ANALYSIS OF TLRs GENES EXPRESSION AND DEFB1 POLYMORPHISMS ASSOCIATION IN CHILDREN WITH BRONCHIAL ASTHMA

Zaitseva MA1, Bragvadze BG1, Svitich OA1,2, Namazova-Baranova LS1, Gankovskaya LV1

1 Department of Immunology, Biomedical Faculty, Pirogov Russian National Research Medical University, Moscow, Russia
2 Laboratory of Molecular Immunology, Mechnikov Research Institute of Vaccines and Sera, Moscow, Russia
3 Scientific Center of Children’s Health, Moscow, Russia

Bronchial asthma (BA) is one of the most common respiratory system diseases. The role of innate immunity components in the pathogenesis of bronchial asthma is studied widely, with particular focus on the antimicrobial peptides. Those include beta defensins that prevent pathogen intrusion into the respiratory tract mucosa, the most active of such pathogens being β-defensin-1 (human beta defensin-1, HBD-1) encoded by the DEFB1 gene. We studied the association of three single nucleotide polymorphisms in the 5’-untranslated region of the gene, namely, rs11362, rs1799946 and rs1200972, with bronchial asthma in children. We also evaluated gene expression of toll-like receptors TLR2, TLR4 and TLR9. The experimental group included 48 patients of 3 to 7 years of age with BA and 70 healthy children. The AA genotype of the rs11362 polymorphism and the CC genotype of the rs1799946 polymorphism were reliably associated with the disease, while the GG genotype of the rs1799946 polymorphism and the AA genotype of the rs120097 polymorphism were found protective. Also, the AA genotype of the rs11362 polymorphism was associated with the reduced expression of DEFB1, the human beta defensin-1 encoding gene, while the AG genotype was associated with its increased expression. In children with BA, TLR2 expression increased 19.5 times in comparison with the controls; TLR9 expression increased 9.5 times, while TLR4 expression increased 8.3 times.

Keywords: bronchial asthma, human beta defensin-1, toll-like receptors, DEFB1, TLR2, TLR4, TLR9, single nucleotide polymorphism, polymorphic marker

Correspondence should be addressed: Margarita Zaitseva
ul. Svyazistov, d.10, kv. 68, Krasnoznamensk, Moscow oblast, Russia, 143090; astice@list.ru

Recieved: 14.06.2016 Accepted: 23.06.2016

АНАЛИЗ ЭКСПРЕССИИ ГЕНОВ TLRs И АССОЦИАЦИИ ПОЛИМОРФИЗМОВ ГЕНА DEFB1 У ДЕТЕЙ С БРОНХИАЛЬНОЙ АСТМОЙ

М. А. Зайцева1, Б. Г. Бргавадзе1, О. А. Свитич1,2, Л. С. Намазова-Баранова3, Л. В. Ганковская1

1 Кафедра иммунологии, медико-биологический факультет, Российский национальный исследовательский медицинский университет имени Н. И. Пирогова, Москва
2 Лаборатория молекулярной иммунологии, Научно-исследовательский институт вакцин и сывороток имени И. И. Мечникова, Москва
3 Научный центр здоровья детей, Москва

Бронхиальная астма (BA) — одно из наиболее распространенных заболеваний органов дыхания. Активно исследуется роль элементов врожденного иммунитета в патогенезе бронхиальной астмы, в частности, противомикробных пептидов. К ним относятся β-дефенсины, предотвращающие вторжение патогенов в слизистую оболочку респираторного тракта, наиболее активным из которых является β-дефенсин-1 (human beta defensin-1, HBD-1), кодируемый геном DEFB1. В исследовании была изучена ассоциация трех однонуклеотидных полиморфизмов в 5'-нетранслируемой области гена — rs11362, rs1799946 и rs1200972 — с бронхиальной астмой у детей. Также оценивали уровень экспрессии генов толл-подобных рецепторов TLR2, TLR4 и TLR9. В опытную группу включили 48 пациентов в возрасте 3–7 лет с BA и 70 здоровых детей. Генотип AA полиморфизма rs11362 и генотип CC полиморфизма rs1799946 достоверно ассоциированы с заболеванием, а генотип GG полиморфизма rs1799946 и генотип AA полиморфизма rs1200972 являются защитными. Генотип AA полиморфизма rs11362 также ассоциирован с повышенной экспрессией, а генотип AG — с повышенной экспрессией гена β-дефенсина-1 DEFB1. У детей с BA выявили повышение уровня экспрессии гена TLR2 в сравнении с контрольной группой в 19,5 раз, TLR9 — в 9,5 раз, TLR4 — в 8,3 раза.

