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The content of mediators of allergic inflammation in the blood serum of patients with atopic asthma, depending on the polymorphism 2258G / A gene TLR2

Introduction. Atopic asthma (AA) is a multifactorial disease characterized by the imbalance of the regulation of T-helper (Th) and the cytokines IL-4, IL-5 and IL-13, which are crucial for the induction of allergic asthma symptoms. It is proved that the regulatory T-cells (T-reg) operate suppressive role in the allergic inflammation and manifestation of AA. These studies provide that T-reg cells can be one of the pharmacological intervention targets for the development of this disease. The role of Toll-like receptor (TLR) was showed in the pathogenesis of several diseases: atopic asthma in children [5], bronchiolitis [19], urogenital infections [4], inflammatory periodontal diseases [6], herpes virus infection [3], diabetes [9], rheumatoid arthritis [1], atherosclerosis [2], chronic sarcoidosis. [14] Effect of TLR activation by T-reg is ambiguous for example: TLR-4 and -5 increase their suppressive capacity [12], while activation of TLR-8 leads to inhibition of function of T-reg. TLR-2 increases the number of T-reg [15] cells, but at the same time loses suppressor activity in vivo and in vitro [18]. Genetic variations – single nucleotide polymorphisms (SNPs) – in these pattern-recognizing molecules have been widely studied, but there is not the common point of view of their effect on the activity of T-reg with AA in adults [16].

The aim of our study was to clarify the mechanisms of immune regulation in patients with AA according to the polymorphism 2258G / A gene TLR2 (rs5743708).

Materials and methods

We examined 45 people with AA. The diagnosis of the AA and its severity was accordance with the international recommendations GINA, 2011. The basis of this research was allergy and pulmonary department of the Poltava Regional Hospital. History data was collected by interrogation using a special questionnaire. All patients with AA were held general clinical laboratory, instrumental and allergy testing. The survey was conducted in the absence of the patient's worsening primary or concomitant chronic, acute lack of intercurrent infections and severe comorbidity, which could affect the results of the study. As a control, were 90 DNA samples healthy individuals with no allergic history to base Institute of genetic and immunological basis for the development of pathology and pharmacogenetics «Ukrainian Medical Dental Academy». The study was conducted according to the provision of a written survey and determine the commission on ethics and bioethics issues of the institution. Isolation of genomic DNA was performed by phenol-chloroform extraction. The definition of polymorphism 2258G/A TLR2 gene was carried by the polymerase chain reaction [19]. Lymphocyte's phenotypes was analyzed by determining the level of expression of cell surface antigens using the monoclonal antibodies, CD4, CD25 (production of «Sorbent», Russia), and intracellular protein FoxP3 («eBioscience», USA) by flow cytometry at EPIX LX-MCL (Beckman Coulter, USA) using a program System II TM software. Level of IgE, IL-4, 10 (OOO «Ukrmed Don» Ukraine) in serum was determined by indirect IFA. Mathematical processing of the data was performed using the program «STATISTICA 6.0» (StatSoft Inc).

Results and discussion

5 (11,1 %) patients had intermittent nature of the AA, 23 (51,2 %) patients had mild, 17 (37,7 %) patients – the moderate severity of the disease. The study of the peripheral blood of 45 surveyed revealed that the level of white blood cells is an average of $6 \pm 0,45 * 10^9$ / L, eosinophils $0,06 \pm 0,008 * 10^9$ /l, lymphocyte count of $1,56 * 10^9/l \pm 0,13$, that does not extend beyond the parameters of healthy individuals. In the study of Th cells indicated that the average level of $CD4^+$ was $0,68 \pm 0,06 * 10^9$ / L, $CD4^+$ / $CD25^+$ was $0,17 \pm 0,02 * 10^9$ / L, which does not extend beyond ratios

practically visual people. The expression level of molecules of $CD4^+$ / $CD25^+$ / $Foxp3^+$ was 0,07 ± 0,01 * 10^9 / l. IgE was 164,9 ± 13,9 IU / ml (from 27,9 to 440,4 IU / ml), IL-4 – 59,5 ± 82 pg / ml (range 59,3 to 313.8) (Fig. 1), is higher than in healthy individuals. This confirms the fact of conventional IL-4-dependent activation of B-lymphocyte immunity [7]. Our data confirm the determination of the AA as a disease with chronic persistent course, even in remission. [10] The level of IL-10 in patients with AA was 0,45 ± 0,02 pg / ml (fig. 1). We didn't found significant differences in groups of patients with different disease severity.

