International Journal of Neurorehabilitation
ISSN: 2376-0281

Akhmatova et al., Int J Neurorehabilitation Eng 2015, 2:3
DOI: 10.4172/2376-0281.1000166

Research Article Open Access

Immunological Effects of Masgutova Neurosensorimotor Reflex Integration in Children with Recurrent Obstructive Bronchitis

Nelli K Akhmatova[1]*, Svetlana K Masgutova[2,3], Irina Zh Shubina[4], Elvin A Akhmatov[1], Vasily V Khomenkov[1], Ekaterina V Sorokina[1], Elena S Korovkina[1] and Mikhail P Kostinov[1]

[1]*I I Metchnikov Research Institute for Vaccines and Serum, Laboratory of Therapeutic Vaccines, Russian Academy of Medical Sciences, Moscow, Russia*
[2]*International Dr. Svetlana Masgutova Institute of Movement Development and Reflex Integration, Warsaw, Poland*
[3]*Svetlana Masgutova Educational Institute for Neuro-Sensory-Motor and Reflex Integration, Melrose, Florida, United States*
[4]*N N Blokhin Russian Cancer Research Center, Laboratory of Cell Immunity, Russian Academy of Medical Sciences, Moscow, Russia*

Abstract

Damage to the mechanisms of immune system regulation contributes to the development and recurrence of chronic inflammatory respiratory diseases in millions of children worldwide. Treatment for those diseases has been primarily pharmacological to date, although some dietary, nutritional, and supplemental therapies have been used. We investigated the effects of a combination of standard drug treatment and therapy using the Masgutova neurosensorimotor reflex integration program, which is based on the activation of the primary motor system, compared with the effects of drug treatment alone in children with recurrent obstructive bronchitis. Our results revealed that combining MNRI with standard drug treatment normalized the number of T lymphocytes (CD3, CD4, CD8) and natural killer cells, the metabolic function of leukocytes, and the levels of regulatory and anti-inflammatory cytokines more effectively than standard drug treatment alone. We also found that the combination of MNRI and standard drug treatment was more effective than drug treatment alone in stimulating immune system function and strengthening the polarization of the immune response, both of which decrease the incidence of respiratory system diseases and prolong the intervals between recurrences.

Keywords: Masgutova neurosensorimotor reflex integration; MNRI; Recurrent obstructive bronchitis; Lymphocytes; Cytokines; Natural killer cells

Introduction

Masgutova neurosensorimotor reflex integration and respiratory conditions

Chronic obstructive pulmonary disease is a leading cause of death and disability worldwide. Since 2000 in Russia and Europe, the incidence of chronic inflammatory respiratory diseases and the frequency of hospitalizations required to treat them have increased [1-3].

Bronchitis is one of the most frequent infectious diseases of the respiratory tract in children. At present clinical forms of bronchitis include acute, recurrent and chronic [4]. Acute (simple) bronchitis is the form of acute inflammation of mucous tunic of bronchi with no signs of lung affected tissue. Most often bronchitis develops as a result of acute respiratory disease of various etiology, such as viral, bacterial, fungal, parasite, or mixed.

Acute obstructive bronchitis is characterized by narrowing and/or congestion of respiratory tract as a result of edema and hyperplasia of mucous tunic, over-secretion of mucus or bronchospasm that determines specific clinical picture. At the age of 3 obstructive syndrome is determined mainly by over-secretion of viscous thick mucus and hyperplasia of mucous tunic. Bronchospasm develops more often in children over 4 years old. In case of repeated bronchitis with broncho-obstructive syndrome (over 2-3 per year) the form may be defined as recurrent obstructive bronchitis (ROB).

Disorders of immune system regulation are thought to contribute to the development and recurrence of chronic inflammatory respiratory diseases [5,6]. For example, in children, ROB is associated with atopic and infectious inflammatory diseases, and in pediatric patients, the anatomical and physiological characteristics of the respiratory system, vegetative regulation, and immature mucous respiratory tract barriers often impede the diagnosis of bronchial asthma.

Recurring respiratory inflammation is associated with immunopathological activity and the malfunction of the hypophyseal adrenal system [7,8]. Acute adrenal insufficiency, which is a factor in the development of diseases such as ROB, can develop in individuals who experience frequent bouts of respiratory disease. It is therefore important to study the interaction of the nervous system and the immune system, because that relationship affects the levels of proinflammatory and anti-inflammatory cytokines and hormones (particularly cortisol) in individuals with a respiratory disease like ROB. In those patients, conventional therapy is often ineffective and may not result in prolonged remission. Since 1999, the use of immunomodulators in the treatment of ROB has been emphasized, although other therapeutic methods have also been effective [9-11].

We investigated the effects of one such alternative treatment. We compared the effects of standard drug treatment plus MNRI with those of drug treatment alone on immune system function and cortisol levels in children with ROB. Diseases such as ROB make it hard to exhale all of the air from the lungs. The etiology of ROB plays a role in the persistence of respiratory viruses such as respiratory syncytial virus and adenoviruses, and it is now playing an increasing role in the development of bronchial infections caused by Mycoplasma pneumoniae and Chlamydia pneumoniae. Bronchial obstruction in children is largely due to the anatomical and physiological characteristics of their respiratory system: the narrowness of the airways, the friability

***Corresponding author:** Nelli Akhmatova, I. I. Metchnikov Research Institute for Vaccines and Serum, Laboratory of Regulation Mechanisms of Immunity, Russian Academy of Medical Sciences, Moscow, 115404, Russia, Tel: 007 (495) 917-5774; Fax: 007 (495) 655-7855; E-mail: anelly@mail.ru

Received February 22, 2015; **Accepted** June 22, 2015; **Published** June 29, 2015

Citation: Akhmatova NK, Masgutova SK, Shubina IZ, Akhmatov EA, Khomenkov VV, et al. (2015) Immunological Effects of Masgutova Neurosensorimotor Reflex Integration in Children with Recurrent Obstructive Bronchitis. Int J Neurorehabilitation 2: 166. doi:10.4172/2376-0281.1000166

and hydrophilicity of the mucous membrane of the bronchial tree, its tendency to edema, and hypersecretion caused by any background inflammatory process. Our study population did not include children with asthma or those with severe acute respiratory syndrome (SARS), a bronchitis viral infection that is also a restrictive lung disease in which children have difficulty fully expanding their lungs with air.