Ключевые слова: бронхиальная астма, β-дефенсин-1, толл-подобные рецепторы, DEFB1, TLR2, TLR4, TLR9, однонуклеотидный полиморфизм, полиморфный маркер

Для корреспонденции: Зайцева Маргарита Алексеевна
143090, Московская область, г. Краснознаменск, ул. Связистов, д. 10, кв. 68; astice@list.ru

Статья получена: 14.06.2016 Статья принята в печать: 23.06.2016
Bronchial asthma (BA) is a chronic inflammatory disease of the upper respiratory tract accompanied by bronchial obstruction and hyperresponsiveness. It manifests itself through shortness of breath, wheezing, coughing and choking episodes. It's prevalence is increasing fast in high- and middle-income countries. According to the Russian Respiratory Society, asthma affects as many as 10 million people in Russia; over 20 % of them are children [1].

It was observed that respiratory infections have a more severe course in patients with BA than in healthy individuals [2, 3]. Acute infections of the upper respiratory tract frequently trigger asthma exacerbations: about 85 % of exacerbations in children and 50 % in adults are caused by respiratory viruses [2]. Pathogens damage ciliated epithelium of the respiratory tract mucosa making it more vulnerable for allergens and toxins and maintaining bronchial hyperresponsiveness. Acute exacerbations can be life-threatening regardless of the BA grade of severity [3].

A lot of contemporary research studies focus on the in-depth analysis of BA pathogenesis, including the role of innate immunity components. Of particular interest is a new class of effector molecules (antimicrobial peptides), such as β-defensins. Antimicrobial properties of the latter are due to the electrostatic interactions between negatively charged surface components of the bacterial membrane, such as lipopolysaccharides of gram-negative bacteria and teichoic or lipoteichoic acids of gram-positive bacteria, and a positively charged β-defensin molecule. Critical concentrations of β-defensin on the surface of the target cell trigger pore formation in its membrane followed by cell lysis. Besides, β-defensins exhibit immunoregulatory activity, participating in chemotaxis and adaptive immunity activation, inducing dendritic cell maturation, etc. [4].

The key role in protecting respiratory tract mucosa is played by human β-defensin-1 (HBD-1) synthesized by epithelial cells [5]. β-defensin-1 is encoded by the DEFB1 gene located on the short arm of chromosome 8 (8p23.1) in a highly polymorphic cluster. Due to gene mutations, its expression can be decreased; in turn, insufficient secretion of β-defensins facilitates bacterial adhesion to and invasion of the mucosa and triggers inflammation [6, 7].

Toll-like receptors (TLRs) of the epithelial cells of the respiratory tract mucosa are another important element of the innate immunity. They recognize pathogen-associated molecular patterns (PAMP) of microorganisms and their metabolic byproducts, transmit the signal into the cell and boost leukocyte functional activity, increase pro-inflammatory cytokine and interferon gene expression. The majority of bacterial and viral pathogens are recognized by TLR2, TLR4, and TLR9 that can activate the local mucosal immunity in the respiratory tract.

The aim of this work was to give a comprehensive assessment of the innate immunity markers, namely, the level of expression of the TLR2, TLR4, TLR9 and DEFB1 genes, and to study the association of some single nucleotide polymorphisms (SNPs) in the 5'-untranslated region of the DEFB1 gene with bronchial asthma in children. Three SNPs were studied: rs1799946, rs1800972 and rs11362. They are associated with HIV infection and infections caused by Candida albicans, Pseudomonas aeruginosa and other microorganisms and sepsis development [8, 9], but there are no reports on their association with allergies.

METHODS

The study was carried out in patients of the Rehabilitation
Care Unit for Children with Allergies and Respiratory Tract Diseases of the Scientific Center of Children’s Health (Moscow).
The study included 48 asthmatic children aged 3–7 years. The control group included 70 children without respiratory
conditions, inflammatory and infectious diseases and allergies. Nasal scrapes were collected at the time of BA exacerbations
that were accompanied by an acute respiratory infection.

