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Clinical and immunological characteristic of children with recurrent episodes of acute laryngotracheitis, acute respiratory infections, otorhinolaryngological deseases

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Annotation

Introduction. Researching on pathogenesis and immunopathogenesis of acute respiratory infections in conjunction with recurrent episodes of acute laryngotracheitis (ALT) and otorhinolaringological diseases (tonsillopharyngitis, rhinosinusitis, otitis media) is a high priority task for pediatrics, necessary to create an approach for preventing chronic diseases.

Objective of the present work. Researching on clinical and immunological characteristics and efficiency of treatment with prescription of bacterial lysate (OM-85) for children with recurrent episodes of acute laryngotracheitis (ALT), acute respiratory viral infections (ARVI), otorhinolaringological diseases.

Materials and methods. Fifty children aged 3–9 years old (32 children 3–6 years, 18 children 7–9 years old; 32 boys, 28 girls) in the initial period of monitoring, with recurrent episodes of acute laryngotracheitis (ALT), acute respiratory viral infections (ARI), otorhinolaryngological diseases were examined. The treatment in accordance with pediatric clinical practice guidelines, consultations with a pediatrician, otorhinolaryngologist, allergologist were provided to all children. Twenty five children received a standard treatment (subgroup Ia) and 25 children (subgroup I b) received bacterial lysate (Broncho-Vaxom) in complex treatment. Subgroups I a, I b were similar in clinical symptoms, gender and age related. Follow up period continued three years. The results were evaluated a year and three years after treatment. Experimental group, consisting of 30 children 3–9 years old, was additionally examined. The group had the following characteristics: frequency of acute respiratory infections 3–5 times a year, absence of chronic respiratory diseases, of recurrent episodes of acute laryngotracheitis. Immunological survey included some parameters of innate immunity, serum immunoglobulins, chemiluminescence, interferons (IFN-alfa, IFN-gamma).

Results. In children with repeated episodes of ALT and ARI and otorhinolaryngological diseases the expression of TLR2 and TLR4 on $CD14^+$cells, receptors for IFN- γ ($CD14^+CD119^+$) is significantly higher than in children without ALT and with a frequency of ARI 3–5 times a year, which is accompanied by a decrease in the level of IFN-γ and IFN-α and is associated with the development of dysregulation of the immune system, which decreases with the administration of bacterial lysate (OM-85). Complex treatment of children within 3 years with the prescription of the drug significantly reduces the frequency of ARVI — 2.6 times, tonsillopharyngitis — 1.8 times, rhinosinusitis — 2.2 times, the need for the prescription of antibiotics — 2.2 times. In the group treated by standards after 3 years, the need for antibiotics decreased by 1.6 times, the frequency of ARVI exacerbations — by 1.2 times, tonsillopharyngitis — by 1.5 times, rhinosinusitis — by 1.8 times.

Conclusion. Children with recurrent episodes of ALT, ARVI and otorhinolaringological diseases experience immune dysregulation of innate immunity: increased expression of TLR2 and TLR4 on $CD14^+$cells ($TLR4^+CD14^+$, $TLR2^+CD14^+$), receptors for IFN- γ ($CD14^+CD119^+$), a decrease in the level of IFN-γ and IFN-α. The inclusion of bacterial lysate (OM-85) in the complex treatment reduces immune dysregulation, ARVI frequency, ARVI exacerbation (tonsillopharyngitis, rhinosinusitis, otitis media), the need to administer antibiotics in children

Keywords: acute laryngotracheitis, bacterial lysate, TLR4, TLR2 receptors, innate immunity, recurrent respiratory infections, laryngotracheitis, tonsillopharyngitis, rhynosinusitis.

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Клинико-иммунологические особенности у детей с повторными эпизодами острого ларинготрахеита, респираторных инфекций и ЛОР-заболеваниями

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Аннотация

Введение. Изучение патогенеза и иммунопатогенеза повторных респираторных инфекций (ОРВИ) в сочетании с острым ларинготрахеитом (ОЛТ) и ЛОР-патологией (тонзиллофарингит, риносинусит, отит) — актуальная задача для педиатрии, решение которой важно и для разработки подходов к профилактике формирования хронических заболеваний. Недостаточно изучены механизмы коморбидности с инфекциями и иммунодисрегуляции врожденного иммунитета.

Цель работы. Изучение клинико-иммунологических особенностей и эффективности назначения бактериального лизата (бронхо-ваксом) у детей с повторными эпизодами острого ларинготрахеита (ОЛТ), ОРВИ и ЛОР-заболеваниями.

