Relationship between Immune Status and Activity of the Lymphocyte Energy Supply System in Adolescents Suffering from Frequent Diseases

G. V. Sukoyan, I. G. Mamuchishvili*, and K. I. Pagava*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 6, pp. 664-666, June, 2005
Original article submitted August 23, 2004

Children and adolescents aged 4-16 years with the diagnosis of acute respiratory viral infection with long-lasting fever, manifestations of intoxication syndrome, and catarrhal symptoms were examined. In children and adolescents suffering from frequent diseases and presented with acute respiratory viral infection we found disorders in the immune status (depression of the cellular component, helper/suppressor imbalance, suppressed production of IgA and hyperproduction of IgM, decreased concentration of secretory IgA in the saliva) in comparison with children rarely falling ill. The redox potential and lymphocyte cytochrome C content were decreased in adolescents often falling ill, while the content of cytochrome oxidase did not change. A negative multiple correlation (R=6.8, p<0.005) was detected between the decrease in cytochrome C content and NADP/NADPH redox potential and increase in the immunoregulatory index. ATP content in lymphocyte from adolescents frequently falling ill remained 21% decreased during the first 2 weeks after acute respiratory viral infection, while the ATP/ADP ratio was shifted towards dinucleotide, which also indicated disorders in ATP synthesis in lymphocytes.

Key Words: *immune system; adolescents suffering from frequent diseases; energy supply system*

Respiratory infections, re-infections of the ear, throat, and nose, acute and chronic bronchopulmonary infections are the main diseases of childhood and adolescence [2,6]. According to different authors, 20 to 65% children and adolescents suffer from frequent diseases, which is mainly due to transitory disturbances and age-specific features of the immune system of children and adolescents. A slight increase in the immunoregulatory index was detected in adolescents suffering from frequent disease, but no other changes in cellular and humoral immunity were found [2,3]. On the other hand, these patients are characterized by decreased activities of glutamate, isocitrate, and lactate dehydrogenases in blood lymphocytes and increased level of glycerol-2-phosphate dehydrogenase. The detected decrease in activities of some mitochondrial oxidoreductases in adolescents with chronic bronchopulmonary diseases, suffering from frequent diseases, suggests that impairment of aerobic processes in lymphocytes and increased level of glucose-3-phosphate dehydrogenase can be regarded as a compensatory reaction aimed at additional substrate stimulation of redox reactions. The aim of this study was to evaluate the role of metabolic immunosuppression in inflammatory diseases and elucidation of the relationship between activity of the immune system and adaptation potential of mitochondrial oxidative phosphorylation system in adolescents with frequent acute respiratory viral infections (ARVI).

MATERIALS AND METHODS

The study was carried out in 54 children and adolescents (4-14 years) with the diagnosis of ARVI, long-lasting fever, and other manifestations of the intoxi-

N. V. Karsanov National Center of Medical Biophysics and Introduction of Biomedical Technologies, Tbilisi; *Tbilisi State Medical University

0007-4888/05/1396-0695 © 2005 Springer Science+Business Media, Inc.

cation syndrome (fatigue, weakness, drowsiness, decreased physical activity, poor appetite, adynamia, headache, myalgia, pain in the eyeballs, vomiting), and catarrhal symptoms. Analysis of smears from throat mucosa for microflora detected monocultures in 43% patients, the presence of two and more agents in 49%, combined bacterial and fungal agents in 8%. The incidence and spectrum of isolated microorganisms decreased with the decrease in the incidence of ARVI exacerbations. Antibodies to *Mycoplasma pneumoniae* were detected in 50% children and adolescents, the antigen was detected in 43%; antibodies to *Chlamydia pneumonia* were detected in 30% patients (the infection was confirmed by PCR findings in 40 and 20% patients, respectively), and in 30% patients the infection was mixed. The patients were divided into 2 groups depending on the incidence of repeated infections of the upper airways and ENT organs for at least one year. The main group consisted of 21 patients with a history of combined diseases of the upper airways (more than 6 exacerbations of ARVI over a year, adenoids, tracheobronchitis, rhinosinusitis, pharyngotonsillitis, otitis, tubotitis), with 3-4 exacerbations of concomitant diseases during the latest 6 months. The control group consisted of 17 adolescents with less than 5 exacerbations of ARVI over a year without foci of chronic infection. The group of healthy donors consisted of 16 adolescents. At the beginning of the study all children were examined by allergologist/immunologist, pediatrician, and other specialists, if necessary, including ENT specialist and dermatologist. Children with herpetic infection, autoimmune diseases, renal insufficiency, cardiovascular diseases, and treated with immunomodulating drugs within 6 months before the study were not included.

Clinical biochemical studies of the population and subpopulation composition of blood lymphocytes were carried out using indirect immunofluorescence with $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$, and $CD72^+$ monoclonal antibodies. The $CD4^+/CD8^+$ ratio was estimated for additional characterization of T-cell component of the immune system [1,5]. NADP and NADPH content was evaluated as described previously [8], levels of cytochrome C and cytochrome oxidase using a previously proposed method [7], the level of intracellular ATP was evaluated using luceferin luciferase (5-2500 nM) at absorption wavelength of 259 nm and extinction coefficient 15,400 [4]. The results were statistically processed using STAT Soft software. The significance of differences was evaluated using Student's *t* test.