The pathogenesis of recurrent obstructive bronchitis

Pathogenesis of recurrent obstructive bronchitis in children is complicated. Infectious factors play the major determining role in disease development. Virus effect on immature tissue may lead to chronic inflammation in bronchi even in early childhood. Acute Respiratory Virus Infection facilitates adding bacterial inflammation. Major inflammatory pathogens are considered to be *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*. The studies have revealed that *H. influenzae, S. pneumoniae* can affect ciliary epithelium and worsen cilia function. Microbial reproduction leads to progression of inflammation as a result of self-damaging bronchus structure and activation of the inflammatory cell enzymes. The consequence of these processes is impaired mucociliary clearance that leads to panbronchitis and peribronchitis, mediates formation of bronchitis deformans [12]. Intracellular pathogens, such as chlamydia and mycoplasma can provoke the start of broncho-obstructive disease or cause its exacerbation, chronic condition and severe course of disease [13].

At present there is no doubt that treatment of broncho-obstructive syndrome (BOS) in children with acute respiratory infection should consider disease etiology and pathogenesis of bronchial obstruction. It is well-known that genesis of bronchial obstruction is characterised mainly by inflammatory edema and mucus over-secretion. Therefore pathogenic and symptomatic BOS therapies include anti-inflammatory, broncholytic and mucolytic drugs [14]. However, the treatment should first aim eradication of the disease causes, which led to BOS, i.e. in case of respiratory infection – to eradication of infectious pathogen. The most difficult manipulation, in terms of both diagnostics and therapy, seems treatment of recurrent broncho-obstructive diseases associated with atypical pathogens of respiratory infections (Mycoplasma pneumoniae, Chlamydia pneumonia, etc.), that is due to the persistence ability of such pathogens and unfavorable immunotropic effect.

Non-rational pharmacologic therapy may lead to transition of the disease in the chronic condition, in some cases bronchial asthma (BA) may develop. All that results in decreasing working ability and quality of life of the patients and enhanced economic expenses due to treatment of BA patients. Thus, the present study was aimed at defining most optimal treatment regimens. Recent studies [15] show that SARS may provoke the development of a transient bronchial hyper-reactivity for 4 to 6 weeks after the onset of the disease; viruses penetrate the submucosa of bronchi and lead to irritation of nerve endings. Thus, even after convalescence from SARS, patients may experience symptoms of bronchial hyperreactivity (SAB) for up to 1 month and may be at greater risk for developing ROB. The foci of chronic respiratory infections can lead to a more stable bronchial hyper-reactivity. The risk factors predisposing to the emergence of bronchial hyperreactivity are with bronchiolitis obliterans syndrome, possible immunological abnormalities of a general and local nature, diseases of the nervous system, atopic "mood," and hyperplastic changes of the upper respiratory tract infections. Consequently, in some patients ROB can lead to asthma or at least a high risk of its development. In children with ROB, a history of atopy and 3 or more episodes of bronchial obstruction should raise the clinician's suspicion regarding the presence of asthma.

Symptoms of recurrent obstructive bronchitis

The exacerbation of ROB occurs in the context of SARS and acute obstructive bronchitis. When chlamydial infection is present, there may be conjunctivitis, sore throat with a pronounced grainy texture at the back of the throat, swollen lymph nodes in the neck, persistent cough during a moderate fever, and finally, bronchial obstruction. In contrast, mycoplasma infection is characterized by an increase in body temperature to 38°C or 39°C, symptoms mimicking those of intoxication (lethargy and perhaps vomiting), symptoms of vegetodystonia (pallor, a marbling pattern to skin coloration, sweating), poor oral flushing of bacteria, drying of mucous membranes, decreased mucus production, rhinitis and pharyngitis, and nasal breathing difficulty. Seventy percent of patients have radiographic evidence of changes in the sinuses [16,17], although clinically, sinusitis is weakly expressed. One of the most prominent symptoms of ROB with mycoplasma infections is a painful, dry cough that can cause vomiting and leads to sleep disruption and then develops into an obstructive syndrome with all its manifestations. In 50% of patients with mycoplasma infection, ROB bronchodilators do not provide relief [18-21].

Masgutova neurosensorimotor reflex integration program

MNRI has been used since 1989 in Russia and Europe and, more recently, in other countries to treat individuals with certain types of motor or reflex development deficits, behavior disorders, disorders of speech or language development, or learning disabilities. It has been our clinical experience that MNRI furthers neurodevelopment in impaired individuals and enables them to integrate primary movements, reflexes, coordination systems, and skills that enable optimal functioning, development, and learning. We have found that therapy with MNRI stimulates the reflex patterns that awaken sensorimotor memory, which has been shown to positively affect physical strength, immune activity, cognitive, emotional, social, and motor abilities.

In brief, MNRI is based on exercises and techniques (repatterning, reeducation, and recoding) that involve the repetition of dynamic and postural reflex patterns. The stimulation of those reflexes revives traces of genetic motor memory and activates the innate defensive mechanisms of the body–brain system. MNRI exercises stimulate innate resources such as self-regulation, stress resistance, and immune system regulation. Repatterning, which is also facilitated by the exercises, involves the stimulation of the "defense" functions of the lower brain regions, the extension of links between neurons, the growth of neural nets, myelinization, and the creation of new nerve routing, as described by Sechenov [22-25] Virella et al. [26] and Anokhin [27,28].

We investigated the hypothesis that MNRI can also enhance immune system function in children with a chronic respiratory disease such as ROB. We have found that such patients often exhibit developmental motor deficits, poor primary motor skills, and/or aberrant reflexes. In these individuals, shallow breathing leads to hypertonus or hypotonus of the muscles and ligaments and produces inadequate protective and survival mechanisms expressed in nonintegrated concrete reflex patterns. The interrelationship of respiratory, muscle, and primary motor systems is impaired, and the development of reflex patterns in these patients is poor.

Materials and Methods

Study participants

From September 2004 through September 2005, we evaluated the effects of several MNRI programs (neurostructural, motor, and reflex

integration; tactile therapy; reflex repatterning, breathing reflex, and visual and auditory reflex integration) in 60 children (30 girls and 30 boys) between the ages of 2 and 13 years) whose ROB had been confirmed and who were treated as either inpatients or outpatients at the Warsaw clinics of the International Svetlana Masgutova Institute and at the Cuvatov Republic Clinical Hospital in Ufa, Russia.