Материалы и методы. Обследовано 50 детей (22 мальчика и 28 девочек) в возрасте 3–9 лет на начало наблюдения (32 ребенка — в возрасте 3–6 лет и 18 детей — 7–9 лет) с повторными эпизодами ОЛТ, ОРВИ и ЛОР-патологией, частота ОРИ 6–12 раз, частота ОЛТ 3–8 раз в год. Все дети консультированы педиатром, ЛОР-врачом. Дети консультированы и наблюдались аллергологом-иммунологом. 25 детей получали лечение по стандартам (подгруппа Iа) и 25 детей (подгруппа Iб) получали в комплексном лечении бактериальный лизат (бронхо-ваксом), обе подгруппы сходны по полу и возрасту, клиническим проявлениям. Срок наблюдения 3 года, эффективность оценивали через год и через три года лечения. Дополнительно обследована группа сравнения из 30 детей в возрасте 3–9 лет (21 — в возрасте 3–6 лет и 9 — в возрасте 7–9 лет) с частотой ОРИ 3–5 раз в год, без очагов хронической инфекции респираторного тракта и повторных эпизодов ОЛТ. Иммунологическое обследование включало показатели системного и врожденного иммунитета ($CD3^-HLA\text{-}DR^\pm$, $CD3^+HLA\text{-}DR^\pm$, $CD3^-CD16^\pm$, $CD3^+CD16^\pm$, $TLR2^+CD14^\pm$, $TLR4^+CD14^\pm$, $CD14^+CD119^\pm$ клетки), сывороточные иммуноглобулины (A, M, G, E), показатели хемилюминесценции, интерфероны (ИФН-α, ИФН-ɤ).

Результаты. У детей с повторными эпизодами ОЛТ и ОРВИ экспрессия TLR2 и TLR4 на $CD14^+$- клетках ($TLR4^+CD14^+$, $TLR2^+CD14^+$), рецепторов к ИФН-γ ($CD14^+CD119^+$) достоверно выше, чем у детей без ОЛТ и с частотой ОРВИ 3–5 раз в год, что сопровождается снижением уровня ИФН-γ и ИФН-α и связано с развитием дисрегуляции иммунной системы, которая уменьшается при назначении бактериального лизата (бронхо-ваксом). Комплексное лечение детей в течение 3 лет с назначением препарата достоверно снижает частоту ОРВИ — в 2,6 раза, тонзиллофарингита — в 1,8 раза, риносинусита — в 2,2 раза, потребность в назначении антибиотиков — в 2,2 раза. В группе, получавшей лечение по стандартам, через 3 года потребность в назначении антибиотиков снижалась в 1,6 раза, частота обострений ОРВИ — в 1,2 раза, тонзиллофарингита — в 1,5 раза, риносинусита — в 1,8 раза.

Заключение. У детей с повторными эпизодами ОЛТ, ОРВИ и ЛОР-патологией наблюдается иммунодисрегуляция врожденного иммунитета: повышение экспрессии TLR2 и TLR4 на $CD14^+$-клетках ($TLR4^+CD14^+$, $TLR2^+CD14^+$), рецепторов к ИФН-γ ($CD14^+CD119^+$), снижение уровня ИФН-γ и ИФН-α. Включение в комплексное лечение бактериального лизата (бронхо-ваксом) у детей снижает иммунодисрегуляцию, частоту ОРВИ, обострений ЛОР-заболеваний (тонзиллофарингита, риносинусита, отита), потребность назначения антибиотиков.

Ключевые слова: острый ларинготрахеит, бактериальный лизат, TLR2, TLR4 рецепторы, врожденный иммунитет, повторные ОРВИ, тонзиллофарингит, риносинусит.

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Comorbidity with respiratory viral infections in frequently ill children, including obstructive laryngitis (OLT) (ICD 10 — J05.0) and acute laryngotracheitis (ALT) (ICD 10 — J04.2) are considered to be relevant interdisciplinary problems. The severity of clinical symptoms, laryngeal stenosis in OLT and other complications point to the need for urgent and emergency care [1, 2]. The pathogenesis involves viruses of influenza, parainfluenza, adenoviruses, respiratory-syncytial virus, bocavirus, viral-viral associations, microbial-viral associations, contributing to the formation of inflammation in the subglottic part of the larynx [1, 2, 3]. The study of OLT immunopathogenesis indicates the presence of allergic component that is confirmed by an increase in total IgE, IL-4 [4, 5]. OLT disappears in most children by age 7; acute laryngotracheitis (ALT) with no laryngeal stenosis is more common in older children [6]. It is important to study immunopathogenesis of repeated ALT using modern methods of allergy and immunodiagnostics, including indicators of innate immunity. We examined children with recurrent ALT and ARVI; bacterial and viral infections, acute ENT pathology (adenoiditis, otitis media, chronic rhinosinusitis, chronic tonsillopharyngitis, etc.) were reported in some of them. Bacterial lysates are known to be effective in chronic diseases of the ENT organs and ARVI [4, 7]. We chose Broncho-Vaxom, there were no studies of its intended purpose for patients with recurrent ALT, ARVI and ENT-pathology in the available literature.

OBJECTIVE

Researching on clinical and immunological characteristics and efficiency of prescribing bacterial lysate (OM-85) to children with recurrent acute laryngotracheitis (ALT), ARVI and ENT diseases.

