

# ABSTRACTS

## SECOND MOSCOW MOLECULAR ALLERGOLOGY MEETING (MMAM 2019)

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# ON THE ROLE OF INTESTINAL MICROBIOTA IN THE DEVELOPMENT OF FOOD ALLERGY FROM THE PERSPECTIVE OF MODERN INTESTINAL MICROBIOLOGY

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Food allergy is an adverse immune reaction to food that can be mediated by IgE or other immune mechanisms. The IgE-mediated food allergy aetiopathogenesis of which still remains unknown becomes more and more widespread [1, 2]. There are a couple of ideas as to what forms a food allergy: genetic and environmental influences that are defined as mediators of risk of food allergy development.

The IgE-mediated food allergy affects about 5% of adults and 8% of children [3]. Food allergy is a disease that manifests itself at an early age. Research on the influence of early food introduction on the development of food allergy has revealed sensibility and clinical reactivity to eggs and peanuts in infants as early as 4 months old [4, 5, 6]. Ecological factors of food allergy risk probably play the main role in antenatal or in early postpartum periods.

The modified risks of food allergy include delivery type, breast, bottle or mixed feeding, presence of older siblings, visiting preschool institutions and domestic animal influence [7]. The research results confirm the “hygiene” theory where the lack of proper bacteria exposure at an early age leads to allergic diseases. There are data that each of these factors affect the intestinal microbiota structure [8-12]. In this regard, it is safe to assume that microbial colonization aetiopathogenetically may cause increased susceptibility to food and play a certain role in the mechanism of food allergy.

T. Yatsunenکو and others used 16S RNA data received from fecal samples of geographically various populations to show that the greatest interindividual variability of microbe structure occurs within the first 3 years of child’s life [13] regardless of the fact that some of the microbes that are found in meconium prove colonization of the child at birth, when microorganisms from the body surface of the mother meet those of the surrounding environment [14]. There is a model of microbe colonization at an early age turning from aerobic to anaerobic. In newborns predominance of proteobacteria (*Escherichia*, *Shigella*) which progresses into dominating actinobacteria (for example, bifidus bacteria), with the subsequent acquisition of adult domination of firmicutes and bacteroids [9, 15, 16] is observed. The maturation of the baby’s microbiota is primarily due to the cessation of breastfeeding. Speed of maturing of microorganisms depends on some environmental factors. [17, 16].

# STUDY OF THE IMMUNOGENIC PROPERTIES OF GRANULATED FORM OF THE MIXED-ALLERGEN FROM HOUSE DUST MITES DERMATOPHAGOIDES PTERONYSSINUS AND DERMATOPHAGOIDES FARINAE

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Sublingual immunotherapy (SLIT) is not only a clinically effective, but also a safe method of treatment of patients with atopy. SLIT is connected with the exposure of allergen in sublingual mucous. Clinically it is presented by on early tranzitorny increase the level antigen - specific IgE, moderate increase antigen - specific IgG4 in blood serum and permanent increase secretory IgA in secretion mucous; increase IL-10 and transforming growth factor beta. Of this research was studying properties of new medical preparation which is deposited on sugar granules and represented the mixed-allergen from Dermatophagoides pteronyssinus (Der.p.) and Dermatophagoides farina (Der.f.). This experimental medical allergen (EMA) is intended for SLIT.

**OBJECTIVE:** To estimate immunogenicity of the received EMA.

**METHODS:** Two groups of Guinea pigs were examined. The first group of animals (n=10) was orally immunized by EMA. The second (control) group of animals (n=10) received the sugar granules. Level of specific IgG to Der.p. and Der.f. in serums of animals was determined by ELISA. The results were processed using a package of statistical software "Microsoft Excel" version 2010. The reliability of differences in groups was estimated by Fischer dispersion analysis method. Results By the ELISA is shown that in animals serum receiving EMA we can see a statistically significant increase of optical density level (O.D.) of specific IgG to Der.f. (O.D.-1,381±0,322) in comparison with control group (O.D. – 0,515±0,28), p <0.01. Similar results were received also when the level of optical density of specific IgG to Der.p. in serum of animals, receiving EMA (O.D – 1,434±0,4), was compared with serum of animals from control group (O.D – 0,549±0,305), p <0.01.

**CONCLUSION:** The received EMA has an immunogene activity that is especially important for achievement of high therapeutic effect when carrying out ASIT.

# EVALUATION OF IGE+ B LYMPHOCYTES IN ALLERGIC PATIENTS AFTER ALLERGEN EXPOSURE

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The characterization of IgE-producing cells in allergic patients has been hampered by their low number and difficulties to identify them by immunological staining protocols due to the presence of several IgE-positive cell populations in the blood of allergic patients. Allergy to birch pollen and in particular to the major birch pollen allergen Bet v 1 is the most common form of allergy in the Moscow region of Russia due to the high prevalence of birch trees and the heavy exposure to birch pollen in this region. In our study we have developed a staining protocol for IgE-producing lymphocytes and assessed the appearance of IgE-positive cells in patients allergic to Bet v 1 and control subjects with allergy to other allergen sources and non-allergic individuals before, during and after the birch pollen season in the Moscow region.

**METHOD:** Peripheral blood mononuclear cells were isolated from whole blood by density-gradient centrifugation from Bet v 1-allergic donors and control subjects. IgE-producing cells were identified by staining with CD19-FITC and Omalizumab-APC because Omalizumab does not react with cells containing IgE bound to FcεRI or CD23.

**RESULTS:** The described staining conditions allowed us to detect an extremely small B lymphocyte subpopulation of IgE+ cells (approximately 0.23%, from B lymphocytes). In birch pollen allergic patients the identified B lymphocyte subpopulation was low before the pollen season and transiently increased during the pollen season whereas it remained low in patients allergic to allergens other than Bet v 1 and in non-allergic subjects. Our results are similar to those obtained for subjects who underwent vaccination and showed increases of the number of plasmablasts shortly after vaccination indicating stimulation of antigen-specific memory cells and appearance of plasmablasts in the circulation.

**CONCLUSION:** We developed a staining protocol for identifying a small B cell subpopulation which seems to be responsible for IgE production in allergic patients after allergen-specific stimulation. Further work will be dedicated to the development of protocols for identifying the allergen-specificity of these cells to study their role in the secondary allergen-specific IgE response to discover new targets for treatment of allergy.

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## LOW-DOSE MODEL OF ALLERGY IN MICE

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The development of type I allergy, mediated by the formation of IgE to safe respiratory and food allergens, is directly related to the use of an adequate animal model. We previously showed that patients with allergy had significantly increased titers of specific IgE, but not IgG or IgA, compared to healthy controls. Current mouse models of allergy use high-dose adjuvant priming, which leads to the formation of both IgE and IgG to the target allergen. Since natural sensitization is induced by very low concentrations of allergens, it is necessary to develop a model of mouse sensitization without high-dose priming. To develop such a model, we used sensitive BALB/c and resistant C57BL/6 mice, which were immunized with allergens in a phosphate buffer in the withers, intraperitoneally (ip) or subcutaneously (sc) in doses of 0, 1, 10, 100, 1000 and 10000 ng/injection two to three times a week for a long time. Ovalbumin (OVA) or recombinant proteins of house dust mites (Der f 2), fungus *Aspergillus fumigatus* (Asp f 2) and cow's milk (Bos d 4) were used as the model allergens. No IgE were detected in resistant C57BL/6 strain. No IgE antibodies were formed during sc immunization of BALB/c mice. The formation of IgE in BALB/c mice was registered during ip immunization or after immunization into the withers, starting after 17-20 injections. IgE titers increased upon more prolonged immunization. Maximal IgE titers were observed after immunization into the withers and in the dose of 100 ng/injection for all model allergens (IgE titers - 100-10000 depending on the allergen), which decreased by an order of magnitude at immunization dose of 10000 ng/injection. When immunized with a dose of 10 ng/injection, IgE appeared only in some mice. Formation of IgG was recorded in both BALB/c and C57BL/6; it directly depended on the immunizing dose and appeared starting from 100 ng/injection (IgG1 titers 2E3, 20E3 and 1000E3 in the control and groups 100 or 10000, respectively). The dominant class was IgG1; IgG2a titers were an order of magnitude lower, and IgG2b and IgG3 were absent. Accordingly, to form a humoral response, as close as possible to the one observed in patients with allergies, it is recommended to inject an allergen without adjuvant into the withers of BALB/c mice at a dose of 50-200 ng/injection two to three times a week during 2-4 months, which will result in the appearance of a specific IgE in titers 100-1000 and IgG1 in titers (50-100)E3.

## EOSINOPHILS IN ASPERGILLUS FUMIGATUS-INDUCED AIRWAY INFLAMMATION: ALLERGY OR NOT?

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Eosinophils are considered to be allergic inflammation markers. However, non-allergic eosinophilic airway inflammation is also described. The present study aimed to identify the nature of the eosinophil-mediated inflammation in the *Aspergillus fumigatus* extract-induced asthma model.

To induce allergic airway inflammation mice were treated with multiple oropharyngeal applications of low doses of *A. fumigatus* extract. A total number of *A. fumigatus* extract applications was 3 for one group and 6 for the other. Blood of mice was collected at 72 hours after each *A. fumigatus* extract application. To induce acute neutrophil-mediated inflammation mice received one high dose oropharyngeal *A. fumigatus* extract application. Bronchoalveolar lavage (BAL) cell numbers were measured by both flow cytometry and cyto-spin staining. Cytokine/BAL fluid levels were measured by ELISA. Allergen-specific immunoglobulin-mediated responses in BAL fluid and peripheral blood: allergen-specific IgG and IgA and total and allergen-specific IgE, were estimated using ELISA.

No eosinophil blood level elevation was observed during the first week of the experiment. However, at 72 hours after the 3rd low dose *A. fumigatus* extract administration, the number of eosinophils in the blood significantly elevated and reached 20% of total blood cells. BAL and bone marrow cell analysis also demonstrated significant eosinophil elevation. While bone marrow eosinophil count reached 20% of total bone marrow cells after the 3rd administration and did not change significantly after 6 administrations, the percentage of BAL eosinophils increased significantly between the 3rd and the 6th administration:  $50.69 \pm 2.83$  versus  $74.23 \pm 5.62$ , respectively. Serum allergen-specific IgG and IgE and BAL fluid allergen-specific IgA were slightly but not significantly elevated both after 3 and after 6 administrations. Significant elevation of BAL fluid IL-4 was detected only after 6 low doses *A. fumigatus* extract applications.

Thus, we showed that the local application of *A. fumigatus* extract induces eosinophilia without strongly pronounced Th2-mediated response activation.

The study was supported by RFBR № 18–315–00166.



# EVALUATION OF THE DATA OF COMPONENT DIAGNOSTICS AND AEROPALINOLOGICAL MONITORING OF POLLEN IN THE ROSTOV REGION OF SOUTHERN RUSSIA IN PATIENTS WITH SEASONAL ALLERGIC RHINITIS

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**BACKGROUND:** According to epidemiological data on the prevalence of allergic diseases in Rostov and Rostov region occupies a special place allergic rhinitis. In this regard, to date, the greatest interest is, as the evaluation of data component diagnostics, so the study of qualitative and quantitative levels of allergenic pollen and fungal spores in the air using palynological analysis.

**AIM:** In the study for the past year in patients suffering from seasonal allergic rhinitis, the profile of sensitization was studied.

**METHODS:** physical methods of examination, molecular diagnostics of allergen extracts by Phadia IDM «ImmunoCAP-100», impact-volumetric trap «Burkarg Pollen Trap».

**RESULTS:** 671 patients were examined, of which 0.35 and more results were revealed: w230 (n Amb a 1) 85 people – 13,0%; w 231 (n Art v1) 60 people-8,9% m 229 ( r Alt a 1) 78 people -11,6%; g 213 ( r Phl p1/r Phl p 5b) 51 people -7,6 %; t 215 ( r Bet v 1) 24 people-3,6%. g 214 ( r Phl p 7/ r Phl p 12 ) 6 people – 0,9%; t 213 ( r Bet v 2/ r Bet v 4) 2 people – 0,3%. It should be noted that Alt a 1 – the main allergen of Alternaria - is associated with the development of asthma. According to our aeropalino logical monitoring in the atmosphere prevails concentration: Alternaria alternata (109 in 1 m<sup>3</sup> of air) and Cladosporium herbarum (6082 in 1 m<sup>3</sup> of air) - on average per year. Spores of fungi are ubiquitous in the air, it is almost impossible to avoid exposure. Cross-reactivity is observed between Alternaria and Cladosporium due to the presence of homologous allergens (Alt a 10 and Cla h 3), but only about 50% of patients respond to both Cladosporium and Alternaria (Valenta R., 2012). Cladosporium herbarum spores are more common worldwide, and are the dominant volatile spores in many territories (Vijay H.M., 2004).

**CONCLUSION:** Given that mold sensitization is a powerful risk factor for severe rhinitis, asthma development, as well as the dominance of volatile Cladosporium herbarum spores in the atmosphere revealed by component diagnostics and aeropalino logical monitoring, it is recommended to further study the Cladosporium allergen and its effect on the occurrence and course of allergic rhinitis and bronchial asthma.

# THE POSSIBILITY OF COMPONENT DIAGNOSTICS IN CLARIFYING THE STRUCTURE OF SENSITIZATION IN PATIENTS WITH BRONCHIAL ASTHMA AND ALLERGIC RHINITIS LIVING IN THE SOUTH OF RUSSIA

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The patients, who have asthma (As) accompanied with allergic rhinitis (AR), oftener suffer from As exacerbations and more frequently have to go to polyclinics and hospitals because of As than asthmatic patients without AR, as a rule, the more severe rhinitis, the more severe the course of As itself.