The composition of the study groups was as follows:

- Group 1, 15 healthy children (8 boys and 7 girls) who served as the control group
- Group 2, 30 children (16 boys and 14 girls) who served as a baseline, before treatment or any therapy
- Group 3, 15 children (8 boys and 7 girls) who were initially part of group 2 and went on to receive standard drug treatment
- Group 4, 15 children (8 boys and 7 girls) who were initially part of group 2 and went on to be treated with both MNRI and standard drug treatment

The study was examining 60 patients at the age of 2 – 13 years, including 32 boys and 28 girls with the proved diagnosis of recurrent obstructive bronchitis. Control group included 15 healthy children (8 boys and 7 girls).

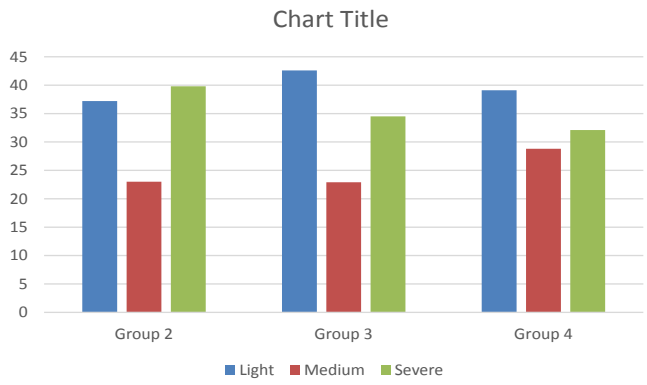

Figure 1: Data shown as means±SD. The significance of differences between groups was determined using Mann-Whitney Rank Sum test.

The following patients groups were formed to analyze treatment effectiveness:

Group 1 – control group, 15 children

Group 2 – 30 children, the condition was assessed prior to therapy

Group 3 – 15 children, who received conventional medicinal therapy

Group 4 – 15 children, who received combined treatment by medicinal therapy and MNRI.

All groups were equal in sex and age (Figure 1).

The analysis of risk factors for recurrent obstructive bronchitis in the examined children considered compromised history of antenatal period, mother's smoking status in pregnancy and compromised heredity of broncho-lung pathology; also the analysis considered compromised heredity in atopia and allergic diseases in children, unfavorable in-house micro-ecology and passive smoking from the first life months (Table 1).

Diagnosis verification was made according to the complex of routine criteria: anamnesis, clinical symptoms of respiratory infection (low-productive cough, dyspnea with difficult exhalation, diffuse auscultative disorders, such as dry and/or moist rales) and parameters of bronchi patency by peakfluorometry and/or spirometry. The rate of clinical symptoms of bronchial obstruction was evaluated by a 4-score system (Table 2). The disorders of bronchial obstruction were documented by peakfluorometry and/or spirometry data at the start and in the dynamics of the treatment. Table 3 presents children subpopulations within the groups in accordance with the severity of the broncho-obstructive syndrome.

To evaluate the reverse character of broncho-obstruction the study used peak fluorometry and/or spirometry with broncodilatation probes: before and 40 min after inhalation of Berodual's solution (ipratropium bromide + fenoterol hydrobromide) through nebulizer.

n	Group 1 (control)	Group 2	Group 3	Group 4
	15	30	30	15
sex (m/f)	8/7	16/14	8/7	8/7
age, years	6.8±4.2	7.2±4.8	7.6±5.1	7.3±5.2
Pathology of antenatal period, %	15	48	51	45
Active mother's smoking during pregnancy, %	40.5	67.3	58.7	61.2
Early start of respiratory infections, %	5.3	10.2	12	9.8
Atopia in child, %	10.4	12.5	15.2	11.1
Compromised heredity of allergies, %	14.2	26.7	32.8	36.2
Compromised heredity of bronch-lung pathology, %	9.8	56.9	62.3	58.4
In-house micro-ecology (old house, mould, pets), %	20.6	70.7	69.4	68.7
Passive smoking, %	43.2	87.4	90.1	88.9

Table 1: Characteristics of the children.

Score	Day symptoms	Night symptoms
0	none	none
1	Short-term, disappear rapidly	Appear when excited, do not cause early wake-up
2	Short-term, repeat during the day	Cause wake-up in the night time or early wake-up
3	Most time of the day do not interfere in the child's activity	Cause two or more wake-ups in the night time
4	Marked most part of the day, interfere in the normal child's activity	Significantly affect the night's sleep

Table 2: Evaluation of the clinical symptoms of bronchial obstruction.

Severity Grade of bronchial obstruction, %	Group 2 n 30	Group 3 n 15	Group 4 n 15
Light	37.2	42.6	39.1
Medium	23	22.9	28.8
Severe	39.8	34.5	32.1

Table 3: Children's subpopulations in groups according to the severity grade of bronchial grade of bronchial obstruction.

ROB in the study participants was diagnosed by physicians at hospitals either at the International Svetlana Masgutova Institute of Movement Development and Reflex Integration in Warsaw, Poland, or at the Cuvatov Republic Clinical Hospital in Russia on the basis of symptoms and findings from clinical examination, laboratory testing, and radiographic analysis [25,26] using the classification of bronchitis forms and lung diseases in children [27] accepted at the Pediatric and Pulmonology Congress of Specialists in Moscow in 1995 [28–35]. Of the 60 participants, 8 had fathers who smoked. However, the fathers reported never smoking indoors or around their children. None of the participants had symptoms of diseases than ROB. Our study was approved by the Russian Academy of Medical Science Institutional Review Board (log number 07.121) on February 15, 2005, and approval was renewed on May 10, 2008. All of the children and their parents provided informed consent for participation in the study.

Standard drug treatment

Study groups 1 and 2 received no drug treatment. The medications administered to groups 3 and 4 consisted of bronchospasmolytic, membrane-stabilizing, or anti-inflammatory agents and/or antibiotics, all of which were approved for use in children by the Russian Department of Allergy. The bronchospasmolytic agents consisted of xanthine derivatives (a 24% solution of aminophylline in 1.0-mL ampoules for intramuscular injection (1.0 mL) once a day or 0.15-g aminophylline tablets (0.15 g) once or twice a day. The duration of effect of the bronchospasmolytic agents varied from 12 to 24 hours. Aminophylline was prescribed in addition to other broncholytic medications (primarily adrenoceptor agonists and anticholinergic drugs, such as 0.3 g of theophylline [Theopec] or 0.1 g of Theobiolong [1–3 times a day]) and was administered via inhalation.