MATERIALS AND METHODS

50 children (22 boys and 28 girls) aged 3–9 years old in the initial period of monitoring (32 children aged 3–6 years and 18 children — 7–9 years) with recurrent ALT (3–9 times a year), ARVI (6–12 times a year) and ENT-pathology (adenoids of I-III degree — in 16 (32 %)), chronic tonsillopharyngitis — in 40 (80 %), chronic rhinosinusitis — in 18 (36 %), recurrent acute moderate serous otitis media — in 8 (16 %)) were examined. The children were randomly divided into 2 groups Ia and Iб and received standard treatment; 25 children (subgroup Iб) additionally got bacterial lysate (Broncho-Vaxom) outside the acute period: 10 days a month, 3 months; 4 courses курса in 3 years; 1 courses in 1st year, 1 course in year 2 and 3. The subgroups are similar in sex, age and clinical manifestations of the disease. Clinical and immunological screening was held within 6 months prior to treatment after the course of bacterial lysate (Broncho-Vaxom) and in 3 years (group Ia did not receive the drug). The children were examined outside ALT, ARVI and acute ENT-pathology, did not received antibacterial therapy while taking bacterial lysate, further— according to the indications. There was further examination of a comparison group of 30 children at age 3–9 years (21 children at age 3–6 years and 9 children — 7–9 years) with ARI frequency 3–5 times a year without chronic respiratory tract infection and recurrent ALT. All children received standard treatment, follow-up period was 3 years. The study was conducted in “SRC Institute of Immunology” FMBA of Russia, all the protocols were approved by the Ethic Committee.

Before consulting an allergist-immunologist at the beginning of the study the children were diagnosed acute stenosing laryngotracheitis (OLT), they had been observed by an ENT doctor and pediatrician for 2 years. Thus, in the opening, the children aged 3–6 years old had: in 25 (50 %) — OLT of I degree, mild course (score of 3–5), according to the classification [1]; in 25 (50 %) — OLT of II degree, moderate course (score of 5–8) with inpatient treatment. Examination and monitoring of such patients are important and relevant. OLT diagnosis was made by an ENT doctor in accordance with the recommendations [8, 9, 10]. Further patient follow-up did not mark laryngeal stenosis symptoms, at an exacerbation accompanied by characteristic clinical manifestations; ENT diagnosed acute laryngotracheitis (ALT) [8-11]. Laryngitis and laryngotracheobronchitis are characterized by rough cough and hoarseness; there is no laryngeal stenosis phenomena and respiratory failure. ARVI can be accompanied by the following nosological forms: acute nasopharyngitis, acute pharyngitis, acute laryngi-

tis, acute tracheitis, acute laryngotracheitis, acute laryngopharyngitis. ARVI symptoms can last on average 10–14 days [9, 12, 13]. In acute ARVI period the children were monitored and treated by a pediatrician at the place of residence. With recurrent ALT (3–8 episodes a year) the children were referred for an allergist-immunologist consultation outside the acute period. The children were examined by a pediatrician, allergist-immunologist, ENT doctor; pathology of the larynx and trachea is excluded. The diagnosis of ENT diseases (tonsillopharyngitis, rhinosinusitis, otitis media) was made by an ENT doctor [10, 14]. All the children received rehabilitation of the foci of infection according to indications at the place of residence; antiseptic drugs were prescribed to irrigate the mucous of the tonsils and pharynx — benzyldimethyl [3-(myristoilamine)propyl]ammonium chloride monohydrate 0,01 % solution twice per day for 7 days. Considering the sensitivity, antibiotics were prescribed in the acute period (tonsillopharyngitis, rhinosinusitis) in the presence of complications, sowing from the pharynx, nose or tonsils mucosa *Streptococcus haemoliticus β, Streptococcus pneumonia*, according to the recommendations [8, 9, 10, 14].

The study included laboratory and instrumental methods: clinical blood test, urinalysis, biochemical blood test (according to indications), bacterial inoculation on flora and the sensitivity of microorganisms to antibiotics from the mucous of the pharynx and tonsils. Immunological indicators were determined by flow cytometry as instructed; monoclonal antibodies with mono- and double-label Beckman Coulter: $CD3^-HLA\text{-}DR^+$-, $CD3^+HLA\text{-}DR^+$-, $CD3^-CD16^+$-, $CD3^+CD16^+$-, $TLR2^+CD14^+$-, $TLR4^+CD14^+$-, $CD14^+CD119^+$-cells were used. TLR2 and TLR4 expression were determined using monoclonal antibodies by Hycult Biotech. The standard method of direct immunofluorescence was applied using monoclonal antibodies, labeled by a fluorescein isothiocyanate (FITC) or biotin, or phycoerythrin as instructed. The samples were analyzed using the flow cytometer Epics PROFILE-II (Cultronics) (FC 500 Beckman Coulter). Total IgE was defined by the ELISA method (Hema, Russia). Serum immunoglobulin concentration (A, M, G) was determined by the method of performing radioimmunoassay in Mancini gel [15]. Chemiluminescence study (spontaneous, induced, stimulation index) was conducted following the methology [16].

Statistical processing of the results was carried out using Standart program with Statistica 6,0 software package. Types of statistical analysis evaluating the reliability by Student's test, an interval estimate of standard deviation and dispersions of the normal distribution by χ^2 criterion, Mann-Whitney U-test were used.

REFINED INCLUSION CRITERIA FOR THE STUDY:

— children with ALT (3–8 episodes a year), with OLT I–II in anamnesis, aged 3–9 years old at the beginning of the strudy;

— disease duration more than 1 year;

— ARVI frequency 6–12 times a year;

— ENT disorders (chronic tonsillopharyngitis, rhinosinusitis).