RESULTS

Disorders in the immune status presenting as suppression of the cellular component, helper/suppressor imbalance, suppressed production of IgA, and hyperproduction of IgM were detected in all children suffering from frequent diseases. The study of local immunity factors in these patients showed decreased content of

TABLE 1. Immune Status and Lymphocyte Energy Supply Systems in Children and Adolescents Suffering from Frequent Diseases (*M*±*m*)

Parameter	Normal	Rare diseases	Frequent diseases
Leukocytes, 10^9/liter	6.9±0.8	7.1±1.1	5.3±0.8
Lymphocytes, 10^9/liter	3.5±0.4	4.1±0.5	3.0±0.4
$CD3^+$, 10^9/liter	2.35±0.25	2.24±0.21	1.67±0.15
$CD4^+$, 10^9/liter	1.25±0.15	1.4±0.2	1.43±0.08
$CD8^+$, 10^9/liter	0.9±0.1	0.94±0.06	0.83±0.08
$CD16^+$, 10^9/liter	0.51±0.04	0.52±0.05	0.49±0.05
$CD4^+/CD8^+$	1.4±0.1	1.48±0.09	1.72±0.12*++
$CD72^+$, 10^9/liter	15.4±0.6	16.7±0.5	17.5±0.8
NADP, nmol/liter	17.6±1.2	17.0±0.6	16.7±1.1
NADPH, nmol/liter	18.9±1.2	19.4±0.8	22.7±1.6
NADP/NADPH	0.94±0.05	0.88±0.04	0.74±0.07*++
Cytochrome C, nmol/liter	0.75±0.06	0.71±0.04	0.63±0.05
Cytochrome oxidase, nmol/liter	0.65±0.05	0.61±0.04	0.60±0.05
ATP, pmol/10^3 cells	5.1±0.3	4.9±0.2	3.85±0.4*
ADP, pmol/10^3 cells	2.1±0.5	2.3±0.2	2.65±0.12*
ATP/ADP	2.4±0.5	2.1±0.2	1.45±0.15*+

Note. *$p<0.001$ compared to healthy subjects; +$p<0.01$, ++$p<0.001$ compared to children rarely falling ill.

secretory IgA in the saliva (15.4±0.8 mg%) in comparison with children rarely falling ill (23.1±1.9 mg%, $p<0.01$). Activity of lysozyme in nasal discharge was slightly decreased (21.4±0.6 μg/ml in frequent diseases and 25.6±0.7 μg/ml in rare diseases, $p<0.05$). The level of IgA and its fixation on the mucosa are considered as the most important factors providing infection resistance. Decreased production of IgA and lysozyme indicates immunodeficiency in the local immunity system in this category of patients. The study of the functional activity of neutrophilic leukocytes in NBT test showed a decrease of this parameter (20.2± 2.8% in frequent diseases and 8.2±1.8% in rare diseases); dysimmunoglobulinemia was detected in 75.9% children. Decreased absolute counts of B-lymphocytes were detected in 21% children.

Despite a slight decrease in the content of NADP and its reduced form NADPH, the redox potential of energy supply system decreased (Table 1). This was paralleled by a decrease in the content of the key enzyme of the oxidative phosphorylation chain (cytochrome C) in the mitochondria, but not of cytochrome oxidase (cytochrome oxidase is more resistant to hypoxia and other factors than cytochrome C). A negative multiple correlation was detected between the decrease in the content of cytochrome C, NADP/NADPH redox potential, and increase of the immunoregulatory index (R=6.8, $p<0.005$). The decrease in the redox potential in combination with decreased level of cytochrome C (the key enzyme in electron transfer chain) attested to disorders in oxygen utilization system in the cascade of reactions of ATP formation (the main source of energy in the cell) in lymphocyte mitochondria. This results in a 21% decrease of ATP content in lymphocytes during the first 2 weeks after ARVI in adolescents frequently falling ill. Moreover, ATP/ADP ratio shifts towards the dinucleotide (Table 1), which also indicates impaired processes of ATP production in lymphocytes. The results prompt the use of drugs aimed at arresting the development of bioenergy insufficiency in children and adolescents suffering from frequent diseases.

REFERENCES

1. A. M. Zemskov and V. M. Zemskov, *Klin. Lab. Diagn.*, No. 3, 34-35 (1994).
2. T. P. Markova and D. G. Chuvirov, *Ros. Med. Zh.*, No. 3, 3-10 (2002).
3. G. I. Savitskii, S. V. Grishchenko, V. F. Krylov, *et al.*, *Vopr. Virusol.*, No. 3, 29-32 (1988).
4. A. A. Savchenko and L. N. Suntsova, *Lab. Delo*, No. 11, 23-25 (1989).
5. R. Richter, W. Braundsadter, and S. Rachkow, *Diagnostische Laboratorums Method. Zbl Phfarm.*, **118**, 949-955 (1979).
6. R. Y. Webster, W. J. Bean, O. Yorman, *et al.*, *Microbiol. Rev.*, **15**, 272-275 (1992).
7. J. N. Williams, *Biochem. Biophys. Acta*, **162**, 175-181 (1968).
8. C. R. Zerez, S. J. Lee, and K. R. Tanaka, *Anal. Biochem.*, **164**, 367-373 (1987).