The anti-inflammatory drugs used were a 3% acetaminophen (Efferalgan) solution (syrup), 15 mg/kg 3 times a day, and mefenamic acid tablets, 0.25 g 3 times a day. The antibiotics administered were cephalosporin (Dardum), 50 to 200 mg/kg once a day via intramuscular injection, and macrolide (Oletetrinum) tablets, 0.012 g 4 to 6 times a day. All drugs were used for 7 to 10 days, depending on each patient's condition.

Complex therapy of obstructive syndrome included:

- Antibacterial drugs (combined amoxicillin and clavulanic acid (Amoxi-Clav), azithromycin, clarithromycin);
- Inhalation β2-antagonists of rapid effect (Ventolin) or combination of β2-antagonist and anticholinergic drug (Fenoterol+Ipratropium bromide);
- Inhalation glucocorticosteroids (Budesonide).
- Inhalation of medicines was done by nebulizers Pari (Germany) and Omron (Japan).

The order and extension of ROB therapy corresponded to its severity grade and was provided according to the following principles:

- The patient's condition was evaluated and symptoms were assessed by scores;
- The previous treatment was revised (the dosage of broncholytics, administration route and the time after the last administration);
- The extension of therapy corresponded to the severity of bronchial obstruction. In the course of examination the severity grade could be revised;
- Monitoring of clinical symptoms and peakfluorometry and/or spirometry (initially and during the course of treatment on days 1, 5 and 10).

Treatment of recurrent obstructive bronchitis

The medication class we used to treat a ROB relapse was the same used for acute obstructive bronchitis: bronchodilators. One of the bronchodilators was theophylline. The name *theophylline* is from the Latin word tea, meaning "tea bush" and the Greek word *phyllon*, meaning "sheet." Methylxanthine, another bronchodilator that we used, is a purine derivative and is a heterocyclic alkaloid of plant origin found in the Chinese *Camellia sinensis* and in Paraguayan holly (*Ilex paraguariensis*), both of which are used in preparing tea. Bronchodilators

- Inhibit phosphodiesterase and increase accumulation of cyclic adenosine monophosphate in the tissues, blocking adenosine (purine) receptors
- Reduce the flow of calcium ions through the channels of cell membranes, thus reducing the contractile activity of smooth muscles; this relaxes the muscles of the bronchi, blood vessels (mainly cerebral blood vessels), skin, and kidneys
- Induce peripheral vasodilation
- Stabilize the membrane of mast cells, inhibiting the release of mediators of allergic reactions
- Increase mucociliary clearance, stimulate contraction of the diaphragm, improve respiratory function and the function of the intercostal muscles, and stimulate the respiratory system
- Normalize respiratory function and promote blood oxygen saturation and lower concentrations of carbon dioxide
- Enhance ventilation in hypokalemia
- Stimulate the activity of the heart, increase the heart rate and the strength and regularity of the heartbeat, and increase coronary blood flow and myocardial oxygen demand
- Decrease blood vessel tone (mainly cerebral blood vessels and blood vessels in the skin and kidneys)
- Reduce pulmonary vascular resistance and lower blood pressure in the microcirculatory system Increase renal blood flow and have a mild diuretic effect
- Extend the extrahepatic biliary tract

- Inhibit platelet aggregation (inhibit platelet-activating factor and prostaglandin E2α) and increase resistance to deformation of red blood cells (improve the rheological properties of blood), reducing blood clots and normalizing microcirculation

Bronchodilators were prescribed for bronchial obstruction of any origin: bronchial asthma (the drug of choice for asthma, including exercise-induced asthma; they are also used as an additional tool in other forms of asthma), chronic obstructive pulmonary disease (chronic obstructive bronchitis, emphysema), pulmonary hypertension, pulmonary heart, edema in renal disease (as part of combination therapy), and sleep apnea.

The following drugs were used to treat COPD when the patient's condition was stable:

- Ipratropium bromide (Atrovent)
- Salbutamol (Ventolin)
- Fenoterol (Berotec)
- Long-acting drugs: tiotropium bromide (Spiriva), salmeterol (Serevent), formoterol (Symbicort)
- Inhaled steroids: beclomethasone dipropionate (Beclazone), fluticasone (Flixotide), budesonide (Pulmicort)
- Mucolytics: acetylcysteine (Fluimucil)

The following were used to treat acute illness:

- Bronchodilators
- Antibiotics: combined amoxicillin and clavulanic acid (Amoxi-Clav), azithromycin, clarithromycin
- Steroids (prednisone)
- Oxygen

Application of Masgutova neurosensorimotor reflex integration

All study participants underwent an initial MNRI evaluation during which their responses to 34 reflex patterns, including asymmetrical tonic neck, hand-pulling, leg-cross flexion–extension, spinal Galant, spinal Perez, Moro, and Robinson hand grasp. Test clusters included 15 to 25 checks of each reflex. Reflex basic patterns were evaluated in each of five parameters: integrity of the physiology circuit, sequence and direction of movement, timing and speed, intensity and symmetry, as follows:

- Physiology circuit: The three-part reflex circuit should activate a motor response exactly like that of the inherent genetically encoded pattern associated with a specific sensory stimulus.
- Sequence and direction: Each reflex consists of a specific sequence of movements that culminate in a posture or are continued as movement in a specific direction. The muscle system coordinates these postures and movements.
- Timing and speed (latency): The reflex circuit involves sensory input, brain processing, and motor response. The motor response must begin a fraction of a second after sensory stimulation. It must also complete its pattern quickly enough to fulfill its protective function. A delayed or slow reflex response could result in injury.
- Intensity: The intensity of a reflex is the amount of physical energy supplied by a system of muscles and ligaments in response to a stimulus. The strength of that response should reflect the intensity of the stimulus. Hyperactive, hypoactive, and areflexive responses are inadequate.
- Symmetry: Symmetry can be seen in the bilateral organization of a pattern, direction, timing, and intensity of the reaction.
- Programming movement actions particular to the reflex scheme
- Motor performance
- Accuracy of sensory perception and motor response
- Direction of reflex response (action of the proprioceptive system—dysfunctional, pathologic, or absent)
- Response level, which depends on the tone and activity (normal, hyperactive, hypoactive, or areflexive) of muscles and ligaments
- Symmetry of reflex patterns between the right and left sides of the body

Each participant's response was assigned a baseline score ranging from 0 to 20, as detailed in Table 1. During the study, groups 1, 2, and 3 received no MNRI therapy. In group 4, MNRI (neurostructural, motor, and reflex integration; tactile therapy; reflex repatterning; and both visual and auditory reflex integration) was performed immediately after the standard drug treatment (already described) had been administered, modified according to each participant's clinical status.