CRITERIA OF PATIENT EXCLUSION FROM THE STUDY:

— children with primary immunodeficiencies;

— children with severe somatic diseases at the sub- and decompensation stage (hemorrhagic syndrome, diabetes, disease of liver, endocrine system, kidney and other internal organs; autoimmune diseases, active and latent tuberculosis);

— children with severe allergic diseases (severe BA, diffuse AtD, severe AR, nasal polyposis II–IV);

— children with ALV in the neonatal period, BPD; — children with diagnosed gastroesophageal reflux;

— children, receiving immunotropic drugs for 6 previous months.

RESULTS AND DISCUSSION

When examining indicators of clinical and biochemical blood tests (total bilirubin, ALT and ACT), pathology of the general urine analysis was not found.

Infection persistence in children with recurrent ALT, ARVI and ENT pathology is presented in Table 1.

In children with recurrent ALT, ARVI and ENT diseases monoculture is identified in 42 %, 2 and more causative agents — in 54 %, *Candida albicans* — in 10 %, combined bacterial and fungal flora is marked in 10 % of examined children. Flora growth was not found in 16 % of children. Quan-

Table 1. **Detection of pharynx microbiota**
Таблица 1. **Микрофлора, высеваемая из зева**

Pathogen	Children, ARI frequency 3–5 times/year, n=30	OLT, ARI frequency 6–12 times/year, n=50
	Qty/% children	
Staphylococcus, including: *Staphylococcus aureus* *Staphylococcus haemoliticus*	12/40* 8/26,6* 4/13,4	30/60 28/56 8/16
Streptococcus, including: *Streptococcus pneumonia* *Streptococcus haemoliticus-β*	8/26,6* 5/16,6* 3/10	22/44 18/36 5/10
Neisseria perflava	5/16,6	7/14
Corinaebacterium pseudodiphtheriae	2/6,6	3/6
Klebsiella pneumonia	2/6,6	5/10
Candida albicans	1/3,3*	5/10
Flora growth is not found	8/26,6	8/16

* $p<0,05$; $\chi^2>3,8$.

titative indicators ranged from 4×10^4 CFU/ml to 6×10^6 CFU/ml. The results point to infection persistence on the pharynx mucous in children with recurrent ALT, ARVI and ENT diseases. When compared to the children with ARVI frequency 3–5 times a year with no OLT and foci of chronic respiratory tract infection there is significantly lower frequency of *Staphylococcus aureus, Streptococcus pneumoniae, Candida albicans* ($p<0,05$; $\chi^2>3,8$) sowing and less pronounced infection persistence. Quantitative indicators ranged from 3×10^3 CFU/ml to 4×10^5 CFU/ml. The groups are similar by sex and age.

Table 2 presents the comparison of indicators in children with recurrent ALT, ARVI and ENT pathology and children with ARVI frequency 3–5 times a year, no ALT and ENT pathology. The children are presented without subdivision into age as dynamics of indicators were similar. There was a higher level of IgG, IgA, indicators of spontaneous and induced chemiluminescence, spontaneous production of IFN-γ cells, induced synthesis of IFN-α and IFN-γ in children without ALT and ARVI frequency 3–5 times a year with no ENT pathology ($p<0,05$). Significant difference in the number of $CD3^-CD16^+$-, $CD14^+$-cells, stimulation index, IFN-α in serum and spontaneous production by IFN-α were not found. Children with ALT have a significant lower level of IgE, expression of TLR2, TLR4, CD119 receptors on $CD14^+$-cells. Most children with recurrent ALT experience increased expression of TLR2, TLR4, CD119 receptors on $CD14^+$-cells ($p<0,05$). The results were obtained for the first time.

Table 4 presents clinical efficacy data of prescribing bacterial lysate (BL) (broncho-vaxom) in children with recurrent ALT, ARVI and ENT diseases over 3 years of follow-up.

In the first follow-up year children received 2 courses of the drug, they experienced acute ENT-pathology, ARVI and recurrent ALT. In the 2nd and 3rd year there were fewer cases of acute ENT-pathology, ARVI and recurrent ALT. Thus, 1 course of BL (broncho-vaxom) a year was prescribed. In 3 years the control group had a significant decrease in the frequency of acute tonsillopharyngitis ($p<0,05$), reduction in the need for antibiotics is significant on the Mann-Whitney test ($p<0,05$), dynamics of other indicators were not reliable.

Group Iб, that received BL, showed a significant decrease in the frequency of tonsillopharyngitis and ARVI ($p<0,05$) in the 3rd year, all the indicators were lower, compared to Ia group ($p<0,05$), ARVI frequency is 2,6 times as low.

