DOI: 10.5281/zenodo.1186168
UDC: [616.211+616.216]-002.2-053.2

Cell therapy in the complex treatment of chronic rhinosinusitis in children

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Abstract

Background: Determination of the general immune status and the effectiveness of the complex treatment with local immunostimulation (autologous mononuclear cells) of chronic rhinosinusitis in children.
Material and methods: The general immunity status and the effectiveness of the complex treatment with local immunostimulation were examined by the application in the maxillary sinuses of activated autologous mononuclear cells in 19 children with chronic rhinosinusitis and 116 healthy children.
Results: The presence of allergic reactions by increase in IgE and CD4/CD8 index was determined in patients examined. The sensitization to streptococcal antibodies was assessed by the cell sensitization data and the presence of streptococcal antibodies according to ASL-0 data. The inhibition of the T-cells was functional decrease of T-lymphocytes in the TTBL test with phytohemagglutinin and tendency towards a decrease of the T-CD-3 lymphocytes titer. The compensatory activation of the B-cells (increase in the IgA titer) was determined. The objective follow-up examination of the patients over one year revealed the absence of hyperemia and edema of the nasal mucosa, while an insignificant nasal obstruction was present in 5 children. The rhinomanometric examination demonstrated an increase in total volume indices and a decrease in the total nasal resistance in all treated children. The new method of local immunocorrection of chronic rhinosinusitis in children proved to be clinically effective.
Conclusions: There were determined increased IgE, CD4 / CD-8 index and CIC (PEG-8% with low molecular weight), cellular sensitization to streptococcal antibodies according to TTBL data, inhibition of the T-cells and tendency towards a decrease of T-CD-3 lymphocytes. Also, the compensatory activation of the B-cells (increase in the IgA titer) was determined.

Key words: chronic rhinosinusitis, children, cell therapy.

Introduction

Chronic rhinosinusitis in children is an important problem in otorhinolaryngology. The actuality of the study is conditioned by two factors: disease incidence and possible serious complications (middle ear and central nervous system complications, bronchopulmonary and other organs complications). Some bibliographic sources indicate that the prevalence of recurrent and chronic rhinosinusitis is over 15% of the population. Clikningham MJ, Chiu EJ, Landgraf JM, Gliklich RE [1] mentioned that the number of orbital complications in children constituted lately 12.4% of the total number of patients hospitalized with acute and chronic rhinosinusitis. This fact confirms that chronic paranasal rhinosinusitis has a high incidence and particular risks.

Nowadays due to certain factors, such as the increased resistance of the microbial flora to antibiotics, the continuous allergyzation of the population and the action of the environmental impurities, the drug therapeutics of these diseases is decreasing more and more. Therefore, researchers have expanded their search in other fields as well. The aggression on tissues causes changes in all processes which occur simultaneously in the body. Therefore, when speaking of the reparative regeneration, it is necessary to emphasize that both the general and local immunity are simultaneously affected in the impaired body area, being interdependent with all the physiological and pathological processes, including the restoration processes.

A priority direction in the field of pediatric otorhinolaryngology is the development of new methods of complex local or general immunostimulation therapy in order to restore nasal physiological functions in the case of chronic rhinosinusitis. This allows increasing the treatment effectiveness in children, to reduce complications and to improve the quality of their lives.

In clinical immunology, a new system of peptides of the immune system – cytokines, the secretion product of practically all the cells actively involved in immune processes, is intensely studied. These peptides are involved in inflammatory reactions, regulation of hematopoiesis, tissue regeneration processes, development of cellular and humoral immune reactions, infectious immunity, antitumor and transplantation immunity.

Depending on the nature of the tissue integrity disorders (skin, mucous membranes, connective tissue, vascular endothelium, etc.), the regulating action of cytokines can be directed to cells involved in inflammation (mononuclear and polymorphonuclear phagocytes, T-lymphocytes etc.), regeneration (fibroblasts, endothelial cells etc.), development of the immune response.

The cytokine action is performed according to the principle of complementarity – the information transmitted to

the cell is not only from an individual peptide, but from a complex of mutually stimulating regulatory cytokines, modulating the receptors on the surface of other mediators.