Identification of the level of sensitization to cause-significant allergens in patients with a combination of As and AR living in the South of Russia.

A complex study of 54 patients – 33 (61,1 %) females and 21 (38,9 %) males - with AR (seasonal (SAR) n=37 and chronic (CAR) n=17) and moderately severe As at the age of 19-54. The methods were as follows: physical methods of examination, spirometry («SPIROSFT-3000»), asthma control questionnaire (ACQ-5), endoscopic study of the nasal mucosa, molecular diagnostics of allergen extracts by Phadia IDM «ImmunoCAP-100». For statistical analysis of the obtained data MS Office Excel software was used.

All the patients complained of itching in mucosal coatings of their eyes and nose, nasal watery discharge, periodical nasal stuffiness, difficulty breathing. Oral allergy syndrome (OAS) was diagnosed in 7 cases; results of the test on As signs and symptoms revealed an insufficient control over As (ACQ-5:  $2,75 \pm 0,5$  points). Study of their life history found out an aggravated hereditary background of AR in 21 patients. Clinical manifestations were accompanied with a reduced function of the lungs (FEV1:  $69,0 \pm 3,2$  %). Results of molecular diagnostics were as follows: high titres of Amb a 1 were found out in 12 patients with SAR (32,4%) ( $15,1 \pm 2,5$  kUa/L), 8 cases (21,6%) had a combination of Amb a 1 + Art v 1 ( $19,1 \pm 1,92$  kUa/L and  $17,1 \pm 0,76$  kUa/L), respectively, 5 cases (13,5%) - Phl p 1 ( $16,2 \pm 2,44$  kUa/L) and Alt a 1 ( $4,7 \pm 1,42$  kUa/L) - the main allergen of Alternaria associated with the development of asthma. 17 patients with CAR underwent endoscopic of their nasal mucosa that dryness, hyposmia, hyperplasia of the inferior nasal concha as well as high values of Derp 1 ( $4,2 \pm 1,4$  kUa/L) in 6 cases (35,3%), Alt a 1, n=4 (23,5%) ( $3,8 \pm 1,6$  kUa/L) and of Feld 1 ( $3,5 \pm 1,4$  kUa/L) in 3 (17,6%) ones.

Molecular diagnostic procedures along with standard methods of study of AR and As make it possible to understand the structure of sensitization, improve control over As, alleviate signs and symptoms of AR as well as reduce the necessity of provocative testing and make it possible to provide AIT on a reasonable basis.

# MECHANISMS OF ALLERGIC BRONCHIAL ASTHMA DEVELOPMENT

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Epidemiological data indicates an increase in the prevalence of bronchial asthma. According to the report of the Global Asthma Network, in 2019, more than 330 million people suffer from this disease, of which 14% are children.

Bronchial asthma (BA) is a heterogeneous disease that is characterized by chronic inflammation of the airways and diagnosed by respiratory symptoms, combined with reversible airway obstruction. The most common phenotype is allergic BA, which usually begins in the childhood, it is associated with the presence of other allergic diseases of the patient or it's relatives, it is also characterized by eosinophilic inflammation of the respiratory tract.

The Global Initiative for Asthma recommends distinguishing between atopic allergic BA (due to the development of IgE-dependent reactions) and non-atopic allergic BA (as a result of non-IgE-dependent reactions, including infectious-allergic BA). In most cases, the development of allergic BA is due to an allergic reaction of I type (anaphylactic reaction) according to Gell-Coombs classification, in which IgE and IgG4 are involved.

The pathogenesis of allergic BA includes few phases. In the immunological phase, under the influence of an allergen, B-lymphocytes secrete specific antibodies. In the pathochemical stage the allergen, entering the patient's body again, interacts with antibody reagents on the surface of target cells, then labrocytes and basophils are getting degranulated, eosinophils are activated with the release of a large number of allergy and inflammation mediators, which cause pathophysiological stage, specifically the development of bronchospasm, swelling of the mucous membrane, infiltration of the bronchial wall with cellular elements, inflammation, hypersecretion of mucus.

Sometimes allergic BA can develop as an allergic reaction of type III (immunocomplex type) may occur with the formation of IgG and IgM. Subsequently, an antigen-antibody complex is formed, so complement is activated, the release of lysosomal proteolytic enzymes and inflammatory mediators from macrophages, neutrophils, platelets, initiation of the kinin and coagulation systems. As a result, patients develop edema and inflammation of the bronchi, bronchospasm.

Currently, drugs have been developed that allow achieving remission of BA, but it is not always possible to control the course of the disease. It is necessary to study the molecular mechanisms of BA to identify therapeutic targets and develop new drugs.

# PREDICTING FACTORS OF SUBLINGUAL IMMUNOTHERAPY EFFICACY IN CHILDREN WITH POLLEN-FOOD ALLERGY SYNDROME

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**BACKGROUND:** The prevalence of pollen-food allergy syndrome (PFAS) is continuously increasing. Therefore, it is relevant to study the predictors of the effectiveness of sublingual immunotherapy (SLIT) both in relation to symptoms of hay fever and in relation to food allergies.

**OBJECTIVE:** To study the possibility of prediction of SLIT efficacy among children with birch pollen allergy and PFAS with different IgE profiles to recombinant component-resolved allergens (CRA).

**METHODS:** The study included 161 children (5 – 18 years old) with birch pollen allergy and PFAS. The sIgE levels to birch pollen and to CRA (Bet v1, Bet v2, Bet v4, Bet v6) were identified using Immuno Cap. Children received 2 courses of SLIT by birch allergen extract: 1 group (n=125) with Staloral, 2 group (n=36) with Microgen (non-standardized). For evaluation of SLIT clinical efficacy we used standardized scale recommended by EAACI (total symptoms score (TSS), medication score (MS), combined symptom medication score (CSMS)) and national Ado scale.

**RESULTS:** 46,58% of patients had monosensitization to Bet v1 component. The rest 53,4% had combinations of IgE to Bet v1 and IgE to one (44,09%), two (7,45%), or three (1,86%) minor allergens. The severity of symptoms of seasonal allergic rhinoconjunctivitis and the need for pharmacotherapy decreased in both groups. The difference between the estimated values before and after 2 courses of SLIT in group 1 was: TSS (-1,02); MS (-0,58); CSMS (-1,60) points; in group 2: TSS (-0,88); MS (-0,28); CSMS (-1,16) points ( $p < 0,01$ ). Monosensitization to Bet v1 was associated with high efficiency of SLIT ( $p = 0,0001$ ). Sensitization to Bet v2 and Bet v4 was combined with a low SLIT efficacy ( $p = 0,0002$ ). The presence of IgE to Bet v6 did not reveal a statistically significant difference in the clinical efficacy of SLIT. SLIT by Staloral unlike SLIT by Microgen reduced the severity of both clinical symptoms of hay fever and PFAS ( $p = 0,0001$ ). Daily dosage regimen at the maximum recommended dose was a positive prognostic factor of treatment efficacy ( $p < 0,01$ ).

# THE DIAGNOSIS OF BRONCHIAL ASTHMA IN RISK GROUPS OF YOUNG AND PRESCHOOL-AGE CHILDREN

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**BACKGROUND:** Epidemiological studies held in different countries have shown that the diagnosis of bronchial asthma (BA) in children is late by an average of 4 years and more. Therefore patients don't receive the necessary therapy in a timely manner and don't follow certain recommendations, which may all lead to the progression of the disease. The differential diagnosis of acute obstructive bronchitis (OB) and bronchial asthma, especially with early symptoms, is hard to achieve due to a similar clinical picture, similar X-ray, functional and laboratory (as well as immunological) changes.

**AIM:** to reveal the most informative laboratory criteria of early BA diagnosis in children aged 5 years and younger by using the method of inducing sputum and determining cellular composition in induced sputum.

**MATERIALS AND METHODS:** Throughout the 2006-2016 period children aged 1-5 (n=157 children) were studied: with BA revealed for the first time (n=21), with episodes of acute OB in the anamnesis (n=85), with atopic dermatitis without episodes of acute OB in the anamnesis (n=34), and practically healthy children without allergies and episodes of acute OB in the anamnesis (n=17). All children were tested for the cellular composition of induced sputum. 54 kids from the group with OB in the anamnesis took part in the 18-month cohort surveillance, and then 36 kids from the group with OB in the anamnesis but no BA diagnosis were re-examined in 10 years.

**RESULTS:** An elevated level of eosinophils in induced sputum ( $\geq 2,5\%$ ) was discovered in no less than a third of children in BA risk groups (children with atopic dermatitis and those with obstructive bronchitis in the anamnesis) and in 100% of children with BA revealed for the first time; analyzed immunological parameters of children's induced sputum in these groups was the same. The analysis of data after a ten-year observation of children who had developed bronchial asthma during this period revealed a level growth of eosinophils in induced sputum in the range of  $< 2.5\%$  to  $> 2.5\%$  in 81% of children, which is characteristic for the eosinophilic type of the bronchi's mucous membrane inflammation. For children without bronchial asthma in 10 years from the beginning of the study a decrease in induced sputum eosinophils levels was detected. Thus, a  $\geq 2,5\%$  level of eosinophils in induced sputum can be recommended as an available and reproducible biomarker for early diagnosis of bronchial asthma in children.

# SCREENING DIAGNOSIS ALGORITHM IN CHILDREN WITH BRONCHOOBSTRUCTIVE SYNDROME

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From November 2018 to May 2019 on the basis of State health care institution of the republic of Bashkortostan Ufa city Children's Clinical hospital No. 17, we examined 78 patients aged 5-18 years, hospitalized for inpatient care with diagnoses of bronchial asthma and acute obstructive bronchitis. At the first stage of the checkup, patient selection was carried out (i.e. the presence of signed voluntary consent of the patient's parents; children who were not examined by molecular allergy diagnostics). Also, anamnestic, routine laboratory and laboratory-instrumental information was studied. At the second stage of the study, all children underwent an allergic diagnostics screening test using the Phadiatop ImmunoCap method with interpretation of the results using a semi-quantitative scale divided into PAU / I sensitization classes (kUa / 1). The number of children diagnosed with acute obstructive bronchitis was 46 (59%); with bronchial asthma - 32 (41%). The number of boys in the observation groups was 42 (54%), girls - 36 (46%). The average age was 9 years. In all children, acute obstructive bronchitis was caused by a respiratory viral infection. To assess the history of all children with acute obstructive bronchitis, an analysis of the modified asthma predictive index (API) was performed, according to which 39 (85%) children noted the presence of more than 3 (4) episodes of wheezing respiration in a year; 25 (54%) children have one of the parents had a confirmed diagnosis of bronchial asthma; 21 (46%) children confirmed the diagnosis of atopic dermatitis and / or allergic rhinitis, and 31 (67%) children previously had a sensitivity to inhalation and food allergens. An analysis of the results of determination of specific IgE for respiratory allergens showed that 39 (85%) children with a diagnosis of acute obstructive bronchitis had sensitization to inhalant allergens, and 23 (59%) children had the indicator corresponded to a high and very high level. In children with bronchial asthma, sensitization was found in 27 (84%). Comparing the analysis data of the modified bronchial asthma development index and the screening data, the diagnosis of acute obstructive bronchitis in 39 (85%) children was changed to the diagnosis of bronchial asthma. Thus, the allergy diagnostic test using the Phadiatop ImmunoCap method can be used as a primary diagnostic method in children with bronchoobstructive syndrome for early verification of bronchial asthma diagnosis.

# IMMUNITY IN GASTROINTESTINAL PATHOLOGY IN CHILDREN

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**OBJECTIVE:** to study the state of immunity in gastrointestinal pathology in children.

**MATERIALS AND METHODS:** 120 sick children aged 14 to 18 years were examined. The control group consisted of 30 healthy children of the corresponding age.

**DISCUSSION:** The structure is dominated by peptic ulcer and duodenal ulcer (UD) - 46 (38.3%), chronic gastroduodenitis (CGD) - 33 (27.5%), chronic cholecystitis (XX) - 21 (17.5%). Chronic hepatitis (CG) - 11 (9.2%), chronic enterocolitis (CE) - 9 (7.5%) were found. The immunological parameters of the blood of sick children with ulcer showed a decrease in the concentration of CD3 + and CD4 + lymphocytes to  $41.0 \pm 0.1\%$  and  $23 \pm 1.0\%$  ( $P < 0.05$ ) against the control -  $48.0 \pm 0.1\%$  and  $34.5 \pm 0.1\%$ , respectively. The concentrations of CD8 +, CD16 +, CD20 +, CD23 +, CD25 lymphocytes (in%) and HLA-DR + (in%) were significantly increased in relation to the control group in all groups of examined patients. Consequently, an increase in suppressor and killer activity, as well as the process of antibody formation, is observed during exacerbation of both ulcer and CGD and XX in children. The study of immunoglobulins showed a significant increase in IgG level by 1.3 times with YB -  $565 \pm 11.0 \mu\text{m} / \text{ml}$  ( $P < 0.05$ ) and CGD -  $545 \pm 9.0 \mu\text{m} / \text{ml}$  ( $P < 0.05$ ) and 1, 7 times with XX -  $730 \pm 5.0 \mu\text{m} / \text{ml}$  ( $P < 0.05$ ) against the control -  $420 \pm 13 \mu / \text{ml}$ . Against the background of an increase in IgG in the blood, a decrease in the concentration of IgA is noted ( $P < 0.05$ ). IgM increases with ulcer by 1.3 times -  $175 \pm 1.5 \mu\text{m} / \text{ml}$ , 1.2 times with CGD -  $165 \pm 4.5 \mu\text{m} / \text{ml}$  and reduced to  $112.0 \pm 5.0 \mu\text{m} / \text{ml}$  ( $P < 0.05$ ) with a control ratio of  $135 \pm 3.5 \mu\text{m} / \text{ml}$ . There is an increase in IgE concentration by 22.5 times more with YB -  $450 \pm 11.0 \text{mU} / \text{ml}$  ( $P < 0.001$ ), 16.3 times more with CGD -  $325.5 \pm 1.5 \text{mU} / \text{ml}$ , and even more 32.5 times greater with XX -  $650 \pm 11.0 \text{IU} / \text{ml}$  against control values -  $20.0 \pm 1.2 \mu / \text{ml}$ . A high concentration of reagins in the blood at the same time indicates microbial and parasitic sensitization.