The children with recurrent ALT, ARVI and ENT-pathology experience the persistence of bacterial flora and changes in immunological parameters, see Table 1 and 2. The administration of BL (broncho-vaxom) increases the indicators of spon-

Table 2. **Immunologic indices in children with recurrent respiratory infections and laryngotracheitis and children without recurrent infections**
Таблица 2. **Иммунологические показатели у детей с повторными эпизодами ОРИ, ОЛТ и ЛОР-патологии и в группе сравнения**

		Children with ARI frequency 3–5 times/year	Children with ALT and ARI frequency 6–12 times/year
Pathogen		3–9 years, n = 30	3–9 years, n = 30
IgG (blood), mg/ml		6,8–16,4*	5–11,4
IgM (blood), mg/ml		0,72–2,2	0,48–1,8
IgA (blood), mg/ml		0,62–1,8*	0,23–1,6
IgE (blood), mg/ml		50–102*	50–2000
$CD3^-CD16^+$-cells	%	5–20	5–19
$CD14^+$-cells	%	64,7–94,2	60,5–98,8
$TLR2^+CD14^+$-cells	%	24,3–44,6*	54,1–94,9
$TLR4^+CD14^+$-cells	%	25,2–51,4*	35,5–96,2
$CD14^+CD119^+$-cells	%	28,8–46,3*	98,8–100
Spontaneous chemiluminescence, mV/min.		18–34*	8–26
Induced chemiluminescence, mV/min.		160–380*	100–620
Stimulation index		31–52	30–75
Interferon, pg/ml		n = 20	n = 20
IFN-α level in blood		1–6	1–3
Spontaneous production of IFN-α by blood cells		1–16	1–13
Virus-induced synthesis of IFN-α		265–760*	115–460
Spontaneous production of IFN-γ by blood cells		10–42*	0–38
Mitogen-induced synthesis of IFN-γ		367–1324*	62–1499

* $p < 0,05$, Mann-Whitney test.

taneous and induced chemiluminescence, serum IgG, IgA ($p < 0,05$), dynamics of IgM, IgE were not reliable, see Table 3. There was an increase in the number of activated T-cells ($CD3^+HLA\text{-}DR^+$), NKT ($CD3^+CD16^+$) and NK-cells ($CD3^-CD16^+$) ($p < 0,05$). The relative amount of $TLR2^+CD14^+$- and $TLR4^+CD14^+$-cells significantly reduced ($p < 0,05$). There was a rise in serum IFN-α and synthesis of IFN-γ and IFN-α cells after after BL (бbroncho-vaxom) administration ($p < 0,05$).

Very often staphylococcus sows in children with recurrent ALT, ARVI and ENT-pathology (see Table 1). Strains of staphylococcus and streptococcus were sensitive to antibiotics only in 60 % and 50 % cases, respectively. At the start of the study *Staphylococcus aureus* sowed higher than $10^4 \times$ CFU/ml in Ia group in 14 (56 %) and after a year of treatment (therapy by the standards) — in 12 (48 %) children. In the children who received broncho-vaxom (Iб group), *Staphylococcus aureus* sowed higher than $10^4 \times$ CFU/ml in 15 (60 %), in a year after 2 courses of the drug — in 7 (28 %) children, the difference is reliable, compared to Ia group ($p < 0,05$; $\chi^2 > 3,8$).

In 3 years Ia group (therapy by the standards) had reduction in frequency of acute tonsillopharyngitis per year (in a year frequency in patient $5,2 \pm 0,85$, in 3 years $3,2 \pm 0,6$ ($p < 0,05$)); decrease in rhinosinusitis frequency was not reliable and the need for antibiotics in a patient for 1st year is $5,8 \pm 0,8$ in days, for 3rd year — $4,8 \pm 0,8$ (the Mann-Whitney test $p < 0,05$), dynamics of ARVI frequency are not reliable. The amount of patients with acute otitis media in 3 years did not change (4 people). In the patients with administered BL (broncho-vaxom) after a year of treatment tonsillopharyngitis frequency per year is on average $3,1 \pm 0,52$ in a patient, for 3rd year — $1,8 \pm 0,3$ ($p < 0,05$), after a year of treatment rhi-

Table 3. **Immunologic indices in children with recurrent respiratory infections and laryngotracheitis treated with bacterial lysate (broncho-vaxom)**
Таблица 3. **Влияние бактериального лизата (бронхо-ваксом) на иммунологические показатели у детей с повторными эпизодами ОЛТ, ОРВИ и ЛОР-патологией**

Indicators		Children, receiving bacterial lysate (broncho-vaxom), n=25	
		Prior to the administration	After the first treatment
$CD3^+HLA\text{-}DR^+$-cells	%	14,1±1,1	22,2±0,9*
	× 10^9 /л	0,45±0,09	0,55±0,12
$CD3^+CD16^+$-cells	%	4,9±0,9	9,1±0,8*
	× 10^9 /л	0,15±0,04	0,3±0,05
$CD3^-CD16^+$-cells	%	11,6±0,8	17,8±1,1*
	× 10^9 /л	0,41±0,06	0,48±0,05
$TLR2^+CD14^+$-cells	%	85,4±3,2	65,9±3,9*
$TLR4^+CD14^+$-cells	%	80,1±2,8	57,8±4,9*
IFN-α level in blood, pg/ml		1,4±0,2	5±0,7*
Spontaneous production of IFN-α by blood cells, pg/ml		6,8±1,4	7,3±1,7
Virus-induced synthesis of IFN-α, pg/ml		289±72	538±69*
Spontaneous production of IFN-γ by blood cells, pg/ml		23,6±2,8	25,5±2,7
Mitogen-induced synthesis of IFN-γ, pg/ml		721,8±108	960±98*
Spontaneous chemiluminescence, mV/min.		19,6±1,8	26,2±1,4*
Induced chemiluminescence, mV/min		298±10,4	326±10,8*
Stimulation index		50,5±9,4	55,3±7,6
IgG (blood), mg/ml		9,4±1,3	12,5±1,1*
IgM (blood), mg/ml		1,05±0,09	1,2±0,12
IgA (blood), mg/ml		0,9±0,1	1,3±0,14*
IgE (blood), mg/ml		152,5±14,1	135,4±13,6