Autocells (MAPS – multipotent adult progenitor cells or MSC – mesenchimal stem cells) are a form of stem cells, considered one of the most acceptable grafts in cell therapy and tissue engineering. Currently there are technologies for obtaining stem cells from the peripheral blood. The advantages of using autocells are obvious: lack of immune conflict, reduced probability of patient`s contamination with hemotransmissible diseases, moral and ethical acceptability [3].

The new method of local immunocorrection (with autologous mononuclear cells) showed to be very effective in the conservative complex treatment of chronic compensated tonsillitis in children, the clinically positive effect serving as proof, the normalizing of the preimmune resistance status of the body (CD-16 content, normal antibodies, dynamics of total hemolytic complement activity and ESR), the obvious decrease in elevated indices of allergic reactions (eosinophils, IgE), the decrease in specific cellular sensitization levels to streptococcal and pneumococcal antigens, the increase in total lymphocyte content, the rise of levels and functional activity of T and B lymphocytes, the increase of cytokine profile efficiency, the reduction of the levels of proinflammatory cytokines (TNF-α, IL-8, IL-1β) and the growth of serum levels of anti-inflammatory cytokine (IL-4).

Sandul Al. [9] has demonstrated the advantages of the stimulated auto-lymphocytes action as a principle of correcting the local immunoresistence, which ensures the optimization of repair processes in the trepanation area after radical ear surgeries. The positive effect was accompanied by an increase in the absolute number of T-lymphocytes in the peripheral blood, the normalization of the immunoregulation index, the decrease in B-lymphocytes and in the content of different classes of immunoglobulins. The local use of auto-lymphocytes determines the positive onset of the postoperative period, accelerates the processes of inflammation eradication, increases the nonspecific immunoresistence, reduces numerically and attenuates the microbial population virulence, which, at the same time, becomes more sensitive to antibiotic therapy.

In connection with the above mentioned, we consider that the research of immune particularities in children with chronic rhinosinusitis is very important and constitutes a new direction in pediatric otorhinolaryngology from two perspectives: completing the knowledge of the disease pathogenesis and increasing the efficiency of the pathogenetic treatment, also serving as a basis to make a prognosis as accurately as possible. In this regard, we found it necessary to carry out a study on the cell therapy application in the treatment of recurrent and chronic rhinosinusitis.

Purpose of the research is to determine the general immune status and the effectiveness of the complex treatment with local immunostimulation (autologous mononuclear cells) of chronic rhinosinusitis in children.

Material and methods

The immunological examination group included 19 children with chronic rhinosinusitis and 116 healthy children.

Table 1

Distribution of patients by gender and age

Patients	No - 19
Boys	66.6%
Girls	33.3%
Age (years)	1112.0±2.3

By gender, the boys predominated in the examination group (2/3) (tab. 1), the girls constituted 1/3. The mean age was 12.0 ± 2.3.

All patients underwent complex immunological examination. The subpopulations of T and B lymphocytes (CD3, CD4, CD8, CD16, CD20) were determined by the Flow Cytometry method (Partec PAS I). To determine phagocytic cells, the phagocytic index and phagocytic number were used (Pavlovich S. A., 1998) [10]. The phagocytic activity of neutrophils was determined by the NBT (Nitro-Blue-Tetrazolium) test (B. H. Park et al., 1968). The content of circulating immune complexes was determined according to the procedure described by Grinevich I. A. and Kamenets L. I. [5] (Grinevich I. A. and Kamenets L. I. (1986) in the version adapted by S. Ghinda et al., (2008)). The Paull-Bunnell reaction was performed according to the procedure proposed by S. Ghinda (1984). Antistreptolysin-O, rheumatoid factor and C-reactive protein were determined by Humatex ASO, Humatex RF, Humatex CRP agglutination tests (manufacturer Human, Germany). The content of immunoglobulins A, G, M was determined by the solid-state immunoenzyme assay, using the reagents of OOK «Vektor BEST» (Russia), according to the attached instructions. Total IgE was estimated by the solid-state immunoenzyme assay using reagents (UBI Company), according to the attached instructions.