The frequency of «wild-type» genotype of TLR2 (GG) was 97,8 %, the frequency of the heterozygous genotype (GA) – 2,2 %, genotype AA has not been identified in 90 people of the control group. Patients with AA corresponding results were next: GG – 88,9 %, GA – 11,11% and the AA – was not found. Between the frequencies of genotypes (GA) in the control group and in patients with AA were statistical significant difference (p = 0,04). The frequency of the mutant allele among the control group was 1.1 %, and among patients with AA – 5,6 %, which did not differ significantly (χ^2 = 3,1, p = 0,078) (table 1). Distribution of mutant alleles in the groups of patients with varying degrees of severity was held as follows: in the group with mild severity – in 4 people, with intermittent in one person, is not statistically significant. In the group with an average weight of TLR2 SNPs are found. It is possible to confirm the absence of data on the correlation between the severity of the ABA and said genetic variant [13]. When comparing the levels of immunological parameters in patients with AAA (table 1) carriers' wild allele TLR2 gene and mutant alleles of a statistically significant difference was observed only in the concentration of cytokines (table 2). This, high levels of IL-4 (63,7 ± 8,7 pg / l) ware observed in the group without evidence of polymorphism (Mann-Whitney U (n_1 = 40; n_2 = 5) 2.79, p = 0,005) and IL-10 levels were significantly elevated in heterozygous carriers embodiment genome TLR2 (Mann-Whitney U (n1 = 40; n2 = 5) 33,0, p = 0,01). It was noted that patients with SNPs had lower concentration of IgE than in healthy subjects and was (96,7 ± 34,8 IU / ml) – this is quite logical, given the high levels of IL-10 and its effect on Th2. Reducing the concentration of pro-inflammatory cytokine IL-4 in patients with changes in the genes of TLR2 may mean shifting the immune response to the classical B-dependent on T-reg dependent confirming below shows the correlation relationship. The correlation analysis in the group with TLR 2 SNPs (Table 3) showed stable relationship of T cells with other immune cells and cytokines (IL-4, IL-10) as well as IgE, confirming pathogenetic links of allergic inflammation AA. Carriers A mutant alleles observed formation of new correlation pairs ($CD4^+$ / 25^+ / $Foxp3^+$ and lymphocytes), and increase the coefficient of correlation between $CD4^+$ and $CD4^+$ / 25^+ / $Foxp3^+$ (table 3). Pathogenesis and functionally important are strong and reliable data communication pair, correlation can be seen as a specific phenotype of immune cells [8]. The controllability of the current AA in heterozygous genetic apparatus TLR 2 possibly due to the direct interaction of T-regulatory cells from other types of lymphocytes, strikes a balance between effector and regulatory mechanisms of the

Table 1

The Frequency Distribution of Genotypes and Alleles 2258G/A gene's TLR2

Genotypes, alleles	Control group (n = 90)	Patients with atopic asthma (n = 45)
GG	97,8 (88)	88,9 (40)
GA	2,2 (2)	11,1 (5)*
AA	0	0
G	98,9 (178)	94,4 (85)
A	1,1 (2)	5,6 (5)

* – p ≤ 0,05 in comparison with the control group

Table 2

Immunological parameters dependency Genotypes 2258G/A

	Index	Homozygous (GG), (n = 40)	Heterozygous (GA), (n = 5)
1.	$CD\ 4^+$, G/l	0,67 ± 0,07	0,82 ± 0,07
2.	$CD\ 4^+/25^+$, G/l	0,17± 0,03	0,21 ± 0,03
3.	$CD\ 4^+/25^+/Foxp3^+$, G/l	0,07± 0,01	0,08 ± 0,01
4.	Leucocytes, G/l	6,69 ± 0,49	8,44 ± 0,49
5.	Eosinophils, G/l	0,05 ± 0,01	0,1 ± 0,04
6.	Lymphocytes, G/l	1,49 ± 0,13	2,09 ± 0,6
7.	IgE, IU / ml	173,4 ± 14,6	96,7 ± 34,8
8	IL10 pg/l	0,42 ± 0,02	0,7 ± 0,05*
9	IL 4 pg/l	63,7 ± 8,7	24,1 ± 6,28*

* – p ≤ 0,05 in comparison with the homozygous

immune response. Elevated levels of IL-10 inducible T-reg cells can confirm their role in the diagnosis and in the long term treatment of atopic conditions, particularly in patients with functional genetic disorders .

Conclusion

1. Single nucleotide polymorphism 2258G/A gene TLR2 was observed more frequently in patients with atopic asthma than in Poltava healthy population.

2. A characteristic feature of the compensation currented atopic asthma in patients who are homozygous allele G is a wide range of positive mutual correlations against high levels of IL-4.

3. Heterozygous variant of TLR2 gene in patients with atopic asthma compensation contributes to an imbalance in the immune system characterized by the activation of IL-10, significant decrease in the number of mutual correlations of immune structures and direct linear relationship between the natural regulatory T-cells, T-helper cells and lymphocytes.