* $p<0,05$ when comparing indicators before and after broncho-vaxom course.

nosinusitis frequency is on average 1,3±0,3 in a patient, for 3rd year — 0,9±0,2 ($p<0,05$ comparing to Ia group in 3 years), for 1st year of treatment the administration of antibiotic is on average 3,5±0,7 in a patient, for 3rd year — 2,2±0,4 days ($p<0,05$ and compared to Ia group in 3 years of treatment). ARI frequency also decreased ($p<0,05$). The amount of patients with recurrent acute serous otitis media reduced in 3 years (4 people at the beginning and 2 people in 3 years). In 3 years all indicators in the group with the administered bacterial lysate (broncho-vaxom, 4 courses) were lower compared to Ia group ($p<0,05$), ARVI frequency is 2,6 as low, tonsillopharyngitis frequency — 1,8 times, rhinosinusitis — 2,2 times, the need for administration of antibiotics — 2,2 times. In Ia group in 3 years the need for administration of antibiotics decreased by 1,6 times, ARI frequency — 1,2 times, tonsillopharyngitis — 1,5 times, rhinosinusitis — 1,8 times.

When prescribing BL (broncho-vaxom) there is an increase in indicators of innate immunity: spontaneous and induced chemiluminescence, NK- and NKT-cells, INF-α level in blood, synthesis of IFN-α and IFN-γ by cells, IgG and IgA levels in blood. There was no increase in serum IgE, allergic reactions. The number of $CD3^+$-, $CD4^+$-, $CD8^+$-cells did not rise, however, there is an increase in the amount of activated T-cells (CD^3+HLA-DR^+). There was a reduction in the expression of TLR4- and TLR2-receptors on $CD14^+$-cells that may be associated with the decrease in the persistence of bacterial flora and the number of ARVI. The ac-

Table 4. **Frequency of recurrent diseases**
Таблица 4. **Частота обострений заболеваний**

Indicators	Ia group		Iб group (received bacterial lysate)	
	1st year	3rd year	1st year	3rd year
Frequency of acute tonsillopharyngitis in a patient a year	3,6±0,6	2,4±0,4*	2,5±0,3	1,3±0,2** ***
Frequency of acute rhinosinusitis in a patient a year	2,2±0,4	2,0±0,3	1,3±0,3	0,9±0,2***
Need to administer antibiotics in a patient a year (days)	5,8±0,8	4,8±0,8	3,5±0,7	2,2±0,4***
Number of ARVI in a patient a year	5,6±0,6	4,5±0,5	3,2±0,4	1,7±0,3***
Number of patients with no ALT	3	10	5	15

* $p < 0,05$ by comparing the data in a year and 3 years in the control group.

** $p < 0,05$ by comparing the data in a year and 3 years in the group that received the bacterial lysate.

*** $p < 0,05$ by comparing the data in the control group and the group that received the bacterial lysate in 3 years.

tivation of innate immunity with chronic and recurrent infections, particularly TLR-receptors, is the starting point in the protection development. After activation of TLR-receptors there is a signal transmission to the nucleus, activation of the synthesis of proinflammatory and anti-inflammatory cytokines, acute phase proteins [17, 18, 19]. TLRs family includes 5 subfamilies (TLR2, TLR3, TLR4, TLR5, TLR9). Sensitivity to a pathogen for tuberculous meningitis depends on TLR2 polymorphism [20]. The interaction of lipopolysaccharides and CD14-receptors through TLR4 leads to activation of cytokine synthesis [21].

We studied the expression of TLR2- and TLR4-receptors on $CD14^+$-cells ($TLR4^+CD14^+$, $TLR2^+CD14^+$), receptors to IFN-γ ($CD14^+CD119^+$) with recurrent ALT, ARVI and ENT diseases for the first time. Children with no ALT and with ARVI frequency 3–5 times a year had significantly lower IgE, lower expression of $TLR2^+$, $TLR4^+$, $CD119^+$ receptors on $CD14^+$-cells. The results were obtained for the first time. It is known that combined activation of IFN-γ synthesis and TLR (TLR4) expression results in activation of STAT1, IRF-1 transcription factors and cytokine genes (TNF, IL-6, IL-12) that triggers the immune system activation [22]. According to our data, IFN-γ decrease combined with a rise in the expression of TLR4 and TLR2 receptors on $CD14^+$-cells in most patients points to dysregulation of immunological interactions. At the same time, there is reduction in IFN-α synthesis in 30–40 % of children of different ages that decreases antiviral protection. Children with no ALT and ENT-pathology with ARVI frequency 3–5 times a year have less pronounced changes in the immune system. These results are not found in the available literature.

