Original Article:

Transforming Growth Factor B: Clinical Significance and Peculiarities of Inheritance in Children with Atopic Dermatitis

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Citation

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Abstract: Transforming growth factor β1 (TGF-β1) plays an important role in the pathogenesis of many allergic diseases, including atopic dermatitis (AD). Objective: to study the role of TGF-β1 in the pathogenesis of AD in children. Materials and methods. A survey was conducted of 34 patients with AD and 20 healthy children. TGF-β1 in serum were determined by ELISA using kits of Human TGF beta1 Platinum ELISA. Definition Arg25Pro gene polymorphism of TGF β1 was carried out using allele-specific polymerase chain reaction. Results. It was found that patients with AD showed a significant increase in the concentration of TGF-β in the serum (222.37±68.58 PG/ml) compared with the control group (3,03±0,41 ng/ml) [p = 0.001]. The concentration depends on the severity and duration of the illness. Frequency of genotypes for the polymorphism Arg25Pro TGF β1 gene among patients with AD had significant differences from the control group (p < 0.05). Moreover, among patients who are heterozygotes for the gene Arg25Pro TGF β1, was significantly more observed moderate (77.78 %) and severe (33.33%) the course of the disease. Conclusion. The obtained results confirm the importance of TGF-β1 in the pathogenesis of AD.

Key Words: Atopic dermatitis, Transforming growth factor β (TGF-β), Children, Genetics, Gene polymorphism, Pathogenesis

Introduction:
Atopic dermatitis (AD) is a chronic allergic inflammation of the skin triggered as atopic and autopic mechanisms. It is proved that atopic dermatitis is the first manifestation of atopy, and in some children is the beginning of “Allergic March”. The increasing prevalence of AD observed in many countries of the world. In developed countries the incidence of children ranges from 13 to 37 % in the US – roughly 17 %. In Russia this indicator makes the regions from 5.9% to 15.5 % [1]. The pathogenesis of AD is dysfunction of cellular immunity, a defect which is observed in quantitative (reduction of the number of T-cells) and functional (impaired production of cytokines and cretaceous paleogene reactions) levels [2]. Growth factors, including transforming growth factor β (TGF-β) have a significant impact on the processes occurring in inflammation [3,4]. This cytokine controls the proliferation, cellular differentiation and other functions in most cells, including immune-mediated. Growth factor β (TGF-β) is a homodimer. The molecular weight of one its chain is about 25 kDa. The factor exists in three isoforms: TGF-beta1, TGF-beta2 and TGF-beta3 (in the immune system the most common TGF-β1) and its producers is the high number of cells, including stromal cells and macrophages, TGF-β is produced in an inactive form containing a primary dimer, and fragments of additional chains of molecules predecessor. Its activation occurs upon cleavage of these fragments under the action of proteases (plasmin, cathepsin, etc.) [5]. It is known that TGF-β regulates the functions of B-lymphocytes, NK-cells, dendritic cells (DC), macrophages, mast cells and granulocytes, but the greatest influence on T-cells. This cytokine has anti-proliferative effects on T-cells: inhibits the secretion of IL-2 to T-lymphocytes, enhances the activity of cell cycle inhibitors (p15, p21, p27) and reducing expression of the gene c-myc that control normal cell proliferation. Also TGF-β regulates the differentiation of T-cells, prevents the development of cytotoxic CD8+lymphocytes, T-helper 1 and 2 type. In addition, it stimulates differentiation of a population of T-helper cells 17, which are characterized by the secretion
of proinflammatory cytokine IL-17 [6]. It is established that TGF-β also increases the expression homing-receptor that interacts with E-selectin in the dermis and responsible for transport of T-lymphocytes in the skin [7]. In recent years, it has been proven that increased education leads to increased synthesis of collagens and, therefore, not only to accelerate tissue repair, but their remodeling process of chronic allergic inflammation too.

Because of the variety of functions performed by this cytokine it needs to be particularly studied in children suffering from atopic dermatitis.

Materials and Methods

There were examined 34 children with AD. Inclusion criteria are: the presence of a confirmed diagnosis of AD; no concomitant chronic pathology of other organs and systems. Exclusion criteria: patients with other chronic diseases of the skin; patient age 18 years or older. The control group consisted of 27 children I and II health groups, matched by sex and age. The average age of patients is 8.82±4.9 years. Examination of children was conducted in the pediatric Department of the Rostov state medical University.

All patients underwent complex clinical and laboratory examination, including collection of anamnestic data, physical examination, determination of total and allergen-specific IgE. Blood tests for immunological parameters was carried out using the method of enzyme immunoassay (ELISA) using kits of Human TGF beta 1 Platinum ELISA, the production of “Bender Medsistems GmbH”. The level of total IgE was determined in fresh serum using solid-phase enzyme immunoassay kits “VedaLab” (France).

Molecular genetic study was conducted in the Department of genetics, Southern Federal University. The study Arg25Pro gene polymorphism of TGF β1 was carried out using allele-specific polymerase chain reaction. DNA was extracted using the reagent “DNA-Express-blood” (Lytech, Russia). Allelic variants was investigated using sets of reagents SNP-Express (Lytech, Russia).