Variables evaluated

In all participants, we assessed changes in the parameters of neutrophil phagocytosis activity, subpopulations of lymphocytes, nitroblue tetrazolium (NBT) level, cytokine level in peripheral blood mononuclear leukocytes (PBMLs), and blood plasma cortisol level, both before the start of MNRI therapy and the day after MNRI therapy was stopped.

Neutrophil phagocytosis activity

Neutrophil phagocytosis activity was evaluated to determine the percentage of phagocytes, the absolute phagocyte number, the phagocyte number, the average number of intracellular latex particles (the result of the division of all phagocyte particles by the number of cells involved in phagocytosis), the phagocytic index, and the number of latex particles counted for 1 phagocyte. Latex particles size ranged from 1.3 to 1.5 mm. We estimated the ability of phagocytes to absorb NBT in a spontaneous NBT probe and a stimulated NBT probe (using zimozan [Zycanum]) [36]. Zymosan is a biopolymer-membrane yeast, *Saccharomyces cerevisi* that consists mainly of polysaccharides.

Subpopulations of lymphocytes

Subpopulations of lymphocytes were identified by flow cytometry performed by Caltag Laboratories (Invitrogen, Carlsbad, CA, USA). The cells were washed in cold phosphate-buffered saline and were stained with antibodies conjugated to fluorescein isothiocyanate and to phycoerythrin according to the manufacturer's instructions. The suspension was then washed twice in cold phosphate-buffered saline. The results were measured with a flow cytometer (FACSCalibur, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The cell-population gate was determined by front and side light scattering and cell size; there were 10000 cells per gate. Statistical analyses were performed with WinMDI (version 2.8; Microsoft, Redmond, WA, USA).

Cytokine level

The cytokine level in PBMLs was evaluated using an immunoenzyme assay with standard complexes for the definition of cytokines. We incubated PBMLs (1×10^6 cells) in culture medium (RPMI-1640) for 24 hours with 5 μg/mL of phytohemagglutinin (PHA). The control group of PBMLs did not contain PHA (spontaneous induction of cytokines).

Blood plasma cortisol level

Blood plasma cortisol levels were assessed before and after therapy in all 4 groups using an immunofluorescence assay (Immulite 2000 cortisol kit, DPC, Los Angeles, CA, USA).

Statistical evaluation

All statistical evaluations were performed with the Mann-Whitney U-test, using Statistica (version 6.0; Stat Soft Inc, Tulsa, OK, USA). P values (M ± Sd) less than 0.05 were considered significant.

Results

Neutrophil phagocytosis activity

ROB was caused by an acute respiratory virus infection in 94% of study participants and was complicated by a bacterial infection in 72% of those individuals. Members of group 3 exhibited a statistically significant decrease in the level of phagocytosing neutrophils and their absorbing ability (Table 4) after standard drug treatment only. In addition, a statistically significant increase of phagocytosing cells and in the phagocytosis index was noted in group 4 after standard drug treatment plus MNRI (Table 5). Members of group 3 exhibited a statistically significant decrease in the bactericidal activity of leukocytes as a result of the inhibition of oxygen-dependent microbicidal mechanisms (Table 5) after standard drug treatment only, and, when compared with group 1, group 3 showed a decrease in the index of leukocyte stimulation in patients with ROB, which indicates the inhibition of immune system reactivity. That index reverted to values within the normal range after the completion of MNRI therapy in group 4.

Sub-populations of lymphocytes

Assessment of the lymphocyte subpopulation structure in groups 2 and 3 revealed a decreased number of CD3, CD4, CD8, natural killer, and natural killer T cells before and after standard drug treatment (Table 6). An increased number of cells expressing molecules of early (CD25) and late (human leukocyte antigen DR-1 [HLA-DR]) activation and an increase in B-lymphocyte and T-regulatory cells numbers were noted again in group 3 before and after standard drug treatment. After the members of group 3 had completed standard drug treatment, their cell phenotype began to normalize gradually, although enhanced immune system reactivity was still noted. In group 4, the reactivity disappeared after MNRI therapy. The levels of CD3 and natural killer cells in group 4 (after MNRI plus

Cytokines pg/mL	Group 1 (control group; n = 15)		Group 2 (baseline; n = 30)		Group 3 (standard drug therapy only) (n =15)		Group 4 (standard drug therapy + MNRI; n = 15)		Reliable difference between groups
	S	PHA	S	PHA	S	PHA	S	PHA	
IL-1β	53.8 ± 2.1	314.4 ± 5.4	12 ± 0.95	50 ± 2.5	23.5 ± 1.1	97.4 ± 4.8	43.3 ± 1.7	281.8 ± 8.8	P_S, P_{PHA} 1 and 2, 3, 4 < .01
									P_S, P_{PHA} 1 and 3 and 4 < .01
IFN-γ	66.9 ± 2.8	426.2 ± 21.1	10 ± 0.7	73.14 ± 3.4	36.9 ± 1.5	109 ± 6.3	56.2 ± 0.7	387.7 ± 11.9	P_S, P_{PHA} 1 and 2, 3 < .01
									P_S, 1 and 4 < .01
									P_S, P_{PHA} 3 and 4 < .01
IL-12	37.8 ± 1.9	113.2 ± 7.6	14.2 ± 1.06	49.2 ± 2.5	28 ± 1.98	93.47 ± 6.7	33.4 ± 1.8	97.3 ± 7.2	P_S, P_{PHA} 1 and 2 < .01
									P_S 1 and 3 < .01
IL-4	41.7 ± 2.2	218.4 ± 9.6	82.9 ± 5.7	443.5 ± 23.1	66.6 ± 3.2	313.4 ± 14.7	52.6 ± 3.1	252 ± 16.6	P_S, P_{PHA} 1 and 2, 3 < .01
									P_S, 1 and 4 < .05
									P_S, 3 and 4 < .01
									P_{PHA} 3 and 4 < .05
IL-10	61.3 ± 2.8	214.3 ± 10.5	95.4 ± 4.3	480.9 ± 10.2	85.2 ± 6.1	384.6 ± 11.6	64.7 ± 2.8	248.85 ± 6.12	P_S, P_{PHA} 1 and 2, 3 < .01
									P_{PHA}, 1 and 4 < .05
									P_S, P_{PHA} 3 and 4 < .01

IFN, interferon; IL, interleukin; MNRI, Masgutova neurosensorimotor integration; PHA, phytohemagglutinin-induced probe; ROB, recurrent obstructive bronchitis; S, spontaneous probe.