TLR-receptors are known to recognize conservative patterns on pathogens and to launch protection mechanisms, activating signaling pathways and cytokine genes. Imbalance of TLR-mediated IFN-α production is found in patients with CVID. According to M.V. Horeva, the number of $TLR2^+CD14^+$-cells in children aged 10–15 years was 29,6 ± 9,5 %, in adults aged 20–40 years old — 39,7 ± 11 %; $TLR4^+CD14^+$-cells in children aged 10–15 was 7,5 ± 2,4 %, in adults aged 20–40 — 6,8 ± 1,3 %б respectively. The expression of TLR2- and TLR4-receptors increased in acute myocardial infarction with inflammation [23]. According to our data, the number of $TLR2^+CD14^+$-cells and $TLR4^+CD14^+$-cells in children with recurrent ALT, ARVI and ENT disorders was higher with the diagnosed persistence of bacterial flora and infectious inflammation. Reduction in ARVI frequency and a decrease in the persistence of bacterial flora lower TLR2 and TLR4 expression that coincides with the data of other researchers [24].

Viral-bacterial association, specifics of microbiome [25] and local immunity [7, 26] often occur with respiratory infection. Our study shows that recurrent course with the drug (broncho-vaxom) over 3 years of follow-up help to reduce the frequency of acute tonsillopharyngitis, rhinosinusitis and the

need for antibiotics that confirms the possibility and feasibility of its administration in the complex treatment of recurrent ALT, ARVI and ENT-pathology.

The administration of the bacterial lysate can activate innate immunity through TLR2/6- and TLR9-receptorp, signaling pathways and IFN-γ, β, α synthesis and lead to the decrease of ARVI frequency, obstruction episodes in children and adults with COPD [27, 28] that correlates with our results on the administration of the drug (broncho-vaxom) with recurrent ALT, ARVI and ENT-pathology.

We have shown previously that patients with recurrent ALT and ARVI may experience allergic diseases that requires observation and treatment by an allergist-immunologist [29]. Thus, patients with an allergic pathology and the development of ENT diseases may be identified among children with recurrent ALT and ARVI that allows to choose a differentiated treatment approach.

CONCLUSION

1. In children with recurrent ALT, ARVI and ENT-pathology the expression of TLR2 and TLR4 on $CD14^+$-cells ($TLR4^+CD14^+$, $TLR2^+CD14^+$) and receptors to IFN-γ ($CD14^+CD119^+$) are significantly higher than in children with no ALT and ARVI frequency 3–5 times a year, accompanied by a decrease in IFN-γ and IFN-α synthesis that is associated with the development of the immune system dysregulation.

2. The expression of TLR2 and TLR4 on $CD14^+$-cells ($TLR4^+CD14^+$, $TLR2^+CD14^+$) and receptors to IFN-γ ($CD14^+CD119^+$) reduce when administrating the bacterial lysate (broncho-vaxom) in the complex treatment of children with recurrent ALT, ARVI and ENT-pathology.

3. The administration of the drug (broncho-vaxom) in the complex treatment of children significantly decreases ARI frequency — 2,6 times, tonsillopharyngitis — 1,8 times, rhinosinusitis — 2,2 times, the need for antibiotics — 2,2 times. In 3 years with the treatment by the standards the need for administrating antibiotics reduced 1,6 ptimes, frequency of acute ARI — 1,2 times, tonsillopharyngitis — 1,5 times, rhinosinusitis — 1,8 times.