Ethical examination

The study was conducted in compliance with all ethical standards. All parents of children and adolescents aged 15 years and older signed informed written consent to participate in research approved by the Local ethics Committee of the Rostov state medical University Protocol ?1 from the city of 20.01.2016.

Statistical processing of the data

The calculation was performed using the applied programs of Microsoft Office and the computer program "STATISTICA 6.0". Under normal law of distribution the significance of differences between groups on the arithmetic mean values was determined by student’s t test – t, with the difference of the distribution parameters from normal used nonparametric Mann-Whitney (M-U). Reliable considered the result for t > 2, where p < 0.05. The degree of relation between pairs of features and the closeness of the connection was evaluated using the rank correlation coefficient P. Spearman. According to the distribution of genotype frequencies equilibrium Hardy-Weinberg equilibrium was determined using the Hardy-Weinberg equilibrium calculator in the program www.oegge.org/software/Hardy-Weinberg [8]. Evaluation of differences in distribution of allelic variants of genes in the examined groups was performed by the Z2 criterion. Statistically significant differences were considered at p < 0.05. About the risk of development of AD was judged by the odds ratio (odds ratio – OR). OR is specified with a 95% confidence interval (CI) [9]. For all groups of the examined patients was observed according to genotype frequencies equilibrium Hardy-Weinberg equilibrium, therefore, the sample can be considered representative enough. Descriptive statistics for quantitative traits was performed using the "mean and standard deviation (M±s)".

Results

Analysis of clinical and anamnestic data showed that the majority of the examined children revealed moderate course of the disease (53.84%), mild at 30.77% and severe from at 15.39% of patients. Among the examined patients children's form was diagnosed in 78.57% and teenage form in 21.43%. At the same time 71.43% noted common pathological process, 14.29% diffuse and 14.28% limited AD. All patients were examined in the period of exacerbation of the disease. The presence of concomitant allergic diseases in anamnestic data were registered at the 53.84% of children (allergic rhinitis was noted in 13.77%, bronchial asthma – at 23.08% and one child had a single episode of acute urticaria accompanied by angioedema). Almost all patients (92.30%) AD has manifested before 1 year of life was associated with allergic reactions to food. At 15.38% of the children were also observed pollen sensitization, 7.69% of patients with fungal and 7.69% of the response to the epidermal allergens. Average values of total IgE amounted to 287.02±86.54 IU/L.

In our study, it was revealed that the children suffering from AD, there is a significant increase in the concentration of TGF-β in the serum (222.37±68.58 PG/ml) compared with the control group (3.03±0.41 ng/ml) (p = 0.001). However, it was revealed a significant inverse correlation between disease duration and content of this cytokine in the serum (r = 0.79).

It was found that patients with low disease duration show a more significant increase in TGF-β in the serum (231.42±86.43 PG/ml) compared with older patients, long suffering from atopic dermatitis (189.20±71.01 PG/ml). Special attention deserves the fact that in patients with AD, the concentration of TGF-β depends on the severity of the disease. In patients with mild severity of disease, the concentration of this factor is 39.76±18.02 PG/ml, whereas moderate disease – 234.41±128.28 PG/ml and heavy – 241.83±110.33 PG/ml. Obtained in the course of the study, the data correspond to the results of a study of our foreign colleagues [10, 11].

The analysis of TGF-β in the serum of patients with different amount of damage to the skin it was found that the maximum high values have been reported in patients with diffuse forms of AD (332.40±143.40 PG/ml), while in advanced cases, its concentration amounted to 183.98±80.98 PG/ml and a limited – 10.68±12.54 ng/ml.

According to immunological studies, the average values of total IgE in the serum of patients with AD is 349.6±90.26 IU/ml. It is important to note that there is an inverse correlation between levels of total IgE and the concentration of TGF-β in serum (r = -0.39). Perhaps these changes are due to the fact that TGF-β has the ability to suppress the synthesis of immunoglobulin E [10].

Analysis of clinical and anamnestic data showed that most patients had concomitant allergic disease: 52.94% of patients had BA, 41.18% allergic rhinitis, 14.70% had episodes of urticaria in anamnestic. It was found that in patients with concomitant allergic disease the values of TGF-β in blood serum was significantly higher than in patients with only AD (p = 0.034).

A detailed analysis of anamnestic data revealed that children with burdened allergoanamnesis have records of TGF-β significantly higher than in patients with uncomplicated heredity (p = 0.023). While more common allergopathology from the mother, while the father's side there is only 11.76%.

The results of the genetic studies not showed that the frequency of genotypes for the polymorphism Arg25Pro TGF β1 gene among patients with AD had significant differences from the control group (p > 0.05) [see Table. 1].
It was found that among children suffering from AD, homozygotes for the polymorphism 25Arg/Arg gene TGF β1 was more than half of the patients (58.82%), whereas heterozygotes Arg25Pro was in 41.18% of the cases. Among healthy children, the frequency of these genotypes was the same: and homozygotes 25Arg/Arg heterozygotes and Arg25Pro gene TGF β1 was noted in 48.1%, while the genotype Pro Pro was one child. However, it should be noted that among patients who are heterozygotes for the gene Arg25Pro TGF β1, was significantly more observed moderate (77.78%) and severe (33.33%) the course of the disease. While among homozygotes for Arg-alleles severe disease was only 14.30% of the examined children.

