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THE CHANGE OF ANTI-INFLAMMATORY CYTOKINES IN PATIENTS WITH PARAINFLUENZA

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The **aim** was to determine the concentration of anti-inflammatory cytokines (IL-1Ra, IL-4 and IL-10) in the serum of patients with parainfluenza. We **examined** 19 patients with moderate parainfluenza aged 15-54 years who have the disease run a positive, without complications. Blood were taken during the acute period of disease and reconvalescent. Served as control blood of healthy donors ($n = 18$). **Results.** Defined increase in all investigated cytokines in the acute period of disease and during convalescence. **Conclusion.** We set the activation level of anti-inflammatory "cytokine network" throughout the disease, indirectly indicating the high activity of effector cells of blood, synthesizing data cytokines. The results were used to create an expert system "Quick diagnosis and prognosis of ARVI".

Keywords: *parainfluenza, interleukin-1 receptor antagonist, interleukin-4, interleukin-10.*

Introduction. Anti-inflammatory cytokines - cytokines, which, even at the minimum (picogram) concentrations, inhibit the activity of cells involved in inflammation, resulting in the inhibition of the general inflammatory response. For anti-inflammatory cytokines include IL-4, IL-10, IL-13, TGF- β . In addition, anti-inflammatory have receptor antagonists of pro-inflammatory cytokines, soluble receptors for inflammatory cytokines and antibodies to pro-inflammatory cytokines, blocking their effects. Receptor antagonists of pro-inflammatory cytokines, among which the most studied is the antagonist of IL-1 receptor (IL-1Ra), is structurally similar to pro-inflammatory cytokines, so they bind to corresponding receptors and block the further development of the inflammatory response. Have also described a similar effect soluble receptors to IL-1 (sIL-1R), IL-2 (sIL-2R), IL-6 (sIL-6R), TNF (sTNFR) and antibodies to IL-1 and TNF [4, 8].

Of these the most important biological regulators are IL-1Ra, IL-4 and IL-10. According to the structure they are proteins with a molecular mass of 15-36 kDa. IL-1Ra in the acute phase of inflammation produce predominantly macrophages and monocytes, and neutrophils, fibroblasts, hepatocytes, endothelial cells, dendritic cells. The mechanism of action of IL-1Ra is

a cellular receptor blockade is specific to IL-1 α and IL-1 β ; thus is regulation of the activity of powerful inflammatory cytokine IL-1 family in the place of virus implementation and replication, including the adverse effects of the organism in excessive concentration of not only the site of inflammation, but also in the systemic circulation. Thus, it is the optimal balance of IL-1Ra and IL-1 provides an adequate response of the organism to the introduction of a foreign agent, including the virus. But a change in this balance inevitably leads not only to disfunction of cytokine network, but also disfunction of the all immune system [4, 8].

In contrast to IL-1Ra, IL-4 and IL-10 produce predominantly activated Th2-lymphocytes and monocytes, macrophages, B-cells, NK-cells, keratinocytes, mast cells, and others under the influence of TNF- α , IFN- α , IL-1, IL-2, IL-3, IL-6, IL-12, IL-15 and other proinflammatory cytokines. The main function of IL-4 and IL-10 is to modify the immune response from Th1 to Th2 [4]. IL-4 and IL-10 – powerful anti-inflammatory and immunosuppressive factors that inhibit excessive synthesis of proinflammatory cytokines (IL-1 β , IL-2, IL-6, IL-8, IL-12, TNF- α , IFN- γ , IFN- α , etc.), activated macrophages and Th1-lymphocytes, which leads to a weakening of the influence of an excess of inflammatory mediators in the human body and to the simultaneous activation of the humoral response of the body, reflected in the progressive increase in the secretion of Ig E and Ig G. At the same time, the production of inflammatory cytokines inhibit the lipopolysaccharide-activated and IFN- γ monocytes [4, 8].