Table 4: Effects of MNRI therapy on cytokine levels in children with ROB.

Parameters	Group 1 (control group; n = 15)	Group 2 (baseline; n = 30)	Group 3 (standard drug therapy only) n = 15	Group 4 (standard drug therapy + MNRI) n = 15
Phagocytosing cells (%)	63.0 ± 2.64	43.8 ± 1.3[a]	36.4 ± 2.6[a]	47.0 ± 3.35[a,b]
Absolute number of phagocytes (millions/mL)	2.9 ± 0.37	1.6 ± 0.27[a]	1.35 ± 0.38[a]	2.13 ± 0.24[b]
Number of phagocytes	0.95 ± 0.08	0.73 ± 0.03[a]	0.43 ± 0.03[a]	0.64 ± 0.05[a,b]
Phagocyte index	1.6 ± 0.06	1.5 ± 0.06	1.22 ± 0.07[a]	1.47 ± 0.04
Spontaneous NBT probe	0.88 ± 0.05	0.4 ± 0.04[a]	0.47 ± 0.03[a]	0.5 ± 0.03[a]
Stimulated NBT probe	1.31 ± 0.05	0.55 ± 0.06[a]	0.46 ± 0.03[a]	0.65 ± 0.05[a,b]
Index of stimulation in the NBT	1.49	1.37	0.97	1.3

MNRI, Masgutova neurosensorimotor integration; NBT, nitroblue tetrazolium (reduction) test; ROB, recurrent obstructive bronchitis.
[a]Statistically significant differences between the control group and groups 2, 3, and 4.
[b]Statistically significant differences between group 2 and group 4 (Mann-Whitney U test).

Table 5: Effects of MNRI Therapy in Children with ROB.

Cell Type	Group 1 (control group; n = 15)	Group 2 (baseline; n = 30)	Group 3 (standard drug therapy only; n = 15)	Group 4 (standard drug therapy + MNRI; n = 15)
CD3	67.4 ± 3	54.87 ± 1.74[a]	55.33 ± 1.19[a]	62.8 ± 1.44[b]
CD16	14.81 ± 0.8	6.37 ± 0.34[a]	9.07 ± 0.26[a]	12.59 ± 0.4[b]
CD3/CD16	3.18 ± 0.25	1.38 ± 0.18[a]	3.87 ± 0.24	4.6 ± 0.42[a]
CD8	21.2 ± 1.1	12.13 ± 0.73[a]	10.75 ± 0.7[a]	13.1 ± 0.81[a,b]
CD4	37.1 ± 1.33	27.99 ± 1.01[a]	30.61 ± 1.18[a]	34.31 ± 1[b]
CD25	10.96 ± 0.86	24.34 ± 0.93[a]	19.15 ± 1.3[a]	13.54 ± 1.1[b]
CD4/CD25/Foxp3	4.04 ± 0.54	9.99 ± 0.55[a]	8.92 ± 0.64[a]	4.98 ± 0.37[b]
CD19	13.13 ± 1.19	16.07 ± 0.63[a]	17.23 ± 0.81[a]	15.07 ± 0.85
HLA-DR	17.49 ± 0.77	24.3 ± 0.91[a]	30.7 ± 1.39[a]	25.05 ± 1.1[a,b]

HLA-DR, human leukocyte antigen DR-1; MNRI, Masgutova neurosensorimotor integration; NBT, nitroblue tetrazolium (reduction) test; ROB, recurrent obstructive bronchitis.
[a]Differences between the control group and the groups with recurrent obstructive bronchitis. [b]Differences between group 3 and group 4 (Mann-Whitney U test).

Table 6: Effects of MNRI therapy on the subpopulation structure of lymphocytes (percent) in children with ROB.

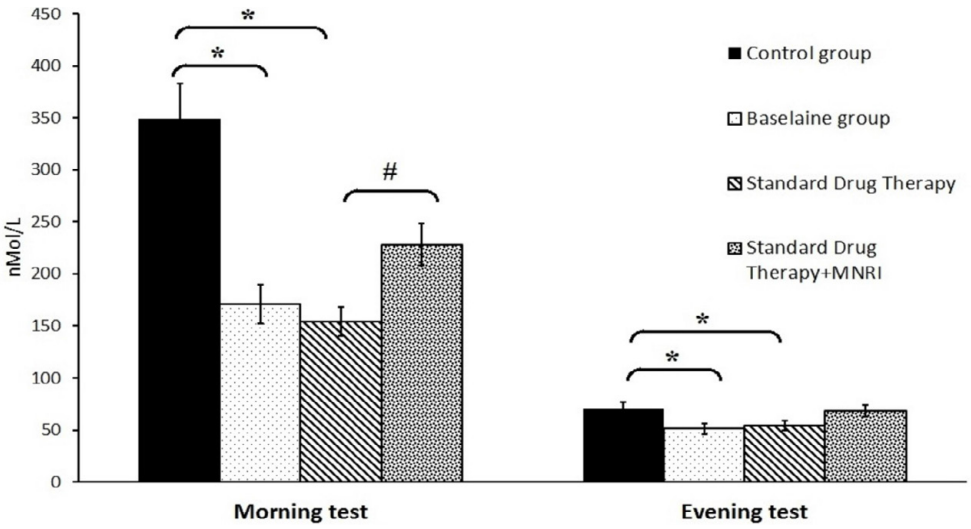

Figure 2: Effects of Masgutova neurosensorimotor reflex integration (MNRI) therapy on cortisol levels in children with recurrent obstructive bronchitis. *p < .01 in comparison with the control group; #p < .01 in comparison with subjects treated only with standard drug treatment.

standard drug treatment) were significantly higher than in group 3 (after standard drug treatment alone), and levels of HLA-DR and T-regulatory cells were lower in group 4 than in group 3. Production of PBML interleukin-1β (IL-1β), interferon-γ (IFN-γ), and IL-12 decreased by a factor of 2.3 in groups with ROB (p<.01). Group 4 did not significantly differ from group 1 (the control group) but did significantly differ from group 3 (standard drug treatment). IL-4 and IL-10 levels were higher in groups 2 and 3. Group 4 (MNRI plus standard drug treatment) did not differ significantly from the control group. Production of IL-4 and IL-10 in group 3 was higher than in group 4 (p< .05), and thus stimulated production of IL-4 was 1.2 times higher in group 3 than in group 4 (p >.05).