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Table 3

The force of correlative connection of immunological parameters

	The pair correlation	Homozygous (GG), (n = 40)	Heterozygous (GA), (n = 5)
1	$CD4^+$ та $CD4^+/25^+$	0,53	–
2	$CD4^+$ та $CD4^+/25^+/Foxp3^+$	0,32	0,97
3	$CD4^+$ and Leucocytes	0,79	–
4	$CD4^+$ and Eosinophils	0,41	–
5	$CD4^+$ and Lymphocytes	0,9	–
6	$CD4^+/25^+$ and Lymphocytes	0,57	–
7	$CD4^+/25^+/Foxp3^+$ and Lymphocytes	–	0,97
8	$CD4^+/25^+/Foxp3^+$та IL10	0,57	–
9	Leucocytes and Eosinophils	0,51	–
10	Leucocytes and Lymphocytes	0,77	–
11	Eosinophils and Lymphocytes	0,49	–
12	IgE та IL4	0,57	–

All of the pairs are statistically significant ($p \leq 0,05$)

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СОДЕРЖАНИЕ МЕДИАТОРОВ АЛЛЕРГИЧЕСКОГО ВОСПАЛЕНИЯ В СЫВОРОТКЕ КРОВИ У БОЛЬНЫХ АТОПИЧЕСКОЙ БРОНХИАЛЬНОЙ АСТМОЙ В ЗАВИСИМОСТИ ОТ ПОЛИМОРФИЗМА 2258G /A ГЕНА TLR2

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Резюме

Введение. *Атопическая бронхиальная астма является мультифакторным заболеванием, которое характеризуется дисбалансом регуляции Т-хелперов (Th) Генетические изменения – однонуклеотидные полиморфизмы – Toll подобного рецептора 2 широко изучаются, но единой точки зрения относительно их влияния на активность Т-reg клеток при атопической бронхиальной астме у взрослых нет.*

Цель. Выяснить механизмы иммунного ответа у больных атопической бронхиальной астмой в зависимости от полиморфизма 2258G / A гена TLR2.

Материалы и методы. *Обследовано 45 больных атопической бронхиальной астмой, кроме обще клинических исследований им проведен анализ фенотипов лимфоцитов путём определения уровней экспрессии поверхностных антигенов клеток с использованием моноклональных антител CD4, CD25 и выявление полиморфизма 2258G/A гена TLR2 методом полимеразной цепной реакции.*

Результаты. *Мы выяснили, что в полтавской популяции больных атопической бронхиальной астмой частота генотипов TLR2 составила GG – 88,9 %, GA – 11,11 % и AA – не обнаружен, что чаще чем в контроле ($p \leq 0,04$); статистически достоверной разницы по частоте алелей в указаних группах нет. Характерным признаком компенсированного течения атопической бронхиальной астмы у больных, носителей аллели G в гомозиготном виде является широкий спектр положительных корреляционных взаимосвязей на фоне повышенного уровня ИЛ-4 (63,7 ± 8,7) пг/л. Гетерозиготный вариант гена TLR2 у пациентов с компенсацией атопической бронхиальной астмы способствует дисбалансу в деятельности иммунной системы, который характеризуется активацией продукции ИЛ-10, значительным уменьшение количества корреляционных взаимосвязей иммунозависимых структур и прямыми линейными связями между натуральными Т-регуляторными клетками с Т- хелперами и с лимфоцитам.*

Выводы. *Полиморфизм гена 2258G/A гена TLR2 играет важную роль в течении атопіческой бронхиальной астмы.*

Ключевые слова: *полиморфизм Toll-подобных рецепторов, Т-регуляторные клетки, атопическая бронхиальная астма.*

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THE CONTENT OF MEDIATORS OF ALLERGIC INFLAMMATION IN THE BLOOD SERUM OF PATIENTS WITH ATOPIC ASTHMA, DEPENDING ON THE POLYMORPHISM 2258G / A GENE TLR2

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Summary

Background. *Atopic asthma is a multifactorial disease that characterized by an imbalance of the regulation of T-helper (Th) and the cytokines IL-4, IL-5 and IL-13, which are crucial for the induction of allergic asthma symptoms. Genetic variations - single nucleotide polymorphisms – of Toll like receptor 2 have been widely studied, but the common point of view with regard to their effect on the activity of T-reg cells in atopic asthma in adults do not.*

Aim. *To clarify the mechanisms of the immune response in patients with atopic asthma, depending on the polymorphism 2258G / A gene TLR2.*

Materials and methods. *The study involved 45 patients with atopic asthma, other than general clinical research they analyzed the phenotype of lymphocytes by determining the levels of expression of cell surface antigens using monoclonal antibodies CD4, CD25 and identification of polymorphism 2258G / A TLR2 gene using PLR.*

Results. *The frequency of genotypes of TLR2 was GG – 88,9 %, GA – 11,11 % and the AA – is not found in cohort of patients with atopic asthma that more often than in the control group ($p \leq 0,04$). A characteristic feature of the compensated flow atopic asthma patients, carriers G allele in homozygous form is a wide range of positive correlation relationship against high levels of IL-4 (63,7 ± 8,7) pg/l. The heterozygous variant of TLR2 gene in patients with atopic asthma compensation contributes to an imbalance in the immune system, which is characterized by the activation of IL-10, a significant decrease in the number of mutual correlations of immune structures and direct linear relationship between the natural regulatory T-cells, T-helper cells and lymphocytes.*

Conclusion. *Polymorphism 2258G / A TLR2 gene plays an important role in the course of asthma.*

Key words: *polymorphism of Toll-like receptors, T-regulatory cells, atopic asthma.*

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