Due to the fact that normally in the human body there is a balance between the activity of Th1- and Th2-lymphocytes, resulting in homeostasis is maintained between the various body systems: immune, hematopoietic, nervous, endocrine and others [1, 4, 8] – , is dysregulated in cytokine-mediated mechanisms of cooperation of T-lymphocyte number of researchers see cause worsening of the clinical picture of various diseases both infectious and noninfectious etiology [5, 8].

Conducted to date, studies of anti-inflammatory cytokine concentrations in acute respiratory infections (ARVI) is sufficiently fragmented. However, many researchers [10-12] indicate that their critical importance in the pathogenesis of ARVI. Thus, elevated levels of major pro-inflammatory cytokines (IL-4, IL-10, IL-1Ra) was detected in nasopharyngeal mucus of young children with severe, but favorable course of respiratory syncytial virus infection [11]. Increasing concentrations of IL-4 in serum was determined in adult patients, not only respiratory syncytial, and adenovirus infection [2]. Increased IL-1Ra and IL-10 in peripheral blood were found in patients with moderate influenza pH1N1, and with severe influenza pH1N1 with the development of acute respiratory distress syndrome [12].

Due to the fact that the available literature we did not encounter studies on the level of anti-inflammatory cytokines in patients with parainfluenza, **aimed this work** was to study the levels of the main anti-inflammatory mediators (IL-1Ra, IL-4, IL-10) in the blood of these patients with explanation of their possible pathogenetic and prognostic role.

Materials and methods. We examined 19 patients with moderate parainfluenza aged 15-54 years who had the disease a positive, without complications. The diagnosis was confirmed by indirect hemagglutination reaction (detection of specific antibodies with increasing titer of 4 or more times).

Blood samples to determine the level of anti-inflammatory cytokines had in the acute period (1-2 days of illness) and the period of early convalescence (for 7-9 days of illness). Cytokine levels were determined in serum by enzyme immunoassay commercial test systems for "Vector-Best", Russia [7]. Control was blood of 18 healthy donors in aged 19-40 years.

Results of clinical and laboratory studies were made in specially designed card of the individual evaluation of patients with subsequent presentation in the form of spreadsheets, Microsoft Excel 2007. Statistical analysis of results of research carried out using the package StatGraphics 15.0.

Normality of variational series (corresponding Gaussian) was checked by goodness of Kolmogorov-Smirnov, Pearson tests and Shapiro-Wilks test, one of the most powerful criteria of normality [3]. When the hypothesis of normal distribution as the point estimate characteristics of the center of the grouping values of a random variable used by the sample mean (M_o). In the case of normal distribution of each of the samples revealed differences between groups with Student's t-test (ST) and Fisher's exact test to compare the variances (F) [3, 6].

The most common distribution of the series did not meet the criteria of normality, which is consistent with published data. Thus, according to some reports [6], only 20% of the distributions of quantitative traits found in biomedical research are approximate normal. When not performing hypothesis of normality of distribution in accordance with the recommendations [6] used methods of nonparametric statistics. In particular, as a point estimate characteristics of the center of the grouping values of a random variable using the median (M_e) – an indicator of the least subject to influence by individual fluctuations characteristic [3, 6]. Between a sample were compared using nonparametric tests: Wilcoxon-Mann-Whitney (WMW) – rank test, well adapted for analyzing small samples and robust to the form of the law of their distribution, as well as two-sample Kolmogorov-Smirnov (KS) [3, 6].

The content of IL-4 and IL-10 in the blood of most patients were below the threshold of sensitivity of laboratory analysis [7], resulting in a sample with the results of laboratory tests had

"0" in most positions. This fact led to a zero value estimates the median ($Me = 0$) and the impossibility of the comparison sample on this parameter [3]. Given that the content of IL-4 and IL-10 in patients' blood was exponentially distributed (in connection with the performance criteria of the exponential distribution the Shapiro-Wilk [3]), as a point estimate characteristics of the center of the grouping values of these factors, we used selective average (Mo) [3, 6, 7].