Further comparison of genetic and clinical data showed that patients with the ProPro genotype carriers twice as often disease, but also adequate control over it. Knowledge of the genetic characteristics of his inheritance opens up opportunities not only for the prevention of this disease, but also adequate control over it.

**Table 1: Frequency of genotype for the polymorphism Arg25Pro TGF β1 gene among children with atopic dermatitis and I and II groups of health**

<table>
<thead>
<tr>
<th>Genotype, allele</th>
<th>Children suffering from AD</th>
<th>Healthy children</th>
<th>( \chi^2 )</th>
<th>( p )</th>
<th>OR</th>
<th>The value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele 1(Arg)</td>
<td>n = 34</td>
<td>n = 27</td>
<td>0.58</td>
<td>0.45</td>
<td>1.48</td>
<td>0.53-4.12</td>
<td></td>
</tr>
<tr>
<td>Allele 2(Pro)</td>
<td>0.206</td>
<td>0.278</td>
<td>0.97</td>
<td>0.62</td>
<td>1.54</td>
<td>0.45-5.24</td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>0.588</td>
<td>0.481</td>
<td></td>
<td></td>
<td>0.75</td>
<td>0.22-2.57</td>
<td></td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>0.412</td>
<td>0.481</td>
<td></td>
<td></td>
<td>0.50</td>
<td>0.02-13.11</td>
<td></td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>0.000</td>
<td>0.037</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

It was found that among children suffering from AD, homozygotes for the polymorphism 25Arg/Arg gene TGF β1 was more than half of the patients (58.82%), whereas heterozygotes Arg25Pro was in 41.18% of the cases. Among healthy children, the frequency of these genotypes was the same: and homozygotes 25Arg/Arg heterozygotes and Arg25Pro gene TGF β1 was noted in 48.1%, while the genotype Pro Pro was one child. However, it should be noted that among patients who are heterozygotes for the gene Arg25Pro TGF β1, was significantly more observed moderate (77.78%) and severe (33.33%) the course of the disease. While among homozygotes for Arg-alleles severe disease was only 14.30% of the examined children.

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**Discussion**

The results of our research have established a considerable increase in the concentration of the transforming factor in the serum of patients with AD. Perhaps this is due to the fact that TGF-β is a critical signal that alter the normal immune response. This factor is secreted by platelets, plays the role of a chemo-attractant for monocytes, promotes their differentiation into macrophages and stimulates the production of the proinflammatory chemokines and TGF-β by macrophages, in addition it changes the effects of IL-4, leading to the formation of the 1st and not the 2nd type CD4+ and CD8+ T cells during the activation of native T-lymphocytes. Therefore, this factor contributes to the chronicity of AD in children.

Some authors have noted that in conditions of allergic inflammation detected T cells with relatively high production of IL-4 and TGF-β, the activation of which leads to an inclusion in the pathological process of T-helper cells type 1, prolonged duration of atopic inflammation, hardening and lichenification skin [12]. And the established inverse correlation between disease duration and content of this cytokine in serum may be related to the dual effect of TGF-β, in that it can be not only a proinflammatory cytokine but also to exert a suppressor effect on the dynamics of the immune response, protecting the organism from excessive production of macrophages and other cells of inflammation cytotoxic compounds. So, in the work of L. S. Freidlin, it is shown that in the initial stage of initiation of inflammation this cytokine can stimulate the production of proinflammatory chemokines and only with the progression of inflammation and the accumulation of TGF-β and its effects become anti-inflammatory [13]. This fact is confirmed by the works of foreign authors, who established that in adult patients with AD also notes the decrease in the level of TGF-β in blood serum, compared with control patients [14, 15].

It is known that TGF-β1 activates the functioning of fibroblasts, stimulating the development of perivascular fibrosis. So probably all the changes of the skin, were detected in the examined patients due to increased release of TGF-β1 of skin cells, which further leads to the increase in the thickness of the basal membrane by increasing the production of collagen I, III, VIII, types, and components of the base material (fibronectin, tenascin). Tissue remodeling in this case can be considered, on the one hand, as a protective and adaptive response, as the morphological marker of the hyperresponsiveness of the skin observed in patients with severe AD.

Our findings prove the need for further survey and study of the genetic aspects of inheritance of predisposition to increased synthesis of TGF-β in children with AD.

**Conclusion**

Thus, the study showed that TGF-β plays a significant role in triggering and maintaining allergic inflammation in AD. Its functions are ambiguous and require further scrutiny. Knowledge of the genetic characteristics of his inheritance opens up opportunities not only for the prevention of this disease, but also adequate control over it.

**Conflict of Interest:** None.

**Ethics:** No ethical issues relate to the present study.

**References**


