, and cannot balance the Th1/Th2 immune response or inhibit the Th2 cell-predominant response. This triggers a series of immunologic cascade reactions, promoting the accumulation of eosinophils, increasing the activity of eosinophils, and promoting the production of IgE, finally resulting in AD.

Increased levels of total IgE in the serum can be found in more than 80% of patients with AD, and the rise of allergen-specific IgE titer can be found in some patients. It has been reported that anti-IgE monoclonal antibody therapy has an obvious efficacy [17]. IgE is bound to tetrameric FcaRI on the surface of mast cells and basophils. When the antigens are bound, the receptor will be cross-linked, causing degranulation, releasing bioactive substances such as histamine, tryptase, and IL-4 and converting naive T cells into T cells that can produce IFN-γ [18, 19]. In turn, IFN-γ can activate natural killer cells and inhibit B cells to produce IgE [20]. Correlation of IFN-γ with IgE was analyzed, and the results show that they were negatively correlated, which proves the interaction between these two cytokines with IgE.

In conclusion, AD is related to immune dysfunction in patients, and IL-2 and IFN-γ have certain effects on the onset of AD. Food and inhaled allergens are the major causes of AD. The detection of IgG and IgE is a method for finding the allergens. IgG, IgE and the exact immune mechanism need to be further studied.

Disclosure of conflict of interest

None.

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