For DNA extraction, the AmpLiPRIME Ribo-sorb kit (InterLabService, Russia) was used. The real time PCR assay
was conducted using SYBR Green I PCR Kit by Syntol, Russia. Data were statistically processed in MO Excel 2007 with
Statistica 10.0 software (StatSoft, USA). Pearson’s chi squared and Odd Ratio were computed (OR >1 indicated genotype
association with BA; OR <1 indicated a genotype protective against BA) [10].

Expression of the DEFβ1, TLR2, TLR4 and TLR9 genes was compared to β-actin gene expression. For RNA extraction,
the AmpLiPRIME Ribo-sorb kit was used. Reverse transcription was performed with the OT-1 kit by Syntol, real time PCR was
carried out using the SYBR Green I PCR Kit. For statistical processing, Mann-Whitney test was applied (p <0.05).

The study was approved by the Ethics Committee of Pirogov Russian National Research Medical University. Participants’
parents gave their informed consent.

RESULTS

Genotype frequency distribution of rs1799946, rs1800972 and rs11362 polymorphisms of the DEFβ1 gene showed that the following genotypes are associated with the risk of asthma in children: AA of rs11362 and CC of rs1799946; while genotypes GG and AA of rs1799946 and rs1200972 are protective against BA (fig. 1). Distribution of DEFβ1 alleles was alike in both groups.

Expression of the DEFβ1 gene was 3.5 times lower in children with bronchial asthma, compared to healthy children (fig. 2). A single nucleotide polymorphism in the promoter region can affect the level of gene expression and the amount of the produced protein. We divided patients of the experimental group into 3 subgroups based on the level of β-defensin-1 expression: low expression (>10,000 times higher than β-actin expression), moderate (10,000–30,000 times higher than β-actin expression) and high (>30,000 times higher than β-actin expression). It was found that AG genotype of rs11362 polymorphism is associated with the increased level of β-defensin-1 expression in epithelial cells. For example, the frequency of AG genotype in subgroups with high and low expression of DEFβ1 was 0.67 and 0.30, respectively. Genotype AA is associated with reduced expression of the β-defensin gene. Other genotypes of the studied polymorphisms are not associated with changes in the β-defensin gene expression. Patients with bronchial asthma showed a 19.5 times increased expression of the TLR2 gene compared to the controls; TLR9 expression was 9.5 times higher, TLR4 expression was 8.3 times higher. Results are presented in the table below.

DISCUSSION

The obtained data can indicate that chronic inflammation of the bronchial mucosa in asthmatic children is partially associated
with mutations in the 5’-untranslated region of DEFβ1. Having assessed the expression of DEFβ1, TLR2, TLR4 and TLR9,
DEFB1 gene are reliably associated with bronchial asthma in children. Genotype GG of rs1799946 and genotype AA of rs120097 polymorphisms are protective against asthma. Genotype AA of rs11362 polymorphism is also associated with the reduced expression of the β-defensin-1 gene DEF B1. Thus, some mutations in DEF B1 cause imbalances in the nasal mucosal innate immunity resulting in frequent exacerbations of BA in the setting of respiratory infections.

Fig. 3. Mechanism of chronic inflammatory response in bronchial asthma
When an allergen first comes in contact with the mucosa, it damages the epithelial barrier, which triggers cytokine secretion, including TSLP, IL-25 and IL-33. In the presence of cytokines, the secondary contact with the allergen induces maturation of dendritic cells (DCs), and their migration to lymph nodes, where DCs in collaboration with major histocompatibility complex molecules (MHC-II) “report” the allergen to Th0 cells (T-helpers) initiating their proliferation and differentiation into Th2 cells. Activated allergen-specific Th2s produce a wide range of cytokines: IL-4 increases proliferation of B-lymphocytes and serves as their growth and differentiation factor, induces B-cell class switching to IgE, IL-5 stimulates proliferation of eosinophils and facilitates release of the major basic protein (MBP) from eosinophils, and IL-9 (activates mast cells). Allergen-specific IgE antibodies bind to high-affinity receptors (FoerR1) of mast cells and basophils and to low-affinity receptors (FoerR2) of eosinophils and macrophages. In case of a repeated allergen invasion, IgE of mast cell membranes binds to the allergen, thus ensuring its degranulation. Not all pathogens can be eliminated by antimicrobial peptides if bacterial load is high. Part of them is recognized by epithelial TLRs of the respiratory tract sustaining bronchial inflammation.

References

Литература