**CONCLUSION:** The observed immunological imbalance in gastroenterology is not accidental, it can occur against the background of food, microbial and parasitic sensitization, in most cases it leads to an aggravation of the course of diseases and, of course, requires not only timely diagnosis of combined lesions, but also adequate complex therapy.

## **MILK-SPECIFIC IGE REACTIVITY WITHOUT SYMPTOMS IN ALBUMIN-SENSITIZED CAT ALLERGIC PATIENTS**

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**BACKGROUND AND OBJECTIVES:** Up to 20% of the population in Russia and Scandinavian countries is sensitized to cat which is one of the most important allergen sources in these countries. In a population study conducted in Moscow, we noted that milk albumin represents a frequently recognized allergen. The objective of our study was to investigate if sensitization to milk albumin may be a result of cross-reactivity to cat albumin and if this sensitization is related with symptoms of milk allergy.

**METHODS:** In a cohort of patients with cat allergy we identified 15 patients with IgE sensitization to cat albumin by tested for IgE reactivity to recombinant and purified cat allergens by ImmunoCAP measurements. These patients were then tested for IgE reactivity to milk allergen extract, milk, albumin and a screening test for food allergy, i.e., fx5 by ImmunoCAP.

**RESULTS:** We found that seven out of the 15 cat albumin positive patients showed IgE reactivity to milk albumin and milk allergen extract and five of them were positive in the fx5 test. None of these patients had any symptoms of cow's milk allergy.

**CONCLUSION:** Our results show that cross-reactivity of IgE from cat albumin sensitized patients with milk albumin can lead to false-positive IgE serology with milk allergen extracts. Therefore, IgE testing for milk allergy should be conducted with purified and/or recombinant milk allergen rather than with milk allergen extract.

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## QUANTIFICATION OF ALLERGEN-SPECIFIC IGE IN PATIENTS WITH BIRCH AND CAT ALLERGY BY ELISA

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**BACKGROUND:** The measurement of allergen-specific IgE (asIgE) levels is important for allergy diagnosis. Quantitative assays such as the ImmunoCAP technology can only be run on special instruments consume rather large volumes of serum and are expensive.

**METHODS:** To establish a standard for the measurement of human asIgE we have expressed and purified a human monoclonal IgE antibody specific for the major birch pollen allergen, rBet v 1 (IgE<sub>moAb</sub>) and the corresponding allergen. Using IgE<sub>moAb</sub> and rBet v 1 we established by ELISA a standard curve allowing to measure and quantify asIgE. Using a set of sera from clinically well-characterized birch pollen and cat allergic patients Bet v 1- and Fel d 1-specific IgE levels were quantified with the IgE ELISA and compared with quantitative IgE ImmunoCAP measurements and results obtained by ImmunoCAP ISAC chip

**RESULTS:** We have established a standard curve for quantification of asIgE with a linear range covering approximately 6 ng/ml to 240 ng/ml. Allergen-specific IgE levels determined by the quantitative IgE ELISA correlated well with quantitative IgE levels determined by ImmunoCAP and ImmunoCAP ISAC. The quantitative IgE ELISA can be performed with simple ELISA equipment and requires less than half of the amount of serum required for ImmunoCAP measurements.

**CONCLUSION:** We have established a simple quantitative IgE ELISA which allows robust quantification of asIgE in the nanogram/ml range.

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# ANALYSIS OF THE NOSOLOGICAL STRUCTURE OF ALLERGIC DISEASES ASSOCIATED WITH THE ACTIVE ATYPICAL PROGRESSION OF CHRONIC MONO- AND MIXED- HERPES VIRUS INFECTION

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**INTRODUCTION:** The incremental increase in the number of allergic diseases (AD) among population necessitates the early detection of clinical and laboratory markers of this pathology which is often associated or going with active progression of chronic viral infections caused by persistent viruses, and in particular the Herpesviridae family viruses.

**MATERIALS & METHODS:** Under our supervision there were 481 people of both sexes from 20 to 79 years suffering from mono- and mixed- atypical chronic active (ACA) HVI. In addition to traditional methods of examination (signs, history, physical examination, blood test, etc.), serologic tests (IgM / IgG VCA EBV, IgM CMV, IgG CMV, IgM HSV1 / 2, IgG HSV1 / 2), PCR method for detecting the genome of viruses in biomaterials (blood, saliva, urine, scraping from tonsils and the posterior pharyngeal wall). For the detection of laboratory markers of an allergic process (total Ig E, special Ig E to various allergens, ECP, etc.) in the patients' blood serum, ELISA and ImmunoCAP (allergic tip) methods were used.

**RESULTS:** According to the data obtained, 49% and 51% of the examined patients suffer from mono- and mixed-Herpes virus infection (HVI), respectively. Among all patients with ACAI and AD 70.4% of cases (342 patients) is detected. At the same time, in patients with mono-HVI, AD is observed in 67.6% and in patients with mixed-HVI – in 73.1% of cases. The dominant nosological forms among patients with both mono- and mixed-HVI are allergic rhinitis, allergic pharyngitis, allergic tracheitis – 43.0 %; atopic dermatitis and allergic skin lesions (acute and chronic urticarial) – 38.9%; drug allergy – 24.5%; food allergy (intolerance) – 23,9%; allergic conjunctivitis – 21.8%; bronchial asthma – 7%.

**CONCLUSION:** Our findings suggest that the prolonged course of ACA HVI is associated with either/as the development or/as active phase of various nosological forms of allergic diseases, which negatively affects the quality of patients' life, contributes to the development of multiple comorbid conditions, leads to polypharmacy and negatively affects the duration and effectiveness of immunotropic therapy.

# **PAPP-A2 BLOOD CONCENTRATIONS IN NON-PREGNANT PATIENTS: POTENTIAL LINK WITH CYTOKINES, OBESITY AND ASTHMA**

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The pregnancy-associated plasma protein A2 PAPP-A2 is homolog of PAPP-A (its sequence identity with PAPP-A is 46 % and it can cleave IGFBP-5 (insulin-like growth factor binding protein) not IGFBP4 to enhance IGF signaling. It is a member of pappalysins subfamily of metalloproteinases (Overaard M.T. et al., 2001)) As cytokines can regulate its expression, as release of cytokines can be result of IGF signal.

Our aim was to estimate PAPP-A2 level in blood of patients with different inflammatory and proliferative diseases.

**MATERIALS AND METHODS:** With DRG ELISA kit we have analyzed 80 serum samples from 8 pregnant women 20-40 years old, 5 patients with Hepatitis C, 40 patients with different thyroid diseases, 10 women with osteoporosis and 3 with adrenal dysfunction. Also 2 men with prostatic hyperplasia and 2 men with liver cirrhosis were included. A control group contained 8 healthy women and 2 men. We modified this ELISA manual to measure low PAPP-A2 concentration in non-pregnant patients.

**RESULTS:** PAPP-A2 in serum of pregnant women was  $10 \pm 1$   $\mu\text{g/ml}$ . The most of another patients and control group have no PAPP-A2 even after serum 5-fold concentration. But we estimate  $0.2 \pm 0.01$   $\mu\text{g/ml}$  (low range of method) PAPP-A2 from 3 patients with autoimmune thyroiditis (AIT), 2 men with prostatic hyperplasia and 2 men with cirrhosis and 2 women with non-renal hyperparathyreosis. 3 PAPP-A2 positive patients have bronchial asthma.

**DISCUSSION:** After that we read IL 6 is associated to IGF-1Ec upregulation (Armakolas A. et al, 2019) from prostate tumors. Similar IL 6 and IGF1 signaling by osteocytes play an important role in the muscle and bone metabolism both in atherosclerosis (Woefle J. et al., 2011). Functional polymorphisms of IL5, IL6, IL13 genes are associated with asthma development (Bulut I. et al., 2018)

**CONCLUSION:** Blood concentration correlates with the PAPP-A2 expression analysis and PAPP-A2 can unite cytokines and signal pathways regulation in inflammatory and proliferative diseases and aging.

## INCREASE BASOPHIL SENSITIVITY TO MAL D 1 EXPOSURE AFTER BIRCH POLLINATION SEASON

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**BACKGROUND:** A detailed characterization of human immune cells is needed to better understand mechanisms associated with allergen capture following oral exposure. Our study aimed to evaluate the predictive value of the basophil activation test (BAT) in pollen-associated food allergy.

**MATERIALS AND METHODS:** 27 patients from 12 to 59 years [Me = 30;  $\pm\sigma=11,12$ ], 17 males and 10 females, were involved in the study and formed 2 groups. Group 1: 13 patients sensitized to birch pollen with oral allergic syndrome (OAS) to apple. Group 2: 14 patients sensitized to birch pollen without OAS to apple.

BAT was determined in blood samples with BeckmanCoulter Allergenecity Kit with 2 purified recombinant allergens (Bet v 1 and Mal d 1, MERC) in 3 concentrations each (1 ng/ml, 10 ng/ml, 100 ng/ml) before and after birch pollination season. Flow cytometry analysis was performed with BD FACS Canto II.

**RESULTS:** Patients from group 1 showed higher basophil sensitivity to Bet v 1 and Mal d 1 than patients from group 2 before and after birch pollination season. Before the season basophils were much more sensitive to Bet v 1 than to the cross-reactive Mal d 1. After the season basophils became more sensitive to Mal d 1 in both groups.

**CONCLUSION:** Birch pollen allergic patients with OAS showed significantly higher basophil sensitivity to Bet v 1 and the cross-reactive Mal d 1 allergen than patients suffering only from respiratory birch pollen allergy without OAS before and after pollen season. There was no significant difference in sensitivity of basophils to Bet v 1 exposure before and after the season. There is tendency to increase in sensitivity of basophils to Mal d-1 exposure after the pollination season.

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# NOVEL ATYPICAL COMBINATION OF CLINICAL MANIFESTATIONS FOR COELIAC DISEASE

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**INTRODUCTION:** Celiac disease (CD) is genetic autoimmune disorder triggered by consuming gluten and is characterized by pathognomonic enteropathy and wide range of clinical manifestations. Extra-digestive manifestations appear more common nowadays and may be associated with a delay in diagnosis.

**OBJECTIVES AND STUDY:** we report a case of an atypical combination of symptoms of celiac disease, resulting in long-term ineffective treatment. We present a clinical case of a 17-year-old girl who from the age of 9 has suffered from erosive HP-associated gastritis and erosive duodenitis. A triple course of eradication therapy was conducted. In this period the indicators of weight and height were age-appropriate. However, sometimes the patient was disturbed by headaches, unstable mood, weakness, fatigue. She did not have any intestinal manifestations. Menarche - at the age of 15. At 16 she was diagnosed with duodenal ulcer, complicated by cicatricial deformity of the bulb. Laboratory examination revealed mild iron deficiency anemia and antitransglutaminase IgA antibody (tTG) 415 U/mL (reference values < 20 U/ml), positive anti-EMA IgA,G; anti-DGP IgA 183 U/ml (reference values < 10 U/ml); anti-DGP IgG 131 U/ml (reference values < 10 U/ml); and severe subtotal villous atrophy on duodenal biopsy (stage 3B of the Marsh-Oberhuber classification).

Histological examination of the duodenal biopsy (Figure 1A) revealed flat surface with severe subtotal villous atrophy (stage 3B of the Marsh-Oberhuber classification). Integumentary epithelium with a diffuse increase in inter-epithelial lymphocytes, Paneth cells at the base of the crypts, an increased density of lymph-plasmocyte infiltration within the lamina propria were detected.

The therapy included adherence to a gluten-free diet, eradication therapy. A partial restoration of duodenal mucosa after 3 months (Fig. 1B).

Fig. Morphological changes on duodenal biopsy before (A) and after 3 months of compliance with a Gluten-Free Diet (B). Bars, 200 mkm.

**CONCLUSIONS:** The peculiarity of this case is an isolated ulcerative lesion of the duodenum without any intestinal manifestations. Efforts are needed to prevent long diagnostic delays and resulting complications, which may affect child development as well as final height and health in adulthood. Lack of awareness on different clinical presentations of CD may contribute to prolonged delays.

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# VITAMIN D AND VENTILATION LUNG FUNCTION IN PATIENTS WITH BRONCHIAL ASTHMA

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Among the various causes responsible for increasing the prevalence of allergic diseases, the role of vitamin D deficiency as a potentially important pathogenetic factor in allergies has also been emphasized in recent years. An indirect evidence of the above vitamin D value is the established fact that the highest incidence of allergies and asthma is recorded among the urban population, in large cities of economically developed countries.