Blood plasma cortisol level

The cortisol levels in the morning and evening blood plasma samples of groups 2 and 3 were significantly lower than those in group 1 (Figure 2). Immunocorrective treatment with MNRI plus standard drug treatment led to a gradual increase in the cortisol level of members of group 4. However, in group 3 the cortisol level from the morning sample after standard drug treatment was significantly lower than that of the sample taken from group 4 after MNRI therapy had been added to the treatment regimen.

Discussion

It has been our experience that patients with chronic respiratory disease often have a disorder of the immune system. After MNRI therapy was added to standard drug treatment in group 4, the following changes in immune parameters were noted: decreased levels of CD3, CD4, CD8, CD16, and natural killer T cells and increased levels of CD19, activation markers CD25 and HLA-DR, and immunoregulatory cells CD4 and CD25. The imbalance in the number of cell subpopulations was regulated by MNRI therapy, after which the population of cells expressing CD3, CD4, CD19, and CD25 markers reached the normal range.

In group 4, an insufficient cortisol level was corrected only after MNRI treatment. In group 3, which received only standard drug treatment, the cortisol level was 1.2 to 1.35 times lower than in group 4. It is well known that the anti-inflammatory and immunomodulating effects of cortisol in a physiological concentration are associated with lipocortin synthesis, which blocks the formation of lipoxygenase and cyclooxygenase (products of arachidonic acid) [37].

The pathological chemical phase of ROB leads to an extensive release of leukotriene B_4 (LTB_4), which has an important role in the formation of bronchial obstructions [38]. The initially low cortisol concentration

in patients with ROB may inefficiently regulate the formation of LTB_4, IgE, and other effectors, thus potentiating the T-helper cell type 2 (Th2) response [39]. That fact is confirmed by the decrease in the production rate of the proinflammatory cytokine IL-1β, regulatory IFN-γ, and regulatory IL-12 and the enhanced level of the anti-inflammatory cytokines IL-4 and IL-10 in peripheral blood mononuclear leukocyte cultures in children with ROB (Table 4). Despite the positive change in cytokine levels in participants who received standard drug treatment, the concentration of those cytokines in the culture medium of leukocytes was significantly different from that in the control group. When MNRI therapy was added to the treatment regimen, the levels of regulatory and anti-inflammatory cytokines normalized.

Therefore, after conventional drug treatment and despite clinical improvement, members of group 3 continued to exhibit the symptoms of insufficient cortisol and the simultaneous inhibition of T-cell immunity. After the addition of MNRI to standard drug treatment for ROB, a statistically significant increase in the absolute number of segmentonuclear neutrophils was noted in group 4 (Table 5). MNRI in combination with standard treatment led to an increase in the absorbing activity of neutrophils and the normalization of leukocyte metabolic function, both of which improved the stimulation index of the NBT test to a value within the normal range (0.97–1.3). In addition, a statistically significant increase was noted in the number of cells expressing differentiation antigens and natural killer cells (CD16). Natural killer cells are the key effectors of innate immunity; they have an important biological role in the mechanisms of immune surveillance (the targeting of tumor cells), in the destruction of viruses and parasite-infected cells, and in the regulation and differentiation of bone marrow cells (they eliminate rapidly proliferating hemopoietic cells) in people with graft-versus-host reaction [40]. In addition to its immunoregulating effect, therapeutic MNRI increased the cortisol level to physiological concentrations in group 4.

IL-1β, which is produced by mononuclear leukocytes, enhances the secretion of adrenocorticotropic hormone and glucocorticoids.38 Our results indicated that MNRI therapy activates mononuclear leukocytes. Thus, adding MNRI to the standard drug treatment regimen for ROB improved the inadequate effects of that therapy on immune system cells and enhanced immune response polarization to the Th1 type.

The children in this study were monitored for 1 year after treatment was completed. ROB outbreaks occurred in each group as follows:

- Group 1 (control group): no ROB outbreaks
- Group 2 (baseline, before treatment): half were moved to group 3 and half were moved to group 4
- Group 3 (standard drug treatment): had ROB an average of 7.8 times a year before treatment; after 1 year of medical treatment, the average decreased to 6 times a year, not a statistically significant difference
- Group 4 (standard drug treatment plus MNRI): had ROB an average of 8 times before treatment

In summary, our study results revealed that the IL-1β level produced by mononuclear leukocytes was significantly higher after MNRI therapy plus standard drug treatment than after standard drug treatment alone. We suggest that MNRI regulates the production of IL-1β and the regulatory cytokines IFN-γ and IL-12 and thus positively affects the interaction of the immune, endocrine, and nervous systems and ultimately homeostasis. We cannot exclude the direct effect of MNRI on circulation and the lymphatic system, because our results revealed a significant decrease in muscle hypertension, hydropic symptoms, vessel spasms, and tissue inflammation after MNRI therapy. We suggest that adding MNRI to the treatment of children with ROB can correct impaired immune system mechanisms, contribute to the resolution of chronic respiratory disease, and enable a longer remission from recurrent disease. However, additional studies of the effects of MNRI therapy on mechanisms regulating immune, endocrine, and nervous system function in children with ROB are required.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Disclosure

Medical editor Katharine O'Moore-Klopf, ELS (East Setauket, NY, USA) provided professional English-language editing of this article.