The level of significance when testing all statistical hypotheses – $p \leq 0,05$ (confidence level > 0.95).

Results and discussion. In the acute period of parainfluenza revealed an increased concentration of anti-inflammatory cytokines compared with controls (see table): IL-1Ra (WMW, KS $p \leq 0,05$), IL-4 (F, KS $p \leq 0,001$) and IL-10 (WMW, KS $p \leq 0,01$). High levels of anti-inflammatory factors, the data was maintained and did not come back to normal for the period of convalescence: IL-1Ra (WMW $p \leq 0,05$), IL-4 (F, KS $p \leq 0,001$) and IL-10 (KS $p \leq 0,001$). Throughout the illness (compared with acute and convalescence periods) increased levels of IL-1Ra were not significantly changed (ST, F, WMW, KS $p > 0,05$). At the same time to the period of convalescence, a statistically significant progressive increase in the concentration of IL-4 (approximately two fold) (KS $p \leq 0,001$) and significant reduction of IL-10 (6, 5 times) (WMW, KS $p \leq 0,01$).

Conclusions. In connection with the prevailing local (distant), but not systemic action of the investigated anti-inflammatory cytokines, rapid destruction and/or their binding to specific receptors (10-15 minutes) – all this causes is that their concentration in peripheral blood is not adequately reflects the processes that occur in the area of inflammation. However, the difficulties of research concentration of anti-inflammatory cytokines and activity of the cell populations according to their synthesis in the inflammatory site are responsible for the need to find indirect evidence that characterize the percolation limit the inflammatory response and activation of humoral immunity, in which data cytokines play a keyrole.

According to the results of the study, patients with parainfluenza revealed significant activation of the anti-link "cytokine network" throughout the period of the disease, which indirectly indicates the high activity of effector blood cells: macrophages and monocytes (according to high levels of IL-1Ra) and Th2-lymphocytes (judging by high concentrations of IL-4 and IL-10). An even greater increase in the concentration of IL-4 to the period of convalescence, apparently due to increased activity progradient Th2-lymphocytes due to their increasing participation in the synthesis of antibodies against parainfluenza virus antigen, first of all Ig G. However, the lack of normalization of the level of IL-1Ra, involved mainly in the early phase of immune response and produced mainly by monocytes/macrophages may be indicative

of retention of high activity, and these blood cells. Thus, in patients with parainfluenza not revealed a complete coincidence of clinical and laboratory recovery, which should be considered when a patient discharged from hospital.

The practical significance. Determined the prognostic significance of high levels of anti-inflammatory cytokines in serum and their dynamics in the disease course in patients with moderate parainfluenza. The data obtained on the status of the major anti-inflammatory factors of cytokine network in normal and in patients with parainfluenza formed the basis of an expert system, developed by us, "Rapid diagnosis and prognosis of ARVI" [9].

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TABLE. Comparative characteristics of anti-inflammatory cytokine concentrations in serum of patients with parainfluenza

Groups of patients		M (for IL-1Ra) or Mo (for IL-4 and IL-10), range (in parentheses), pg/ml	
<i>Period</i>		<i>Acute period</i>	<i>Convalescence</i>
Parainfluenza	IL-1Ra	1275,3 (648,4 - 3000) [#] , n = 17	1371,5 (489,3 - 3000) [#] , n = 16
	IL-4	0,127 [#] , n = 16	0,223 ^{*#} , n = 14
	IL-10	44,319 [#] , n = 18	6,808 ^{*#} , n = 16
Control	IL-1Ra	619,55 (74,7 - 3000), n=16	
	IL-4	0,021 (0 - 0,334), n = 16	
	IL-10	5,970 (0 - 88,951), n=17	

Note:

* – significant differences in the rates of the disease ($p \leq 0,05$).

[#] – significant difference from control ($p \leq 0,05$).

n – number of patients studied.