Our study involved 80 patients with bronchial asthma, of varying severity. The control group consisted of 50 patients without bronchopulmonary pathology. All patients underwent a study of vitamin D and spirometry.

The average level of vitamin D in 80 patients with BA was significantly lower -  $18.83 \pm 3.73$  than in 50 subjects of the comparison group (without bronchopulmonary pathology) -  $27.47 \pm 3.65$  ng/ml ( $p = 0.001$ ). At the same time, the level of vitamin D in patients with BA with a late onset of the disease was significantly lower and amounted to  $15.07 \pm 6.79$  ng/ml than with a duration of BA more than 10 years -  $24.18 \pm 6.57$  ng/ml ( $p = 0,03$ ). Therefore, a low level of 25- (OH) D may be one of the risk factors for BA. The level of vitamin D was significantly lower in the group of patients with severe uncontrolled asthma than in the other groups. Moreover, the minimum vitamin D content in patients with severe BA was 5.088 ng / ml, which is a pronounced deficiency of 25 (OH) D and requires correction. Vitamin D deficiency is a marker of deterioration in the course of BA ( $r = -0.54$ ,  $p = 0.0002$ ), and leads to more and more severe exacerbations ( $r = -0.42$ ,  $p = 0.0003$ ). The correlation between the parameters of the lung ventilation function and the concentration of vitamin D were determined: vitamin D / FEV1 ( $r = 0.34$ ;  $p = 0.01$ ); Vitamin D / MOS50 ( $r = 0.33$ ;  $p = 0.01$ ); Vitamin D / MOS75 ( $r = 0.39$ ;  $p = 0.004$ ); Vitamin D / PSVavr. ( $r = 0.63$ ;  $p < 0.0001$ ). The revealed relationships confirm the results of studies on the effect of vitamin D deficiency on BA and prove the presence of receptors for this vitamin in the bronchopulmonary system.

**CONCLUSIONS:** Vitamin D deficiency can be considered a marker of both severe asthma and a factor that increases the frequency and severity of exacerbations.

# THE ISSUE OF LABORATORY DIAGNOSTICS OF ALLERGIC DISEASES OF OCCUPATIONAL ETHIOLOGY

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The occupational morbidity rate is a commonly recognized criterion for the impact of adverse working conditions on workers' health. A significant proportion in the development of occupational morbidity is of allergic origin. The leading nosological forms are: occupational bronchial asthma (47%), eczema (22%) and dermatitis (10%). The combination of prolonged exposure to adverse air factors of the working area, even at the level of maximum permissible concentrations, increases the risk of sensitization.

**PURPOSE:** To analyze the information content of diverse methods for the specific diagnosis of industrial allergens in patients with occupational allergy.

**Materials and methods:** a retrospective analysis of laboratory performance of 48 patients with suspected occupational allergy admitted to the clinic of Ufa Research Institute of Occupational Health and Human Ecology was performed. Among the examined there were healthcare workers - 17 (35.4%); agricultural workers (poultry farmers) - 11 (22.9%); construction workers (painters) - 5 (10.4%); processing workers (grinders, flour mills, bakers) - 6 (12.5%); electric power workers (insulators, operators) - 4 (8.3%); mining industry workers (operators) - 4(8.3%), others - 1 (2.0%).

To identify sensitization to occupational, food and drug allergens, an enzyme-linked immunosorbent assay for the determination of specific IgG and IgE using liquid biotilinated allergens (Dr. Fouquet, Germany) in blood serum was used.

**RESULTS:** Allergen-specific antibodies were detected in most of the examined individuals - 42 (87.5 ± 12.6%). Moreover, positive results for food allergens were detected in 36 (75.0 ± 10.8%); to drugs - in 4 (11.1 ± 1.6%); occupational - in 13 (27.0 ± 3.9%), including 8 (16.6 ± 2.4%) patients - to chloramine; 5 (10.4 ± 1.5%) - to latex.

**DISCUSSION:** The analysis showed that in 76.1% of cases, sensitization to two or more allergens was detected. Positive results on occupational allergens in 100% were accompanied by polyvalent sensitization to drug and food antigens. The combined presence of specific IgG and IgE antibodies to various allergens was found in 37.5% of samples, which may be associated with the formation of blocking antibodies or with the participation of various (IgE-dependent and non-IgE-dependent) mechanisms for the development of allergic reactions.

## PROLONGED COUGH IN CHILDREN

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In pediatric practice in recent years, the problem of lingering and chronic coughing has become increasingly significant, due to the persistent tendency to increase the number of children with these complaints, the absence of obvious or subclinical manifestations of the disease, the ineffectiveness of therapy, as well as the uncertainty of the prognosis. Of the infectious diseases that can cause a protracted cough, pertussis is the most significant. There is an annual increase in the number of children hospitalized in the department with a diagnosis of whooping cough, reparation period. More than half of the children were vaccinated. This is confirmed by the fact that the traditional DTP vaccine does not now guarantee 100% protection against pertussis, which may be associated with a change in specific proteins - antigens. We examined 1,200 children with a protracted cough who were examined and treated at the Department of Allergology - Immunology, GBUZ JSC CSTO named after N.N.Silishcheva "at the age of 4 - 17 years for 2016 - 2018 years. All children were divided into groups by age (1st group - 3-7 years old, 2nd group - 8-11 years old, 3rd group - 12-15 years old and 4th group - over 15 years old). The examination was carried out: general clinical tests, a study of nasal swabs, pharynx for flora and sensitivity to a / b, ELISA examination in a qualitative and quantitative way (immunoglobulins A, M, G) for pertussis and pertussis, studies on chlamydia, mycoplasmas, x-ray examination (or computed tomography) of the chest and sinus organs (if indicated). If in 2016, of all patients hospitalized in the department, the percentage of children with cough was 22.9%, in 2017 this figure increased to 28.1%, then in 2018 it amounted to 33.2%. Children between the ages of 8 and 11 are predominant, with no significant difference between girls and boys. With a more in-depth examination of children for pertussis, pertussis, there is also a tendency for an increase in the number of patients. In 2016, this diagnosis was made in 9.1% of the total number of children with cough, in 2017 - 11.9%, in 2018 - 18.7%. It is interesting to note that 56% of children with this diagnosis were vaccinated according to the vaccination calendar. This requires further, deeper examination and dictates the need to find new ways to prevent and treat this infection.



## EXPRESSION OF TLR2 AND TLR4 MRNA BY PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENT WITH BRONCHIAL ASTHMA

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TLR2 and TLR4 are well represented on various hematopoietic and non-hematopoietic cells, such as monocytes, macrophages, epithelial cells, etc. These cells are the first that recognize invading microorganisms and various allergens (for example, Der p). Polymorphism of TLR genes, impaired expression of these receptors on the cell, ligand recognition or signal transduction can contribute to the development of various diseases, including bronchial asthma. 30 healthy controls, 30 patient with allergic bronchial asthma, 17 patient with mixed asthma and 11 patient with non-allergic asthma were enrolled in the study. The diagnosis was made in the National Research Centre – Institute of Immunology FMBA of Russia based on anamnesis of the disease, clinical and laboratory examination. All patients received standard therapy prescribed by the patient management protocol. PBMC were isolated from human peripheral blood using Boyum method by Ficoll-Urographen gradient centrifugation. RNA was isolated using RNeasy Plus Mini Kit (Qiagen, USA), reverse transcription reaction was performed using Set for performing reverse transcription reaction (Synthol, Russia). TLR2 and TLR4 mRNA expression in PBMC were evaluated using real-time PCR assays with Reagent kit for real-time PCR in the presence of SYBRGreen I and primers (Synthol, Russia). TLR2 and TLR4 mRNA differential expression were calculated using the  $\Delta\Delta C_t$  method. StatSoft Statistics 6.0 software was used to perform all statistical analyses. Differences in each variable between patients and controls were evaluated with Mann–Whitney U-tests. A p-value <0.05 was considered statistically significant. The expression of TLR2 mRNA in PBMC was significantly higher at number of patients with asthma than in controls (p <0.05). Allergy patients have an increase the expression level of TLR2 mRNA of 2.3 times, mixed asthma patient have an increase of 2.8 times, non-allergic asthma have an increase of 2.5 times. Compared to healthy controls, the expression of TLR4 mRNA in PBMC was statistically by far higher (p <0.05) at patients with mixed asthma (3,4 times), non-allergic asthma (2,5 times) and in patients with moderate allergic asthma (5 times). Our findings suggest that high level TLR2 and TLR4 mRNA expression in PBMC may indicate the involvement of these receptors in the pathogenesis of the diseases, activation of innate immunity mechanisms in the development of bronchial asthma, leading to chronic inflammation development.

# **EFFECTIVENES OF SUBLINGUAL ALLERGEN-SPECIFIC IMMUNOTHERAPY (ASIT) IN PATIENT WITH ADVERS GASTROINTESTINAL REACTIONS IN THE COURSE OF THE THERAPY**

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**INTRODUCTION:** The sublingual ASIT found use as a viable and effective method of therapy. Though, sometimes it occurs that associated gastrointestinal adverse reactions result in refused treatment by patients. Typically, in such a situation an attempt to switch to subcutaneous ASIT is used; but that method has its restrictions, particularly, in pediatric population. In this article we present our successful experience of maintaining the sublingual mode of ASIT in 5 patients: two adults and 3 pediatric patients (5 to 8 years old, male and females).

**MATERIAL AND METHODS:** All the patients were given ASIT with the birch pollen allergen; sensibilization profiles were confirmed by presence of specific immunoglobulins E (IgEs).

During the treatment abdominal pains of various intensities were registered in all the patients. Medical screening did not reveal any pathological changes in gastrointestinal tract. All the patients (and pediatric patients; parents) gave written informed consents for the therapy.

In the course of the ASIT treatment, at the point where the maintenance dose had been established, moderate abdominal pains without oral allergic syndrome emerged, within 30 minutes to 2 hours after taking the medicine, recognized by the physician as related to the therapy. All the patients stated willingness to continue the treatment. Therefore, the traditional therapy regiment of sublingual ASIT were modified. The following therapy regiment were proposed: after a two-weeks interval, the course of ASIT were resumed as 1 puff in the morning, the dosage 10 ir/ml, with the dose escalation every other day by 1 puff, until the maximum tolerated, i.e., not associated yet with any adverse reactions, dose was achieved. After it was achieved, the maximum tolerated dose in a patient remained the same during two weeks, where the medicine was taken every other day. And then, an attempt to increase the dose was undertaken; if the pain syndrome reappeared, the previous treatment was retained. We were able to attain the medicine level as high as 1-2 puffs with the dosage of 300 ir/ml.

**RESULTS:** Children were treated in the course of one year with a significant clinical effect, such as decreased symptoms intensity during the pollination season. Adult patients received ASIT according to the proposed therapy regiment during more than a year, with a high effect.

# FOOD ALLERGEN TRIGGERS AND THEIR ROLE IN THE DEVELOPMENT OF IGE-MEDIATED FOOD ALLERGY AMONG CHILDREN

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Identification the spectrum of allergy sensitivity in patients with food allergy is a necessary part of the work of an allergist.

**OBJECTIVE:** To study the structure of sensitization to food allergens among children in Moscow and Moscow region.

**METHODS:** The concentration and class of specific IgE in blood serum of children with IgE-mediated allergic diseases were examined with RIDA AllergyScreen method.

**RESULTS:** 253 serums of children aged 8 months to 16 years with IgE-mediated food allergy were studied. The frequency of sensitization to food allergens is as follows: cow milk – 48,2%; rye flour - 27.7%; egg albumin– 26,9%; celery – 24,5%; hazelnut – 22,9%; peanuts – 22,5%; carrot – 19,8%; wheat flour - 19.0%; cod fish -13.4%. Egg yolk, fruits of Solanaceae, of walnut, of Rosaceae, of Pedaliaceae and of Rutaceae cause allergic reactions less frequently. Among milk proteins the leading role in sensitization belongs to whey proteins of milk, among which the greatest allergic activity is recognized for  $\beta$ -lactoglobulin. However, our analysis showed that the percentage of sensitization among children in Moscow and the Moscow region to  $\alpha$ -lactalbumin is significantly higher than to  $\beta$ -lactoglobulin, and is 61,5% and 44,9 %, respectively.

**CONCLUSIONS:** According to the study, about half of children with IgE-mediated food allergies in Moscow and the Moscow region have sensitization to cow's milk proteins. Among them sensitization to  $\alpha$ -lactalbumin was detected the most frequently. Changing the sensitization profile to cow's milk proteins may be associated with the use of new adapted mixtures for artificial feeding of infants. Adaptation of the protein component of modern infant formula is in reducing the total protein content, as well as in changing its qualitative composition. Whey proteins in modern infant formulas are  $\alpha$ -lactalbumin. The latter is justified by the presence of this protein in breast milk and the absence of  $\beta$ -lactoglobulin in it. As a result, when feeding with infant formula, the food antigenic load on the child's body is represented by cow  $\alpha$ -lactalbumin, which in the presence of predisposing factors can lead to the formation of food allergy primarily to this protein.