The local immunomodulatory therapy was performed by applying activated autologous mononuclear cells, derived from the patient's venous blood, into the maxillary sinuses after draining and removing the suppurating masses with 0.9% NaCl solution. The autologous cells were separated from the blood sample and activated by an original technology developed in the Laboratory of Tissue Engineering and Cell Culture of *Nicolae Testemitsanu* State University of Medicine and Pharmacy (concentration by gradient separation and centrifugation, then cultivation on special nutrient medium for 5 days in a CO_2 incubator).

The statistical analysis of the materials included operational methods of statistical assessment, including the Student criterion, the alternative variation, etc. (Leah P.E et al., 2006) and Windows 2007 computer operating system utilities.

Results and discussion

The analysis of immune changes in the recurrent and chronic rhinosinusal inflammatory process (according to the CIC titer of various molecular weights) demonstrated that CIC titers (PEG - 2.5%) and CIC (PEG - 4.2%) with high and medium molecular weight were higher in the group of children with rhinosinusitis, but with no significant statistical reliability. The CIC titre (PEG-8.0%) with low molecular weight was significantly higher in sick children compared to healthy subjects (p <0.001) (tab. 2).

Table 2

Characteristic of rhinosinusal intoxication in the study groups

Indices	Healthy children (n - 116)	Sick children (n - 19)
CIC (PEG-2.5%)	5.9±0.33	9.2±3.56
CIC (PEG-4.2%)	24.6±0.65	39.6±8.03
CIC (PEG-8.0%)	121±3.57	293±43.1○

Statistical veracity among studied groups: ○ – sick children and healthy children

Given the fact that immune complexes disappear from the body through the phagocytosis processes of macrophages, monocytes and neutrophils, the pre-immune resistance parameters were analyzed (tab. 3).

Table 3

Parameters of phagocytosis in the groups of examined children

Indices	Healthy children (n - 116)	Sick children (n - 19)
Phagocytic capacity of neutrophils (PhCN)	0.12±0.001	0.12±0.009
Phagocyte number (PhN)	72.2±0.76	71.8±2.83
Phagocyte Index (PhI)	4.1±0.70	4.8±0.77

Statistical veracity among the studied groups: ○ – sick children and healthy children

The ability to destroy engulfed bacteria (tab. 3), analyzed by the test of phagocytic capacity of neutrophils, demonstrated that the number of neutrophils involved in phagocytosis (PhN) and phagocytic index (PhI) in patients with rhinosinusitis and healthy children was equal. The pre-immune resistance parameters, such as phagocytosis, are the most relevant body defense mechanisms and change under exceptional circumstances.

The CD-16 lymphocyte titres (tab. 4) were similar in the groups of patients studied. The number of natural antibodies in children with recurrent and chronic rhinosinusitis was significantly lower than in healthy subjects (p<0.001). This phenomenon is accounted for the natural antibodies, which are the first protection and participate in the respective antigen inactivation.

Table 4

Parameters of pre-immune resistance

Indices	Healthy children (n - 116)	Sick children (n - 19)
CD-16	15.6±0.24	15.2±1.89
Natural antibodies	2.4±0.05	1.8±0.13○

Statistical veracity among studied groups: ○ – sick children and healthy children

Table 5

Allergic parameters

Indices	Healthy children (n - 116)	Sick children (n - 19)
IgE	9.2±0.27	57.9±22.79○
CD-4/CD-8	1.8±0.02	2.1±0.19

Statistical veracity among studied groups: ○ – sick children and healthy children

The analysis of some allergic parameters (tab. 5) in the groups of children examined showed a significantly higher IgE titer in the group of sick children (p<0.05). The CD-4/CD-8 immunoregulation index in children with rhinosinusitis was slightly higher. This fact demonstrates the presence of allergic reactions in children with recurrent and chronic rhinosinusitis.

The ASL-O titre (tab. 6) was significantly higher in sick children than in the control group. The CRP and RF titres did not show any difference between the studied groups.

Table 6

Titers of ASL-O, CRP and RF in the study groups

Indices	Healthy children (n - 116)	Sick children (n - 19)
ASL-O	11.5±2.08	178±96.5
CRP	1.1±0.20	1.22±0.66
RF	1.1±0.24	1.22±0.66

Statistical veracity among groups studied: ○ – sick children and healthy children

The sensitization of T-lymphocytes to streptococcal antigens (tab. 7) was significantly higher in sick children compared to the control group (p<0.001).