References

1. Martinez FJ, Curtis JL, Albert R (2008) Role of macrolide therapy in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 3: 331-350.
2. Löfdahl CG, Postma DS, Laitinen LA, Ohlsson SV, Pauwels RA, et al. (1998) The European Respiratory Society study on chronic obstructive pulmonary disease (EUROSCOP): recruitment methods and strategies. Respir Med 92: 467-472.
3. Egorova NB, Semenov BF, Kurbatova EA, Semenova IB, Kuz'mina LA, et al. (2001) Immune response following administration of multicomponent vaccine VP-4 in elderly patients. [In Russian.] Zh Mikrobiol Epidemiol Immunobiol.
4. Samsigina G A (2014) Antibiotics in the treatment of acute bronchitis in children. [In Russian.] Doctor.
5. Holtzman MJ, Byers DE, Benoit LA, Battaile JT, You Y, et al. (2009) Immune pathways for translating viral infection into chronic airway disease. Adv Immunol 102: 245-276.
6. Bartoli ML, Di Franco A, Vagaggini B, Bacci E, Cianchetti S, et al. (2009) Biological markers in induced sputum of patients with different phenotypes of chronic airway obstruction. Respiration 77: 265-272.
7. Leow MK, Kwek DS, Ng AW, Ong KC, Kaw GJ, et al. (2005) Hypocortisolism in survivors of severe acute respiratory syndrome (SARS). Clin Endocrinol (Oxf) 63: 197-202.
8. Bergeron C, Boulet LP (2006) Structural changes in airway diseases: characteristics, mechanisms, consequences, and pharmacologic modulation. Chest 129: 1068-1087.
9. Semenov BF, Zverev VV (2007) [Concept of inducing of rapid immunological protection against pathogens]. Zh Mikrobiol Epidemiol Immunobiol : 93-100.
10. Andronova TM, Chumakova MM (2002) [Theoretical problems of use of an immunomodulator likopid in clinical practice]. Ter Arkh 74: 70-72.
11. Kurbatova E A, Semenov BF, Egorova NB, Efremova VN, Gruber IM, et al. (2004) Immunostimulating activity of polycomponent vaccine "Immunovac-VP-4" and Grippol after their combined administration.] [In Russian.] Zh Mikrobiol Epidemiol Immunobiol.
12. Zhukova O C (2014) Economic evaluation of treatment of acute and recurrent obstructive bronchitis in children. [In Russian.] Doctor.
13. Bar Meir E, Amital H, Levy Y, Kneller A, Bar-Dayan Y, et al. (2000) Mycoplasma-pneumoniae-induced thrombotic thrombocytopenic purpura. Acta Haematol 103: 112-115.
14. Zhukova O C, Kononova, S C, Kaniskina T M (2012) Clinical and economic components symptomatic treatment of acute bronchitis in children. Farmateka. 15: 89-95.
15. Chu YK, Ali GD, Jia F, Li Q, Kelvin D, et al. (2008) The SARS-CoV ferret model in an infection-challenge study. Virology 374: 151-163.
16. Hui DS, Wong KT, Antonio GE, Ahuja A, Sung JJ (2009) Correlation of clinical outcomes and radiographic features in SARS patients. Hong Kong Med J 15 Suppl 8: 24-28.
17. Korytina GF, Tselousova OS, Akhmadishina LZ, Victorova EV, Zagidullin ShZ,

et al. (2012) [Association of the MMP3, MMP9, ADAM33 and TIMP3 genes polymorphic markers with development and progression of chronic obstructive pulmonary disease]. Mol Biol (Mosk) 46: 487-499.

18. Hosker H, Cooke NJ, Hawkey P (1994) Antibiotics in chronic obstructive pulmonary disease. BMJ 308: 871-872.
19. Saint S, Bent S, Vittinghoff E, Grady D (1995) Antibiotics in chronic obstructive pulmonary disease exacerbations. A meta-analysis. JAMA 273: 957-960.
20. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 152: S77-121.
21. Wilson R, Tillotson G, Ball P (1996) Clinical studies in chronic bronchitis: a need for better definition and classification of severity. J Antimicrob Chemother 37: 205-208.
22. Sechenov IM (1935) Reflexes of the brain. In: Selected works. Moscow: State Publishing House for Biological and Medical Literature.
23. Setchenov IM (1961) Reflexes of the brain. Moscow: Academy of Science of USSR.
24. Rozinova NN (1992) Classification of clinical forms. In: World Health Organization. International statistical classification of diseases and related health problems, 10th revision. Geneva.
25. Setchenov IM (1960) Selected physiological and psychological works. Belsky S, Koschtoyants K (ed.,) Foreign Languages Publishing House, Moscow.
26. Virella G, Goust JM, Fudenberg HH (1990) Introduction to medical immunology. Marcel Dekker, New York.
27. Anokhin PK (1968) Biology and neurophysiology of a conditioned reflex. Medicina Moscow.
28. Anokhin PK (1973) Biology and neurophysiology of the conditioned reflex and its role in adaptive behavior. International series of monographs in cerebrovisceral and behavioral physiology and conditioned reflexes. Elsevier, Rockville, MD.
29. Mizerniski JL, Rozinova NN (2011) Chronic lung diseases in children. Pulmonology Practics, Moscow.
30. Gordienko SM (1983) [Comparative evaluation of the results of the nitroblue tetrazolium reduction test in microscopic and spectrophotometric variants of the method with various tetrazolium salts]. Lab Delo : 21-24.
31. Zakharov P, Rosinova N (2001) Outcomes of chronic inflammatory disease of the lungs in children on the basis of long-term follow-up findings. Russian Journal of Perinatology and Pediatrics 3: 30–35.
32. Classification of clinical forms of bronchitis and lung diseases in children. Russian Bulletin of Perinatology and Pediatrics 2: 52–56.
33. World Health Organization: International statistical classification of diseases and related health problems, 10th revision. Geneva.
34. Musajanova R (2011) Pathogenetic approaches to the treatment of children with chronic pneumonia. Medical and Health Science Journal 7: 87–89.
35. Rozinova NN (1995) Clinical forms of inflammatory lung diseases in children: diagnosis criteria and principles of therapy. “Problem Patient” Journal Archives.
36. Zhang DX, Gauthier KM, Falck JR, Siddam A, Campbell WB (2007) Steroid-producing cells regulate arterial tone of adrenal cortical arteries. Endocrinology 148: 3569-3576.
37. Gordienko SM (1983) [Comparative evaluation of the results of the nitroblue tetrazolium reduction test in microscopic and spectrophotometric variants of the method with various tetrazolium salts]. Lab Delo : 21-24.
38. Erel F, Gulec M, Kartal O, Caliskaner Z, Ozturk S, et al. (2007) Serum leptin levels and lipid profiles in patients with allergic rhinitis and mild asthma. Allergol Immunopathol (Madr) 35: 232-238.
39. Terawaki K, Yokomizo T, Nagase T, Toda A, Taniguchi M, et al. (2005) Absence of leukotriene B4 receptor 1 confers resistance to airway hyperresponsiveness and Th2-type immune responses. J Immunol 175: 4217-4225.
40. Macdonald HR (2007) NKT cells: In the beginning... Eur J Immunol 37 Suppl 1: S111-115.