# THE METHOD OF ISOLATION OF THE MAJOR ALLERGEN FROM THE HOUSE DUST MITE EXTRACT

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In modern literature, you can find a lot of discussions on the advantages and disadvantages of water-salt allergen extracts (WSA). However, we cannot deny the fact that these allergens are the closest to possible to natural allergenic triggers. WSA contain the most complete set of B-cell and T-cell epitopes and natural haptenic components. The high clinical efficiency of allergen-specific therapy using WSA is probably due to the participation of the largest number of sensitized cells and the ability to switch the entire spectrum of compromised lymphocytes to the production of blocking IgG. Multicomponent and enzymatic instability of the WSA complicates the task of its research and standardization.

**OBJECTIVE:** To develop the most effective method of isolation of the main allergens Der.p1 and Der. f 1 from a phenolic free water-salt extract of a mixture of allergens *Dermatophagoides farina* and *Dermatophagoides pteronyssinus* (FWSA).

**METHODS:** The concentration of FWSA protein was determined by the Bradford method. We made precipitation of the mixture of proteins with ammonium sulfate. Then we made dissolution of sediment phosphate buffer with 6M urea. Sample was dialyzed with phosphate buffer. Affinity chromatography of samples of the FWSA using Ni-activated Sepharose (ACN) was held and the samples were washed off the column with 0,3M imidazole. Then we made sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of three FWSA samples: 1 - FWSA; 2 - FWSA after ACN; 3 - FWSA after concentration with ammonium sulfate with subsequent ACN.

**RESULTS:** The concentration of protein in FWSA was -1,383 mg / ml. In the sample, which underwent preliminary concentration using ammonium sulfate and purification by ACN, proteins with a molecular weight of about 26-27 kDa were detected on the electrophoregram. SDS-PAGE of other samples was ineffective due to low protein concentrations.

**CONCLUSIONS:** Detected by SDS-PAGE proteins with this molecular weight correspond to the major house dust mite allergens - Der p1/Der f1. It is known that histidine in the active center of cysteine protease determines its binding to Ni-activated Sepharose. Thus, by affine chromatography we purified a protein with MM equal to 27 kDa, most likely corresponding to house dust mite cysteine protease. This method can be recommended for further study as a promising method for the isolation of cysteine and serine proteinases from a protein mixture.

# THE HEALTH CONDITION OF CHILDREN DURING THE FIRST YEAR OF LIFE, BORN FROM MOTHERS WITH BRONCHIAL ASTHMA

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The aim of the work is to study the frequency and structure of allergic morbidity in of children (during the first year of life), born from mothers with bronchial asthma (BA).

**MATERIALS AND METHODS:** The observation was conducted in 98 children from mothers with BA. The comparison group consisted of 70 children of the same age, born from mothers without bronchopulmonary pathology. In order to value the impact of the level of mothers's BA control during pregnancy on the health of children of the 1 year of life, there were made 2 groups of children. Group I - 55 children from mothers with uncontrolled BA, group II - 43 children from mothers with partially or fully controlled BA.

**RESULTS AND DISCUSSION:** 36 (36,7%) of children were born by caesarean section. 42 (76,3) children from the I group and 14 from the II (32,5%) were discharged from the maternity hospital with cerebral ischemia I-III severity; with the implementation of intrauterine infection (IUI) - 15 children of group I (27,3%) and 8 of II (18,6%). The combination of cerebral ischemia and IUI was observed in 15 (2,3%) of children in group I and 7 (16,3%) of children in II. In the comparison group, these data were 20% and 23,3%, without combined conditions for these diseases. 3 children (5,5%) of group I and 6 children (13,9%) of group II were considered healthy. Throughout the 1 year of life, the following conditions were noted more often than others: hyper-excitability - in 12 (21,8%) children of group I and 7 (16,3%) of group II, hypertension syndrome, respectively, in 8 (14,5%) and in 2 (4,5%), syndrome of motor disorders - in 11 (20%) and in 5 (11,6%), vegeto-visceral dysfunctions - in 10 (18,2%) and 4 (9,3%) children. The delay in physical development was observed in 9 (16,4%) children of the I group and 3 (7,0%) of the II. Throughout the 1 year of life, 56 (57,1%) children from mothers with BA showed signs of allergic diathesis. Most often, children had atopic dermatitis (54,1%) of mothers with BA and in 25,7% in the comparison group.

There were better conditions for the development of the fetus, the health of the newborn and the child of 1 year of life with controlled BA than in the absence of disease control. At the same time, cerebral ischemia was observed 2 times less often, 1,7 times less often - its combination with IUI, 1,9 times less often - delayed physical development, less often stress of adaptation processes was noted – 1,7 times and 3 times more children are considered healthy.

# EXTRAPULMONARY MANIFESTATIONS OF ALLERGY IN PREGNANT WOMEN WITH BRONCHIAL ASTHMA AS PREDICTORS OF ITS UNCONTROLLED COURSE

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The relevance of this study is associated with the constant growth of allergic diseases, including bronchial asthma (BA), which is noted worldwide. The aim of the work was to study extrapulmonary manifestations of allergy in patients with BA depending on its clinical course, severity and level of control.

The clinical and functional features of the course of BA have been researched on 290 patients in the dynamics of pregnancy. 180 (62.1%) patients had mild BA (group I), 86 (29.6%) - moderate BA (group II), 24 (8.3%) - severe BA (group III). Allergic form of BA was diagnosed in 62.4% patients, non-allergic form was diagnosed in 10.4% patients, mixed form - in 27.2% patients. Exacerbations of BA were detected in 218 patients (75.2%).

Burden hereditary of allergic diseases was traced in 118 (40.7%) patients, while in BA - in 103 (35.5%), 61 (59.2%) of them were maternal. 15 patients had BA in three generations, 12 (80%) of them had BA from maternal side. The structure of allergic diseases in pregnant women with BA was determined depending on the severity of the disease. Extrapulmonary diseases of the atopic circle were observed in 125 (69.1%) patients of group I, in 68 (79.1%) patients of group II, in 21 (87.5%) patients of group III. At the same time, allergic rhinitis was noted in 148 patients (51%), fever was noted in 125 (43.1%), urticaria was noted in 57 (19.7%) and atopic dermatitis was noted in 27 (9.3%). Allergic rhinitis, hay fever and urticaria were the most frequent extrapulmonary manifestations of allergy in patients with BA observed in 214 (73.8%) patients, while 143 (66.8%) of them showed a combined allergy. The presence of atopic BA and allergic rhinitis (AR) was detected in 148 (51%) patients, moreover, in 59 patients (39.9%) AR preceded BA, in 89 patients (60.1%) AR manifested simultaneously with BA debut. AR was significantly more common in patients of group III than group II and I ( $p < 0.001$ ). An increase in the frequency of respiratory allergies, which is proportional to the aggravation of the course of BA, confirms the concept of the unity of allergic in the upper and lower respiratory tract.

The presence of extrapulmonary allergic diseases is a factor that aggravates the course of asthma in the gestational period, and a predictor of an uncontrolled course, which dictates the need for constant monitoring of this category of patients and prophylactic measures, with the obligatory correction of the volume of therapy in pregnancy.

# CLINICAL EXPERIENCE OF MOLECULAR ALLERGY DIAGNOSTICS USE FOR EVALUATING THE FORECAST OF EFFICIENCY OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

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**RELEVANCE:** Molecular identification of allergen components increases the sensitivity and specificity of diagnostic tests for atopic diseases to predict the effectiveness of allergen-specific immunotherapy (ASIT).

**OBJECTIVE:** to present the experience of using allergen components of birch pollen and timothy pollen to predict the effectiveness of ASIT.

**MATERIALS AND METHODS:** asIgE to molecular allergic components of birch pollen - rBet v 1, rBet v 2, rBet v 4 (N = 186) and timothy birds - rPhl p1, rPhl p 5b, rPhl p7, rPhl p 12 (N = 132) were determined in 160 adult patients and 158 children suffering from bronchial asthma (N = 105) and allergic rhinitis (N = 213). 267 patients received ASIT, the effectiveness of which was scored (1 - not effective, 2 - low efficiency, 3 - average efficiency, 4 - high efficiency).

**RESULTS:** An increased level of asIgE to rBet v 1 was detected in 87% (<0.35 kUA / l - 9.6%; Grade 1 was not detected; Grade 2 - 16.1%; Grade 3 - 3.2%; Grade 4 - 35, 4%; 5th grade-19.3%; 6th grade-19.3%). The ASIT efficiency of 4 and 3 points for groups with asIgE level 5 and 6 class (N = 37) was 0.47; Grades 3 and 4 - 0.35; Grades 1 and 2 - 0.19 (p <0.05). Sensitization to minor components of birch pollen was detected in 16.1% (asIgE 3 Grade - 9.6%; 4 Grade - 3.2%; 5 Grade - 3.2%). The effectiveness of ASIT 1 and 2 points for groups with Grade 5 asIgE level was 0.29%; Grades 3 and 4 total 0.25% (p > 0.05). An increased level of asIgE to rPhl p1, rPhl p 5b was detected in 80% of patients (result <0.35 kUA / l - 8.3%; Grade 1 level - 8.3%; Grade 2 - 16.6%; Grade 3 - 33.3%; Grade 4 24.0%; Grade 5 24.0%). Medium and high level reactions prevailed. The ASIT efficiency of 4 and 3 points for groups with class 5 asIgE level (N = 37) totaled 0.37; c asIgE 3.4 Grade 0.25 (p <0.05); for groups with a level of 1.2, a total of 0.14 (p <0.05). Sensitization to rPhl p7 rPhl p12 was detected in 16.1% of patients (Grades 1 and 2 were not determined, Grade 3 - 8.3%, Grade 4 - 8.3%). sensitization of medium and high levels predominated to minor components of timothy. The effectiveness of ASIT 1 and 2 points for groups with an asIgE level of grade 3 was 0.39; grade 4 0.32 (p > 0.05).

**CONCLUSION:** according to our study, a higher efficiency of ASIT with a birch and timothy allergen is predicted at very high levels of major components of allergens (grade 5, 6).

## **ADVANTAGES OF MOLECULAR ALLERGY DIAGNOSIS OF CAT ALLERGY OVER SEROLOGY AND SKIN TESTING ALLERGEN WITH EXTRACTS**

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**BACKGROUND:** Traditionally the diagnosis of cat allergy is based on case history as well as skin testing and serology performed with allergen extracts. However, it is very difficult to prepare high quality natural allergen extracts for diagnosis of cat allergy. Often allergens are missing and false positive results have been reported due to contaminations with allergens from other allergen sources such as house dust mites (HDM).

**METHODS:** We have performed allergy diagnosis in 85 subjects who according to questionnaire appeared to suffer from cat allergy by performing skin prick testing with cat allergen extract in 70 patients, IgE serology using cat allergen extract in by quantitative IgE measurements using ImmunoCAP technology and with a panel of seven (Fel d 1, Fel d 2, Fel d 3, Fel d 4, Fel d 6, Fel d 7 and Fel d 8) cat allergen molecules coupled to ImmunoCAPs in all subjects (cut off equal or greater than 0.1 kUA/L).

**RESULTS:** One out of the 85 subjects was negative by SPT. Seventy subjects were positive by SPT and in 14 SPT were not performed. Among the 70 SPT-positive subjects, 62 had specific IgE antibodies to cat allergen extract whereas 64 were detected with the sum of the seven allergen molecules. The subjects without detectable cat allergen-specific IgE were also sensitized to other indoor allergens, in particular to HDM. From the 14 subjects with evidence for cat allergy for whom no SPT was done 11 were confirmed by IgE serology with allergen extract and the allergen molecules.

**CONCLUSION:** The sum of allergen molecules was superior to cat allergen extract in identifying subjects with cat allergen-specific IgE. Cat allergy in 6 of the SPT positive and 3 of the subjects with a case history of cat allergy who had also sensitizations to other indoor allergen sources could not be confirmed by serology suggesting that they are not genuinely sensitized to cat.



## DEFINING THE PANEL OF CLINICALLY RELEVANT CAT ALLERGENS FOR IMMUNOTHERAPY

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**BACKGROUND:** Patients suffering from allergy to cat suffer from severe respiratory symptoms such as asthma. Besides the major cat allergen Fel d 1 seven more allergens are described but their clinical relevance has not been studied in depth.

**METHODS:** Fel d 1, 2, 3, 4, 7 and 8 were expressed in *Escherichia coli* and purified. Fel d 6 containing the same epitope as Fel d 5, Alpha-Gal was purified as natural allergen. Sera were obtained from 84 cat allergic patients whose clinical symptoms were recorded and 4 non-allergic control subjects. IgE levels to each of the cat allergens molecules were quantified by ImmunoCAP technology. For this purpose purified cat allergens were biotinylated and coupled to Streptavidin ImmunoCAPs.

**RESULTS:** For cat allergic patients the following frequencies of IgE recognition were obtained for each allergen molecule: Fel d 1: 97,2% (n=71), Fel d 2: 30,1% (n=22), Fel d 3: 50,6% (n=37), Fel d 4: 52% (n=38), Fel d 5-6: 32,8% (n=24), Fel d 7: 54,7% (n=40), Fel d 8: 42,4% (n=31). Patients who also suffered from atopic eczema in addition to respiratory allergy showed a broader sensitization to the cat allergens than patients suffering only from respiratory allergy.

**CONCLUSION:** Sensitization to Fel d 1 is the most frequently recognized cat allergen but Fel d 3, Fel d 4 and Fel d 7 seem to be also of clinical relevance and may be considered as components in a vaccine for cat allergy.