Table 7

Sensitization of T-lymphocytes to streptococcal antigens

Indices	Healthy children (n - 116)	Sick children (n - 19)
TTBL-streptococcus	1.3±0.07	3.3±0.46○
TTBL- staphylococcus	1.6±0.06	2.1±0.29

Statistical veracity among groups studied: ○ – sick children and healthy children

The sensitization of T-lymphocytes to staphylococcal antigens in patients with rhinosinusitis was relatively higher compared to the control group, but with no statistical difference.

Table 8

Quantitative and functional parameters of T-lymphocytes

Indices	Healthy children (n - 116)	Sick children (n - 19)
TTBL-PHA	73.7±0.41	61.4±1.21○
CD-3	70.0±0.43	66.3±2.89
CD-4	44.0±0.31	46.3±1.47
CD-8	24.5±0.30	23.1±1.99

Statistical veracity among groups studied: ○ - sick children and healthy children

The T-lymphocytes functional activity (tab. 8), assessed by the (TTBL-PHA) blast transformation of lymphocytes by phytohemagglutinin, was lower in sick children compared to healthy ones ($p<0.001$). The T-lymphocytes (CD-3) titre was low in patients with the rhinosinusal disease, but with no statistical difference. The T-helper (CD-4) lymphocytes were higher, while the T-suppressor lymphocytes (CD-8) were lower in sick children than in healthy ones. This suggests the suppression of T-lymphocytes in sick children.

Table 9

Quantitative and functional parameters of B-lymphocytes

Indices	Healthy children (n - 116)	Sick children (n - 19)
CD-20	8.0±0.14	6.0±1.20
IgG	10.6±0.27	10.2±0.59
IgA	1.1±0.03	1.9±0.25○
IgM	1.0±0.02	1.2±0.12

Statistical veracity among groups studied: ○ - sick children and healthy children

The B-lymphocytes count (CD-20) in sick and healthy children did not differ. No differences in IgM and IgG were found in the studied groups. A significant increase in IgA ($p<0.001$) was determined in the group of children with chronic rhinosinusitis, resulting in the activation of B-cells (tab. 9).

At hospitalization, patients had unilateral or bilateral nasal obstruction in 19 cases (100%). The patients presented with purulent nasal discharge – 10 cases (52.6%) and mucopurulent nasal discharge – 9 (47.3%) cases. Quite often, children`s parents reported snoring in 12 cases (63%), and fever or subfebrility during a sinusitis relapse in 13 cases (68.4%). Of major symptoms, headache was present in 100% cases, hypo/anosmia was determined in 9 cases (47.3%). The endoscopic examination revealed changes in the inferior nasal turbinate in 8 cases (42.1%) and various external modifications in the middle nasal turbinate in 11 cases (57.8%). The radiological investigations of the nose and paranasal sinuses revealed a total or partial opacity in the study groups: maxillary sinus – 19 cases (100%), ethmoidal sinus – 12 cases (63.1%) and frontal sinus in 5 cases (26.3%).

The rhinomanometry results were compared between the children with recurrent and chronic rhinosinusitis and the control group. The children in the study group had reduced total volume of the nasal fossa compared to the control group ($P<0.05$). An increase was attested in the total resistance at 150 Pa ($P<0.05$) in the study groups.

The disease course and treatment outcomes of patients in the study group were assessed over one year. The objective examination determined mucosal changes in only three patients (15.7%): hyperemia and edema of the pituitary and pale or violet turbinates. This can be accounted for the allergic and immunologic status changes in these children. The most common clinical sign was nasal obstruction – 5 cases (26%). Headache was rarely revealed – 3 cases (15.7%). Of minor signs, cough, snoring and fever or subfebrility were practically not detected. The rhinomanometric examination demonstrated an increase in the total volume indices and a decrease in the total nasal resistance in all treated children.