REFERENCES /ЛИТЕРАТУРА

1. Tsarkova SA. Acute stenosing laryngotracheitis in children. 10 questions to a specialist. Polyclinic. Infectious diseases of the respiratory system. Special issue. 2015; 2: 14–19. (In Russ.)
2. Geppe NA, Kolosova NG. Acute stenosing laryngotracheitis in children. Farmateka. 2013; 15 (268): 40–43. (In Russ.)
3. Jedrychowski W, Perera FP, Jancowski J, Mrozek-Budzyn D, Mroz E, Flak E, Skarupa A, Edwards S, Lisowska-Miszczyk I. Early wheezing phenotypes and cognitive development of 3-yr-old. Community-recruited birth cohort study. Pediatr. Allergy Immunol. 2010; 21: 550–556.
4. Zaplatnikov AL, Girina AA, Burtseva EI [et al.]. Acute, recurrent and repeat respiratory infections in children: the issues of immunoprophylaxis and immunotherapy. Russian Journal of Woman and Child Health. 2023; 6 (1): 50–59. (In Russ.)
5. Kladova OV, Malinovskaya VV, Suskova VS [et al.]. Clinical and patogenetic significance of immunity in relapsing rump in children. Children Infect. 2004; 4: 6–21. (In Russ.)
6. Ryazantsev SV, Karneeva OV, Garashchenko TI, Gurov AV, Svistushkin VM, Abdulkerimov HT, Polyakov DP, Sapova KI. Acute Laryngitis. Clinical guidelines by the national medical association of otorhinolaryngologist. 2016; 19 p. (In Russ.)
7. Markova TP. Often sick children. Immunologist's view. M.: TORUS PRESS, 2014. — 196 p. (In Russ.)
8. Group in children (acute obstructive laryngitis). MKB-10 J05.0: clinical guidelines. Moscow: Original layout, 2015. — 32 c. (In Russ.)
9. Clinical Guidelines — Acute Respiratory Viral Infection (ARVI) in Children — 2016 (2017–2021, 14.05.2019). — Approved by the Ministry of Health of the Russian Federation. (In Russ.)
10. Palchun VT, Magomedov MM, Luchikhin L.A. Otorhinolaryngology. Textbook. 2-edition, revised and additional. — M.: GEOTAR-Media, 2011. — 656 p. (In Russ.)
11. Clinical guidelines. Acute laryngitis. National Medical Association of Otolaryngologists. Union of Pediatricians of Russia. 2020. 36 p. (In Russ.)
12. Clinical Guidelines — Bronchitis — 2021–2022–2023, 34 c. (28.09.2021). — Approved by the Ministry of Health of the Russian Federation. (In Russ.)
13. Thompson M, Cohen HD, Vodicka TA et al. Duration of symptoms of respiratory tract in children: systematic review BMJ 2013; 347. https://doi.org/10.1136/bmj.f7027.
14. Clinical Guidelines — Acute Tonsillitis and Pharyngitis (Acute Tonsillopharyngitis) — 2021–2022–2023 (28.09.2021). — Approved by the Ministry of Health of the Russian Federation, 30 s. (In Russ.)
15. Mancini C, Iacovoni R, Fierimonte V. [et al.] Evaluation of serum IgG sub-classes in children with recurrent respiratory infections. Minerva Pediatr. 1996; 48 (3): 79–83.
16. Porter CD, Parkar MG, Collins MK, Levinsky RJ, Kinnon C. Superoxide production by normal and chronic granulomatous disease (CGD) patient-derived EBV-transformed B cell lines measured by chemiluminescence-based assays. J. Immunol. Methods 1992; 155: 151–157.
17. Kovalchuk LV, Gankovskaya LV, Meshkova RY. Clinical immunology and allergology with the basics of general immunology [M.: GEOTAR-Media, 2011. — 640 p. (In Russ.)
18. Moore CE, Segal S. Lack of association between Toll-like receptor 2 polymorphisms and to susceptibility to severe disease caused by Staphylococcus aureus. Clin. Diagn. Lab. Immunol. 2004; 6: 1194–1197.
19. Sandor F, Buc M. Toll-like receptors. II. Distribution and pathways in TLR signaling. Folia Biol. (Praga). 2005; 51 (6): 188–197.
20. Thuong TN, Hawn TR, Twaites GE. A polymorphism in TLR2 is associated with increased suseptibility to tuberculous meningitis. Genes. Immun. 2008; 8 (5): 422–428.
21. Da Silva Correia J, Soldau K, Christen U, Tobias TS, Ulevitch RJ. Lypopolysaccharide is in close proximity to each of the protein in its membrane receptor complex: transfer from CD14 to TLR4 and MD2. J. Biol. Chem. 2001; 276: 1129–1135.
22. Qiao Yu, Giannopoulou EG, Chan CH, Park S, Gong S, Ghen J, Hu X, Elemento O, Ivashkiv LB. Synergistic activation of inflammatory cytokine genes by interferon-γ-induced chromatin remodeling and toll-like receptor signaling. Cell, Immunity. 2013; 39 (3): https://doi.org/10.1016/j.immuni. 2013.08.009.
23. Horeva MV. Comprehensive analysis of TOLL-similar receptors in various pathological states of humans. Diss. doctor med. sciences. M., 2004. (In Russ.)
24. Gankovskaya LV, Namazova-Baranova LS, Khoreva MV, Bragvadze BG, Ogurtsova AD, Alekseeva AA, Gankovsky VA, Svitich OA. Features of Toll-like receptor 2 and Toll-like receptor 4 expression in children with bronchial asthma. Medical immunology. 2017; 19 (4): 431–440. (In Russ.)

25. Lee JT, Frank DN, Ramakrishnan V. Microbiome of the paranasal synuses: update and literature. Am. J. of Rhinology & Allergy. 2016; 30: 3–16.
26. Brandtzaeg P. Mucosal and glandular distribution of immunoglobulin components: differential localisation of free and bound SC in secretory epithelial cells. J. Immunol. 2017; 198 (5): 1768–1774.
27. Coviello S, Wimmenauer V, Polack FP, Irusta PM. Bacterial lysates improve the protective antibody response against respiratory viruses through Toll-like receptor 4. HumVaccin Immunother. 2014; 10 (10): 2896–902.
28. Luan H, Luan H, Zhang Q, Wang L, Wang C, Zhang M, Xu X. OM85-BV induced the productions of IL-1β, IL-6, and TNF-α via TLR4- and TLR2-mediated ERK1/2/NF-κB path way in RAW264.7 cells. J Interferon Cytokine Res. 2014; 34 (7): 526–536.
29. Chuvirova AG, Yartsev MN. The role of atopic sensitization in recurrent laryngotracheitis in children. Allergology and Immunology in Pediatrics. 2018; 1 (52): 33–40. (In Russ.)

AUTHORS CONTRIBUTION

A.G. Chuvirova — conducting the study, critical content check.
M.N. Yartsev — development of publication design, verification of the article critical content