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# THE EPIDEMIOLOGICAL OF INSECT ALLERGY IN PEDIATRIC PRACTICE IN MOSCOW

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**BACKGROUND:** there is little data on the prevalence of Insect allergy (IA) in children in Moscow. The incidence of insect anaphylaxis in children is only 0.3–1.0% of all cases of childhood anaphylaxis. Urticaria and other systemic reactions (SR) in children have been also reported. Cockroaches (CR) have been increasingly recognized as an important source of indoor allergens. Sensitization to environmental allergens such as CR may drive shrimp sensitization in children who have never ingested shellfish, or house dust mite (Der. p, Der. f) sensitization because of CR, dust mite and shrimp cross-reactivity. The aim of this study was to investigate the proportion of IA among all allergic diseases in children in Moscow.

**METHODS:** 1000 children from Moscow 0-18 years were asked for having reactions to insect bites and stings. 22% children have reactions of varying severity degrees. They underwent detailed allergologic assessment, including serum-specific outdoor and indoor insect, food and inhaled allergens IgE by ImmunoCAP.

**RESULTS:** the prevalence of IA in Moscow is 2% - 6% in child populations. LLRs to mosquito bites are common. 70% of mosquito bites, 26% of midge bites, 17% of horsefly bites, 26% of wasp bites, 8% of bee bites caused hypersensitivity reactions. Patients aged 5-7 years (33%) and 8-13 years (42%) have IA more often, they have commonly LLRs. Older children (14-18 years, 22%) have IA less often, but children in this group have SR in 8% cases. Children with a personal history of atopy have reactions to bites and stings more often (in 34% cases).

The prevalence of CR sensitization is 31.2 % (based on serum IgE level) in children with allergic diseases, the level of CR IgE is 0.48 – 4.76 IU / ml. CR IgE-positive patients have more frequent contact with CR at home compared with CR IgE-negative (73.7 % vs 35.6 %,  $p < 0.001$ ), they also have less frequent insect bite reactions (20.5 % vs 51.2 %,  $p < 0.005$ ). CR IgE-negative children have zero IgE levels against insect (wasp, bee, mosquito) and house mite (Der. p, Der. f) allergens.

# ALLERGENIC PROFILE OF SENSITIZATION IN PATIENTS WITH ALLERGIC RHINITIS USING RECOMBINANT ALLERGENS FROM TREE POLLEN AND CEREAL GRASSES

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In patients with allergic rhinitis, IgE sensitization to the some allergens of tree and grass pollen is usually detected. Perhaps, it is associated with the presence of cross-molecules in diagnostic allergen extracts. Birch pollen and cereal grasses have protein families containing ubiquitous, highly cross-reactive panallergens (part C of the EAACI Molecular Allergology User's Guide).

In this study we conducted molecular diagnostics using the main and minor allergic components of birch and timothy to prognosis adverse events and clarify the characteristics of the trigger that caused allergic reaction.

This study used the sera of 24 patients with allergic rhinitis. Their sera were analyzed for total IgE and specific IgE to individual birch pollen and timothy allergens (recombinant Bet v 1, Bet v 2/4, Phl p1/p5 and Phl p7/p12) using Phadia CAP System.

The levels of total IgE were higher than the age norm (197 [20.4-114.5] kU/l). The levels of sIgE to rBet v 1 had positive CAP results (28.5 [0.455-70.3] kUA/l). On the other hands, the levels of sIgE to bet v2/v4 were negative (0.02 [0.01-0.125] kUA/l). The levels of sIgE to rPhl p1/p5 were also positive (15.05 [8.12-48.42] kUA/l) and to rPhl p7/p12 - negative (0.02 [0.01-0.272] kUA/l). There was a weak, but significant correlation between the levels of sIgE to rBet v 1 and the IgE levels to rPhl p1 / p5 ( $r = 0.52$ ,  $p < 0.05$ ). In the sera of 5 patients, the levels of sIgE to the birch allergic components were within the normal range, while the levels of sIgE to the major allergens of timothy rPhl p1/p5 were increased.

In most patients with allergic rhinitis, birch pollen, its major allergen bet v 1 is a trigger allergen so far as the levels of sIgE to this allergen is significantly higher than to rPhl p1/p5. Moreover, sIgE to timothy in these patients does not indicate true sensitization to the pollen of this cereal grass, but about cross-reactions to it. Only in 9 patients the levels of sIgE to rPhl p1/p5 were significantly higher, than rBet v 1, that can indicate true sensitization to timothy.

The use of molecular methods of diagnostics allows the selection of the most appropriate drug for immunotherapy in patients with allergic rhinitis. It is very important not only for the diagnosis of the true sensitization and the reasonable appointment of pathogenetic therapy, but also for predicting the risks associated with ASIT.

## **EXPERIENCE IN THE USE OF COMPONENT ALLERGY DIAGNOSTICS BEFORE PLANNING ALLERGEN-IMMUNOTHERAPY**

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Allergic rhinitis is a chronic allergic disease in which IgE-mediated inflammation develops in the nasal mucosa. Allergic rhinitis is manifested by sneezing, itching of the nose, watery discharge from the nose and nasal congestion. From 15 to 40% of the population suffers from allergic rhinitis in different countries. Rhinitis not only violates the quality of life of patients, but is also a high risk factor for the development of bronchial asthma.

The only etiopathogenetic method for treating allergic rhinitis is allergen immunotherapy with a causative allergen. An allergist is faced with the difficult task of selecting patients with allergic rhinitis for allergen immunotherapy. Even with the highest quality certified allergy vaccines, treatment success is 85 - 95%. The discovery by the professor of the University of Vienna, Rudolf Valenta, of the major and minor components in allergens revolutionized allergology.

The study involved 55 people with allergic rhinitis caused by pollen from plants. Of these, children under 18 years old - 15, adults - 40. The diagnosis was made to each patient on the basis of complaints, an allergological history, objective examination data, including an examination by an otorhinolaryngologist, and confirmed by skin allergy tests using the pric-tests with pollen allergens. All patients were scheduled for allergen immunotherapy. To increase the effectiveness of the planned allergen immunotherapy, a component allergy diagnosis was carried out with the determination of the major and minor components of birch pollen, timothy and wormwood using the Immunocap method on a Phadia 250 analyzer. Specific IgE only to the major components of pollen allergens was detected in 47 patients (85.45%) (15 children (100%) and 32 adults (80%)), to the major and minor components in 7 adults (12.73%), only to minor components - in 1 adult patient (1.82%). In 3 adult patients, a high level of LTP was detected. Allergen immunotherapy in patients with specific IgE to the major components of allergens is highly effective, in patients with specific IgE to the major and minor components is moderately effective. In the presence of specific IgE to several major components, its level allows you to choose an allergen for treatment

Thus, the use of component allergological diagnostics makes it possible to select patients for whom allergen immunotherapy will be highly effective and to select an allergen for allergen immunotherapy.

# PROTEOMIC SPECTRUM OF BLOOD SERUM OF CHILDREN WITH BRONCHIAL ASTHMA

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Bronchial asthma (BA) is a multifactorial disease. The pathogenesis of asthma is based on chronic allergic inflammation, associated with significant changes not only at the cellular, but also at the molecular level. Impaired expression of proteins can serve as one of the initiating factors that play a key role in the formation of the disease. Currently, it is the proteomic analysis that is a valuable tool for searching for markers associated with the pathological process. Its task is to detect the entire spectrum of proteins in the sample under study.

The purpose of the study is to investigate the proteomic spectrum of the blood serum of healthy children and patients suffering from bronchial asthma.

17 children were examined (8 healthy patients and 9 patients suffering from bronchial asthma). A comprehensive examination was carried out, which included a proteomic analysis of blood serum for all patients. The protein spectrum was analyzed by high-resolution two-dimensional electrophoresis (Protein IEF Cell and Protean II xi Multi-Cell, Bio-Rad, USA) followed by MALDI-TOF mass spectrometric detection (Microflex RLF, Bruker, Germany) using a peptide analysis algorithm Mascot MS Search fingerprint (Matrix Science, USA) and Swiss-Prot database.

**RESULTS:** A comparative analysis of the protein spectra of the blood serum of patients and healthy children revealed four proteins in the range of molecular masses 13–36 kDa, the expression of which was reduced in BA ( $p < 0.05$ ): glutathione peroxidase 3, transthyretin, C3 and C4b fragments of the complement system. A decrease in the content of the antioxidant enzyme glutathione peroxidase 3 leads to a violation of redox homeostasis in the bronchopulmonary system. Insufficient synthesis of transthyretin can contribute to the accumulation of retinoids in the pulmonary parenchyma, leading to the development of a number of negative reactions: differentiation of epithelial cells by mucoid type, mucus hypersecretion, remodeling of the intercellular matrix, etc. The components of the complement system C3 and C4b are one of the main regulators of inflammatory processes that occur with allergic lung diseases.

**CONCLUSION:** The results of the study allow us to expand our understanding of the molecular mechanisms of BA pathogenesis in children, and, also, showed the need for further study of the proteomic spectrum of blood serum in order to develop modern methods of diagnosis and treatment of this disease.

## ANTIGENIC ACTIVITY OF CATIONIC PEPTIDE DENDRIMERS

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**BACKGROUND:** This research is dedicated to the matter of antigenic presentation of cationic peptide dendrimers, a class of compounds, although seen perspective as gene carriers, microbicides and some other medicines, yet still not thoroughly studied in many fields, one of them being their antigenic properties.

**METHODS:** Peptides: LTP – (((R)2K)2K)2KAC; SA-32 – (((R)2K)2KY)2KPWC; KK-50 – ((R)2KG)2KAWWC; NC-803 – (RRRKK)2KAC; L-1 – RKKKAC; OA-678 – R8; NC-810 – Palmitoyl-CKR11 were obtained by means of chemical synthesis in form of C-amides and purified with HPLC. LTP was covalently attached to hemocyanin from *Megathura crenulata* using N-maleoyl-β-alanine as a linker, giving KLH-NMBA-LTP, and to bovine serum albumin, giving BSA-NMBA-LTP while using the same modification path. BALB/c mice were immunized trice using KLH-NMBA-LTP with Freund's adjuvant, and their blood sera were obtained one week after the last immunization. The sera were analyzed with standard ELISA; BSA-NMBA-LTP was used for evaluation of the mice immune response to LTP, while pure LTP (free base) was used for cross-reactivity studies.

**RESULTS:** ELISA revealed high level of anti-LTP IgG in sera. The sera were tested for cross-reactivity with linear peptides representing fragments of LTP: L-1 structure is derived from LTP α-amide backbone, while OA-678 and NC-810 contain eight and eleven guanidine groups located in close proximity to each other. All three of these peptides were not recognized by antibodies from anti-LTP serum. The serum was also analyzed using 3 other cationic peptide dendrimers – SA-32, KK-50 and NC-803. Interestingly enough, although anti-LTP serum was not able to bind the linear peptides, it showed an intensive signal against SA-32 and KK-50 and a moderate signal against NC-803 peptides.

**CONCLUSIONS:** The data obtained during this research strongly suggests that antibodies against LTP recognize branched 3D structure of other cationic peptide dendrimers, being almost indifferent to their backbones, which is a fact to be taken into consideration while developing and testing new cationic peptide dendrimers.

# WATER-SOLUBLE FULLERENE C60 IS HIGHLY ACTIVE AGAINST HERPES VIRUS INFECTIONS

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**BACKGROUND:** Water-soluble forms of fullerene C60 is known as a promising therapeutic agent due to its antioxidant, anti-inflammatory, antiviral properties and the lack of toxicity. The aim of our research was to assess the antiviral effects of the C60 aqueous nanodispersion (dnC60) against of herpes simplex viruses type 1 (HSV-1) and human cytomegalovirus (HCMV).

**METHODS:** The dnC60 was prepared by a novel tangential ultrafiltration method. The antiviral activities were evaluated in Vero or HF cells, infected with HSV1 (F strain) or with HCMV (AD169). HSV1 experimental model in vivo was induced in mice by the HSV1 suspension application in scarified areas of back. Mice were treated with dnC60 or ACV (Acyclovir) ointment or PBS. To evaluate the level of virus repression under the dnC60 action, the model of HCMV infection in vitro was used and expression levels of HCMV mRNAs of three important viral proteins, IE2 (UL122), pp65 (UL83) and gB (UL55), were assessed. Therapeutic effects were assessed by the severity of the development of virus-specific skin lesions. The IC50 and selectivity index (SI) were calculated.

**RESULTS:** The cytotoxicity of dnC60 was not observed even at the maximum studied concentration. In vitro antiviral study has shown that the dnC60 suppressed HSV1-infection with SI>100. In pre-treatment assay, maximal inhibition effect was shown for dnC60. In post-treatment assay, inhibition of HSV1-infection was 93±5.6% for ACV and 60±4.4% for dnC60. In virucidal assay, dnC60 displayed the high inhibitory activity. Skin damage was minimal in animals treated with dnC60 and ACV. However, the first showed effect 24 h earlier and at molar concentration 500-fold lower than the ACV. At concentrations 50 and 10 µg/ml, dnC60 produced anti-HCMV effect prior to 82±4.6% and 57±1%, respectively, and 80±2.6% and 27±1% after infection. In the tested concentrations dnC60 displayed no virucidal activity. IC50 determined by linear regression was 8.0±0.7 µg/ml for prior-infection and 30±2.8 µg/ml for post-infection assay. Anti-HCMV activity of dnC60 was dose-dependent both in pre- and post-treatment assays. The RT-PCR assay showed that the expression of all the studied HCMV mRNA in the presence of dnC60 was significantly reduced, which indicates the suppression of virus reproduction

**CONCLUSION:** The results suggest that dnC60 exhibits a pronounced therapeutic activity against HSV1- and HCMV-infection and it is a promising candidate for a new antiviral drug.