Conclusions

1. According to the data received on pre-immune resistance, the increase in CIC (PEG-8% with low molecular weight) confirms the presence of the body intoxication signs.
2. The presence of allergic reactions in examined patients was determined by the increase in IgE and the CD4 / CD-8 index.
3. The sensitization of streptococcal antibodies according to TTBL data (cell sensitization) and the presence of streptococcal antibodies according to ASL-O data (humoral sensitization) were assessed.
4. The inhibition of T-cells (according to data on T-lymphocyte functional decline in TTBL test with phytohemagglutinin and tendency of T-CD-3 lymphocytes towards a decrease) was revealed.
5. The compensatory activation of B-cells (increase of IgA) was determined.
6. The new method of local immunocorrection (with autologous mononuclear cells) in the conservative complex treatment of chronic rhinosinusitis in children showed to be effective, demonstrating a clinically positive effect.

References

1. Sedaghat AR, Phipatanakul W, Cunningham MJ. Prevalence of and associations with allergic rhinitisin children with chronic rhinosinusitis. Int J Pediatr Otorhinolaryngol. 2014;78(2):343-7. doi:10.1016/j.ijporl.2013.12.006. PubMed Central PMCID: PMC3966022.
2. Brietzke SE, Shin JJ, Choi S, Lee JT, Parikh SR, Pena M, et al. Clinical consensus statement: pediatric chronic rhinosinusitis. Otolaryngol

Head Neck Surg. 2014;151(4):542-53. doi:10.1177/0194599814549302. PubMed PMID: 25274375.
3. Cunningham MJ, Chiu EJ, Landgraf JM, Gliklich RE. The health impact of chronic recurrent rhinosinusitis in children. Arch Otolaryngol Head Neck Surg. 2000;126(11):1363-8.
4. Danilov L. Amigdalita cronică la copii [Chronic tonsillitis in children]. Chisinau: Medicina; 2014. 210 p. Romanian
5. Danilov L. Particularităţile florei microbiene în patologiile inflamatorii acute şi cronice orofaringiene la copii [Peculiarities of microbial flora in acute and chronic inflammatory oropharyngeal pathologies in children]. Buletin de perinatology (Chisinau) [Bulletin of perinatology]. 2014;61(1):55-61. Romanian.
6. Ababii I, et al. Optimizarea regenerării reparatorii a ţesuturilor şi imunogenezei locale în contextul funcţionarii nanosistemelor naturale [Optimization of regenerative tissue reconstruction and local immunogenesis in the context of the natural nanosystems functioning]. Chisinau: Medicina; 2011. 335 p. Romanian.
7. Koval'chuk LV, Gankovskaia LV. Immunotsitokiny i lokal'naia immunukorrektsiia [Immunocytokines and local immunocorrection]. Immunologiia [Immunology] (Russia). 1999;(1):4-7. Russian.
8. Danilov L. Aspecte etio-patogenetice şi optimizarea tratamentului conservator în amigdalita cronică la copii [Etiopathogenetic aspects and optimization of conservative treatment of chronic tonsillitis in children] [dissertation]. Chisinau: "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova; 2016. 274 p. Romanian.
9. Sandul A. Mastoidoplastia cu autoos spongios combinată cu imunocorecţie pentru tratamentul bolnavilor cu otite medii cronice suppurate [Mastoidoplasty with an autologous cancellous bone in combination with immunocorrection in the treatment of patients with chronic purulent otitis media] [dissertation]. Chisinau: "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova; 1996. 265 p. Romanian.
10. Pavlovich SA. Osnovy immunologii [Fundamentals of immunology]. Minsk: Vysheishaia shkola; 1998. 114 p. Russian.
11. Park BH, Fikrig SM, Smithwick EM. Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet. 1968;2(7567):532-4.
12. Grinevich IuA, Kamenets LIa. Osnovy klinicheskoi immunologii opuholei [Fundamentals of clinical immunology of tumors]. Kiev: Zdorov'ia; 1986. 158 p. Russian.
13. Ghinda S, Sofronie S, Chiroşca V, Privalova E, Calenda O, Smeşnoi V, Lesnic E, Barbuţa A, Rotaru N, Iaconi L, inventors. Metoda concomitenta de determinare a CIC cu masa moleculară joasa, medie şi nalt [Concomitant method of determining CIC with low, medium and high molecular weight] [Certificate of innovation no. 46 registered at "Chiril Draganiuc" Phthisiopneumology Institute of the Republic of Moldova. 2008 May 12]. Romanian.