# IMMUNE FEATURES OF IGE-SENSITIZATION IN CHILDREN WITH CHRONIC URTICARIA WITH AND WITHOUT ATOPY

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**INTRODUCTION:** Chronic urticaria (CU) appears to be associated with atopy in a subpopulation of children. The object of the research was to examine sensitization to allergens and to compare immune mechanisms in children with CU and atopy with and without sensitization to allergens.

**METHODOLOGY:** We observed 65 children  $10,1 \pm 4,8$  years with CU, among them 29 children ( $9,4 \pm 0,72$  years) with CU had atopy. We examined in serum IgE (total, specific), complement components C3a, C4, C1inh, ELISA; basophil activation (CD3-CRTh2+CD203c++), Th2 (CD3+CRTh2+), flow cytometry.

**RESULTS:** Among 65 children 29 had atopy and 36 children did not have clinical symptoms of allergic diseases. Children with atopy were shown: Atopic dermatitis (20%); asthma (15%); allergic rhinitis (13%); drug allergy (6%). Sensitization to allergens was found in 33 (51%) children with CU (in 15 aeroallergens; in 8 food allergens; in 5 pollen allergens; in 2 drug allergens; in 3 polysensitization). Among 29 children with CU with atopy 20 (69%) had sensitization to allergens and 9 (31%) had not. Among 36 children without atopy 13 (36%) had latent sensitization to allergens and 23 (64%) had not. In children with atopy more often were found sensitization to aeroallergens 60%. In children without atopy were dominated sensitization to food 54%. The frequency of the sensitization to aeroallergens in children with atopy was statistically higher compared with patients without atopy (60% and 23.5%,  $p=0.008$ ). On the other hand the frequency of the sensitization to food allergens in children without atopy was statistically higher compared with patients with atopy (54% and 5%,  $p=0.001$ ). Higher serum IgE-levels were demonstrated in CU children with sensitization to allergens compared with children without sensitization ( $285,8 \pm 85,3$ ;  $152,2 \pm 68,7$  ME/ml;  $p=0,02$ ) as well as Th2 ( $0,7 \pm 0,3$ ;  $0,4 \pm 0,1$ ;  $p=0,01$ ); basophil activation ( $15,5 \pm 5,3$ %;  $6,6 \pm 2,5$ %;  $p=0,04$ ); C3a ( $398,8 \pm 36,4$ ;  $254,4 \pm 33,9$  ng/ml;  $p=0,01$ ). Levels of C4 ( $295,6 \pm 51,8$ ;  $260,7 \pm 27,3$  mcg/ml) and C1inh ( $462,5 \pm 50,6$ ;  $475,9 \pm 80,9$  mcg/ml) were not significantly different from each other in both groups.

**CONCLUSIONS:** We showed domination of the sensitization to aeroallergens in children with CU with atopy and prevalence of food sensitization (latent) in children without atopy. Sensitization to allergens influences on pathogenesis mechanisms and shows an IgE-related mechanisms, Th2 orientation of immune response, basophil and C3a activation.



# ANALYSIS OF GENES EXPRESSION OF TRANSCRIPTIONAL REGULATORS FOR T HELPER CELLS IN ALLERGIC RHINITIS BEFORE AND AFTER ALLERGEN-SPECIFIC IMMUNOTHERAPY

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Regulatory T cells (Treg) play an important role for maintaining immune homeostasis and preventing excessive reactions. The main marker of Treg is FOXP3. Four isoforms of FOXP3 have been described in humans, one of them is full-length (FOXP3-FL), and the other three are formed as a result of splicing of some exons - 2, 7 or both 2 and 7 (FOXP3d2, FOXP3d7, FOXP3d2d7). It is believed that the FOXP3d2 form has a suppressor function and is the main isoform that determines the functional activity of Treg. In this study we have analyzed the number of regulatory T cells and the level of FOXP3 isoforms expression in patients with seasonal allergic rhinitis (AR) and their dynamics during allergen-specific immunotherapy (SIT).

We examined 38 patients with AR aged 18 to 64 years (median age – 32.5 years) and 24 healthy donors aged 20 to 65 years (median - 33 years). All patients with AR received 1 course of SIT according to the classical method with water-salt extracts of tree pollen allergens (Microgen) using the accelerated schedule with a target dose at least 6,000 PNU subcutaneously. Blood tests were performed three times: before treatment, immediately after SIT and at the end of the polling season. The level of FOXP3 isoforms expression were measured in peripheral blood by flow cytometry using monoclonal antibodies (eBioscience).

Before the therapy, there was a statistically significant decrease in the total number of Treg - CD4+CD25+FOXP3+ ( $19.8 \times 10^6/L$  (13.9-27.3) in AR and  $26.4 \times 10^6/L$  (21.0-36.5) in control group,  $p < 0.05$ ). The decrease of Treg was mainly due to amount reduction of Treg expressing FOXP3 molecule lacking exon 2 - FOXP3d2 Treg ( $11.7 \times 10^6/L$  (8.4–16.8) in AR and  $17.4 \times 10^6/L$  (11.9–23.0) in healthy donors,  $p < 0.05$ ).

After SIT at the end of the polling season, we observed a 2-fold statistically significant reduction of Treg, expressing full-length FOXP3 isoform. Since the FOXP3-FL molecule is localized mainly in the cytoplasm of the cell and functionally less active as a transcription factor, we can speak about a relative increase in the functional activity of Treg through the accumulation of cells expressing FOXP3 molecule lacking exon 2.

So, the functional activity of Treg is reduced in patients with AR and corrected after SIT. The positive effect of SIT has a long-term character, remaining at least until the end of the polling season.

# RESEARCH AND CHARACTERIZATION OF THE IN-HOUSE REFERENCE PREPARATIONS OF POLLEN ALLERGENS FROM RYE, TIMOTHY AND MUGWORT

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Allergen pollen extract preparations are widely used for diagnostics of seasonal allergy (pollinosis) and therapy by method of allergen-specific immunotherapy (ASIT). According to the requirements of the Russia State Pharmacopoeia the allergenic activity must be established by comparison with the In-House Reference Preparation (IHRP). IHRP must be characterized with biochemical and immunological tests. This report describes the results of research and characterization of candidate IHRPs of allergenic activity of pollen allergens from rye, timothy and mugwort.

Biological activity of candidate IHRPs was assessed by in vivo skin-prick tests (SPT) in allergic individuals. Final allergen preparations and intermediate allergenic extracts of candidate IHRPs of rye, timothy and mugwort were additionally characterized by protein profile (PAGE-SDS and size exclusion chromatography, SEC-HPLC) and allergenic components (western blot). Protein content was determined by Bradford assay. In compliance with general monograph allergen preparations were characterized by protein nitrogen using Nessler's reagent. The assays were performed in comparison with final allergen preparations and intermediate allergen extracts from different years of pollen collection and manufacturing.

Protein profile obtained by SDS-PAGE and SEC-HPLC showed a high concordance between extracts from different years of production of the same origin. Moreover, western blot determined the presence of the major specific allergenic components by reaction with positive sera. To assess allergenic activity of the produced series competitive ELISA of pollen allergens from rye, timothy and mugwort, was developed and validated.

On the basis of the results of SPT biological activity and assessment with biochemical and immunological methods the primary standards (IHRPs) for allergens from rye, timothy and mugwort pollen, were developed and characterized for registration in compliance with general monograph of the Russia State Pharmacopoeia.

# CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS FOR THE TREATMENT OF ALLERGY: THE ELIMINATION OF HUMAN ALLERGEN-SPECIFIC CD4<sup>+</sup> T LYMPHOCYTES WITH RECEPTOR-MODIFIED T CELLS (RMTCS)

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Allergen-specific CD4<sup>+</sup> T cells play a central role in the pathogenesis and maintenance of allergic diseases. Currently, patients suffering from allergic diseases are mainly treated with symptomatic therapy. There are only few and suboptimal causative therapy options available, *e.g.*, allergen-specific immunotherapy aimed at the induction of blocking antibodies or Treg cells. We here aim to develop a novel, causative treatment for allergies based on the redirected killing of human allergen-specific CD4<sup>+</sup> T cells. We created chimeric antigen receptors (CARs) with specificity for allergen-specific CD4<sup>+</sup> T cells. They consist of immunodominant peptides of major respiratory allergens linked to HLA-DR heterodimers chimerized with intracellular signaling tails, *e.g.*, derived from the CD3  $\zeta$ -chain, CD28, 4-1BB or Dap10. Preliminary results showed that CARs are well-expressed on Jurkat e6.1 cells, with 58% to 91% of the cells expressing the different receptor prototypes when introduced by lentiviral transduction. Currently, the different CARs are ligated with mAbs or TCR tg T cells to characterize their capability to activate Jurkat e6.1 T cells, which is determined by CD69 expression. Alternatively, activation of the NF $\kappa$ B transcription factor upon CAR ligation is being examined in Jurkat reporter cell lines. These results will allow us to estimate the signaling capabilities of the various constructs and to select those most suitable for cellular therapy approaches. Furthermore, we are planning to express the selected CARs in effector T cell populations, generating receptor-modified T cells (RMTCs). RMTCs will be activated upon contact with allergen-specific T cells, which is expected to lead to the killing of the latter. We are also planning to assess different effector cell populations (*e.g.*, CD8<sup>+</sup> or  $\gamma\delta$  T cells, Treg cells, NK cells) for their cytotoxic capacity. Treatment with RMTCs might help reducing the systemic burden of allergen-specific CD4<sup>+</sup> T cells in individuals suffering from severe forms of allergies.

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# ALLERGEN-LADEN VIRUS-LIKE NANOPARTICLES (VNP) DECORATED WITH IL-12 MODULATE ALLERGEN-SPECIFIC IMMUNE RESPONSES

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Virus-like nanoparticles (VNP) are safe and efficient platforms to modulate allergen-specific immune responses *in vitro* and *in vivo*. To develop the VNP platform even further, we here created and evaluated VNP, which express the allergen in their interior (i.e., safely shielded by the lipid envelope from the environment) and are in addition decorated on their surface with the Th1-inducing cytokine IL-12. Shielding of the allergen within VNP was achieved by fusing the major mugwort pollen allergen Art v 1 to the viral matrix protein M<sub>Ap</sub>15 of MoMLV. Surface expression of single-chain (p35::p40) IL-12 was accomplished by its fusion to the minimal CD16b GPI anchor acceptor sequence. Interestingly, incubation of bone-marrow derived dendritic cells with VNP did not result in upregulation of the activation markers CD40, CD80, CD86 or MHC class II. These results were confirmed by the absence of the inflammatory cytokines IL-1b, IL-6, IL-23 and IL-27. In contrast, in splenocyte preparations from transgenic “allergy mice”, allergen-specific T cells produced high-levels of IFN-gamma, moderate levels of IL-10 but very low levels of IL-4, IL-5, IL-13 and IL-17A upon incubation with IL-12 expressing VNP. While IL-12<sup>+</sup> VNP tended to inhibit cellular proliferation, the fraction of IFN-gamma<sup>+</sup>CD4<sup>+</sup> T cells was significantly increased and the CD3<sup>+</sup>CD4<sup>+</sup>IFN-gamma<sup>high</sup> T cells co-expressed IL-10, indicative of a Tr1 cell phenotype. IL-12<sup>+</sup> allergen-expressing VNP were even able to polarize naive allergen-specific T-cells towards the Th1 phenotype, which remained utterly stable upon restimulation with allergen. Furthermore, IL-12<sup>+</sup> VNP much more efficiently inhibited the expansion of allergen-specific, IL-13<sup>+</sup> Th2 cells *in vitro* when compared to VNP without IL-12. Upon prophylactic intranasal treatment with IL-12-decorated allergen-laden VNP and subsequent challenge with aerosolized allergen, reduced expansion of IL-4<sup>+</sup> as well as IL-13<sup>+</sup> CD4<sup>+</sup> T cells was observed.

Decoration of allergen-laden VNP with IL-12 enhances their immunomodulatory capabilities and might thus represent a helpful new remedy for the treatment of allergies in the future.

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## SENSITIZATION IN CASE OF INSECT ALLERGY

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**BACKGROUND:** The widespread prevalence of insects in the environment, the unpredictability of contacts with them, frequent and acute systemic reactions, including anaphylactic shock, determine the relevance and clinical significance of insect allergy (IA). Moreover, data on the prevalence and profile of sensitization of patients with IA in the Russian population are practically absent.

**AIM:** to analyze the sensitization profile of patients (pts) with insect sensitization.

**Patients:** There were 36 patients (pts), 20 male and 16 female, aged from 4 to 63 with different severity of IA observed. All sera from these pts were tested to insect allergocomponents (rApi m 1, nApi m 4, rPol d 5 and rVes v 5).

**METHODS:** The presence and level of IgE-aB to by ISAC ImmunoCAP technology (ThermoFisher Scientific, Sweden).

**RESULTS:** For allergic components rApi m 1 (honey bee), sensitization was detected in 9 (25%) patients, of which 1 (2.7%) was low, 7 (19.4%) was medium and 1 (2.7%) was very high. Allergic components of rPol d 5 (paper wasp) caused sensitization in 14 (38.8%) patients, of which 8 (22.2%) were low and 6 (16.6%) were medium. Most frequently the IgE-aB to 25 (69.4%) were positive to rVes v 5 (common wasp), of which 13 (36.1%) were low and 12 (33.3%) were medium. At the same time, 11 (30.5%) patients were sensitized to the components of rPol d 5 and rVes v 5. The components rApi m 1 and rVes v 5 at the same time sensitization was detected in only one patient. Sensitization to only one component was detected in 24 (66.6%) patients. To nApi m 4 (honey bee) not one of the examined patients was sensitized.

**CONCLUSIONS:** Most of the examined patients (66.6%) have monosensitization to only one allergic component of insects, which can indicate a favorable prognosis and high efficiency of SCIT by these allergens. Moreover, co-sensitization is possible for rPol d 5 and rVes v 5, which in turn requires taking this into account when selecting a drug for SCIT in one third of patients. Very high sensitization level was detected in only one patient, which may indirectly indicate the absence of a relationship between the level of IgE-aB calculated in ISU-E for multiplex analysis and the likelihood of anaphylaxis in patients with IA. Perhaps sensitization to nApi m 4 is not typical for Russia, which can be taken into account when forming a set of allergic components for multiplex studies, depending on the population being examined.

# IGE-ANTIBODIES TO THE MITES ALLERGEN COMPONENTS

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**BACKGROUND:** The incidence of sensitization to mite allergens in allergic patients in Russian population is high. There no data about revealing IgE-aB to this allergen components in Russia.

**AIM:** to investigate frequency of IgE-aB to mite allergen components.

**PATIENTS AND METHODS:** The study was conducted 86 patients (pts) that suffered from bronchial asthma, allergic rhinitis and conjunctivitis. The pts were aged from 3 to 58 years old, gender distribution was equal. Specific IgE-aB to *B. tropicalis*, *D. farinae*, *D. pteronyssinus* and *L. destructor* allergen components were determined by means ImmunoCAP ISAC® (ThermoFisher Scientific, Sweden).

**RESULTS:** The frequency of IgE-aB to most commonly identified allergen components were approximately equal: to nDer f 1 – 56,9% (49/86), to rDer f 2 – 67,4% (58/86), to nDer p 1 – 50,0% (43/86) and to rDer p 2 – 54,6% (47/86). To the other components detection rate was minimal: to rBlo t 5 – 5,8% (5/86), to rLep d 2 – 6,9% (6/86) and to rDer p 10 – 18,6% (16/86). Only 2,3% (2/86) patients showed sensitization to all 5 allergen components (rBlo t 5, nDer f 1, rDer f 2, nDer p 1, rDer p 2). Sensitization of varying degrees of intensity to 4 components (nDer f 1, rDer f 2, nDer p 1 and rDer p 2) was detected in 25,5% (22/86) pts. Only two components (rDer f 2 and rDer p 2) respond to 11,6% (10/86) pts. To nDer f 1, rDer f 2, nDer p 1 and rDer p 2 with co-sensitization to rDer p 10 were found in 2,3% (2/86) pts and at very high levels of ISU-E. Sensitization to only one allergen component was detected in 29,0% (25/86) of which 15,1% (13/86) react only with rDer p 10. The component rLep d 2 has always been combined with sensitization to mite allergens *D. farinae* and *D. pteronyssinus*.

**CONCLUSIONS:** Polysensitization to major house dust mite allergens and to rDer p 10 is characterized by very high levels of ISU-E, which may indirectly indicate the severity of allergic symptoms in this category of pts, difficulties or inability to conduct immunotherapy. The profile of pts with sensitivity to mite allergens is quite heterogeneous, although the symptoms may be identical clinically. To select a drug for immunotherapy and to predict its effectiveness, an additional examination using component diagnostics is necessary.

# SIGNIFICANCE OF DIAGNOSIS OF NON-IGE ALLERGY TO VENOM BEE APIS MELLIFERA

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**BACKGROUND:** Hymenopteran venom Allergy (HVA) occupies one of the leading places in the structure of acute anaphylactic reactions, and is often a life-threatening Allergy. In Russia, there are no registered therapeutic and diagnostic allergens of hymenopteran insects for in vivo testing, as a result, in vitro diagnostics plays a leading role. Immunological methods used in clinical practice do not always allow to determine the nature of the reaction in the patient. We conduct research on the epidemiology of HVA and the most effective methods of laboratory diagnosis.

**METHODS:** 900 random respondents answered the questions of a specialized questionnaire developed by us "Allergo\_insect". 55 selected patients with HVA clinic were also examined. Blood and serum of all patients were examined by immunological methods for the content of sIgE venom of Hymenoptera, as well as for the expression of CD63 and CD203c molecules by flow cytometry.

**RESULTS:** According to the survey, 53.3% noted the absence of allergic reactions, and 46.7% reported Allergy symptoms. 315 patients reported increased sensitivity to contact with insects, of which 201 people indicated reactions to Hymenoptera venom. The analysis of the survey results shows that 65.2% of patients showed the development of a physiological local inflammatory reaction when stung, and only 70 patients (34.8%) can be assumed to be Hymenoptera Venom Allergy.

During laboratory examination, the presence of positive sIgE by ELISA was revealed in 51% of patients (n=55), sIgE by ImmunoCap was revealed in 39 (71%) of patients. During the basophil activation test (TAB), CD63 molecule expression and significant CD203c expression under Hymenoptera allergens were observed in all 39 patients. Also, in 16 (29%, n=55) patients with a positive history of TAB with stimulating doses of Hymenoptera venom, CD63 expression was not detected, but a moderate increase in CD203c expression was detected. We consider these patients as a group with non-IgE-mediated HVA.

**CONCLUSION:** Allergy to Hymenoptera venom reduces the quality of life and causes serious socio-economic problems, hyperergic reactions are common, but the proportion of HVA is not high. HVA is characterized by a particular severity and rapid development of life-threatening symptoms after stinging, and can occur not only in the prevailing IgE type, but also in the non-IgE type.

# IMMUNOTHERAPY WITH THE PRES-BASED GRASS POLLEN ALLERGY VACCINE BM32 INDUCES HBV-SPECIFIC PROTECTIVE ANTIBODY RESPONSES: EPITOPE SPECIFICITY AND KINETICS OF ANTIBODY RESPONSES

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There are several unsolved issues in the management of hepatitis B infection (HBV). Approx. 10% of vaccinees are non-responsive to HBsAg-containing vaccines, and therapeutic vaccines for the treatment of chronic infection are not available. Conventional HBV vaccines are based mainly on HBsAg, although the other surface protein preS has been shown to be crucial for the virus' entry into hepatocytes via NTCP. We recently found that immunotherapy with the preS-containing recombinant grass pollen allergy vaccine BM32 induced antibodies which inhibit HBV infection *in vitro*. To further investigate the vaccine's anti-HBV potential, we produced preS in *E. coli* and synthesized three preS-derived peptides: peptide A comprising the epitope, involved in liver cell attachment; peptide B – the epitope thought to be required for inhibition of infection; and peptide C including both regions. Sera from 130 grass pollen allergic patients, who had received 3, 4 or 5 injections of BM32 or placebo in monthly intervals, were tested in ELISA assays. We analyzed levels of IgG as well as IgG isotypes and subclasses. We detected the induction of a robust preS-specific IgG antibody response, consisting mostly of IgG<sub>1</sub> and IgG<sub>4</sub> subclasses; IgG<sub>1</sub> appeared early, while IgG<sub>4</sub> appeared later and was more sustained. Serum levels of IgG<sub>1</sub> and IgG<sub>4</sub> antibodies against preS and preS-derived peptides were quantified. PreS-specific IgG<sub>1</sub> and IgG<sub>4</sub> reacted with all three peptides and thus were directed against the regions of preS important for liver cell attachment and inhibition of infection, which supports our previous findings and indicates that BM32 indeed may protect against HBV.



# APPLICATION OF RECOMBINANT GAMMA-GLIADIN 1 FOR THE CELIAC DISEASE PATIENTS IDENTIFICATION.

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**INTRODUCTION.** Celiac disease (CD) is a hereditary chronic inflammatory disease of the small intestine caused by a hypersensitive immune response to dietary gluten proteins, that affects approximately 0.7% of the population.

**GOAL.** Development of a new diagnostic method for celiac disease identification based on the detection of antibodies to recombinant gamma gliadin.

**MATERIALS AND METHODS.** A synthetic gene encoding gamma-gliadin 1 (GG1) was expressed in *Escherichia coli*. Recombinant gamma-gliadin 1 (rGG1) was purified and used for ELISA analysis of sera obtained from the patients with CD and healthy subjects.

Patients received a diagnosis according to European Society for Paediatric Gastroenterology, Hepatology and Nutrition guidelines (serology and histology).

Group I – 5 healthy patients - control serum (median age, 4 years; range, 2-11 years; male/female sex, 4/1).

Group II – sera were taken from 6 untreated patients with CD (median age, 9 years; range, 1-17 years; male/female sex, 4/2).

Group III – serum samples were obtained from 11 patients with CD after gluten free diet (GFD) 6 months to 81 months (median age, 8 years; range, 4-17 years; male/female sex, 4/7).

## RESULTS.

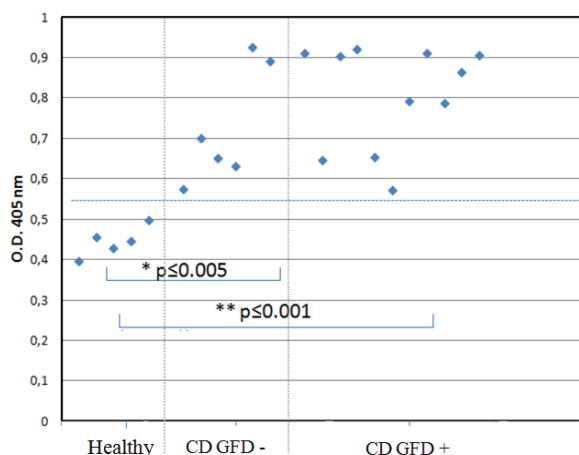


Fig.1 IgA reactivity to rGG1. Sera from healthy persons (I group), untreated patients with CD (II group), and CD after gluten free diet (III group) were tested for IgA reactivity to rGG1 by means of ELISA. The results are shown as a scatter plot, and OD values corresponding to the amounts of bound antibodies are displayed on the y-axes. The dotted horizontal lines indicate the cutoff values, and the dotted vertical lines show the division into I-III groups.

The serum all children were determination of the number of antibodies to rGG1 using ELISA. Group I - the average rGG1 value was  $0.44 \pm 0.04$ , Group II -  $0.73 \pm 0.14$ , Group III -  $0.80 \pm 0.13$ . In children with newly established CD and patients with CD GFD +, a significant increase in rGG1 was established compared with the control group ( $0.44 \pm 0.04$ , versus  $0.73 \pm 0.14$ ,  $p \leq 0.005$ ) and ( $0.44 \pm 0.04$ , versus  $0.80 \pm 0.13$ ,  $p \leq 0.001$ )

**FINDINGS.** Our preliminary results showed that the amounts of bound antibodies to rGG1 in the group of children with celiac disease were significantly higher than in healthy children. Further research is needed to prove the feasibility of using recombinant g-gliadin 1 for the diagnosis of celiac disease.

# MYCOBACTERIUM VACCAE LYSATE INDUCES IL-10 RESPONSE IN HUMAN IMMUNE CELLS

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**BACKGROUND:** *M. vaccae* lysate used in topical emollient have been shown to prevent atopic dermatitis in high-risk infants. However, the mechanisms of action of lysate is not fully understood.

**METHODS:** We characterized consequences of immune responses to sonic lysate of *M. vaccae* strain DSM 43292 (MVSL43292) in a model system containing whole human blood samples from 32 healthy donors, using commercially available *B. longum*, *B. bifidum* and *B. adolescentis* lysate (BL) as a control and PBS as a negative control. The innate and adaptive cytokine responses were evaluated in cell-free supernatants of the whole blood incubations: IL-1 $\beta$ , IL-6, TNF $\alpha$ , IL-8, IL-10, IL-2, IL-4, IL-13, IL-12p70, and IFN $\gamma$ .

**RESULTS:** Exposure to MVSL43292 or BL resulted in the initiation of immune responses and change of secreted cytokines profile. Similar levels were recorded for IL-1 $\beta$ , IL-6, TNF $\alpha$ , IL-8, IL-2, IL-12p70, and IFN $\gamma$ . However, median IL-4, 13 levels were diminished in MVSL43292-incubated blood (1.67 (2.39) pg/mL vs 3.86 (4.05) pg/mL ( $p=0.003$ ); 5.44 (6.98) pg/mL vs 9.38 (8.22) pg/mL ( $p=0.0023$ ), respectively) and IL-10 level was higher (7.03 (7.14) pg/mL vs 1.11 (0.96) pg/mL ( $p=0.0012$ )) compared to blood incubated with BL. Data are presented as median (IQR), Bonferroni-adjusted  $\alpha$ -level was applied for multiple comparisons.

**DISCUSSION:** Atopic diseases are on the rise on urbanized societies. One explanation for this is 'hygiene hypothesis' claiming that reduced exposure to environmental microorganisms leads to Th2-skewed immune cells differentiation and the consequent clinical presentations. One of the microorganisms responsible for the proper immune system development is *M. vaccae*. We have proposed recently its lysate use in topical formulations for the primary prevention of atopic dermatitis. Here we showed that mechanism of action of *M. vaccae* lysate is associated with induction of anti-inflammatory cytokine profile. Further research is needed to elucidate whether these events are responsible for the anti-allergic MVSL43292 effects in early life and whether MVSL43292 or its analogues can be used for the treatment of atopic dermatitis and other allergic diseases.