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DYNAMICS OF IMMUNOLOGICAL INDICES IN WOUND HEALING ACCOMPANIED BY DIFFERENT TYPES REACTIVITY OF THE ORGANISM

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The dynamics of pro- and anti-inflammatory cytokines, circulating immune complexes of different sizes after surgical treatment of spontaneous periodontitis accompanied by normo-, hyper- and hyporeactivity of the organism was studied. It has been revealed that adequate time- and level-balanced changes in the studied indicators form a normoreactive response with synchronization of necrotic and reparative processes. In cases of hyperreactivity of the organism changes of immunological parameters boost strengthening of necrotic and delay of reparative processes during wound healing; in cases of hyporeactivity - changes in pro- and anti-inflammatory cytokines and circulating immune complexes of different sizes contribute to the slowing of necrotic processes, which also leads to delaying of wound healing. The revealed varying dynamics of indicators depending on the condition of reactivity of the organism preconditions necessity of their directed medical correction with reduction of indicators to those of normoreaction.

Keywords: reactivity of the organism, spontaneous periodontitis, wound healing, cytokines, circulating immune complexes.

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ДИНАМІКА ІМУНОЛОГІЧНИХ ПОКАЗНИКІВ ПРИ ЗАГОЄННІ РАНИ НА ТЛІ РІЗНОЇ РЕАКТИВНОСТІ ОРГАНІЗМУ

Вивчена динаміка про- та протизапальних цитокінів, циркулюючих імунних комплексів різного розміру після проведеного хірургічного лікування при спонтанному пародонтиті на тлі нормо-, гіпер- та гіпореактивності організму. Встановили, що адекватні збалансовані за часом і рівнем зміни досліджених показників формують нормореактивну відповідь із синхронізацією некротичних і репаративних процесів. При гіперреактивності організму зміни імунологічних параметрів сприяють посиленню некротичних і затримці репаративних процесів при загоєнні рани; при гіпореактивності - зміни про- та протизапальних цитокінів та циркулюючих імунних комплексів різного розміру сприяють уповільненню некротичних процесів, що також призводить до затримки репарації рани. Виявлена відмінна динаміка показників в залежності від стану реактивності організму обгрунтовує доцільність їх спрямованої медикаментозної корекції з приведенням значень до таких при нормореакції.

Ключові слова: реактивність організму, спонтанний пародонтит, загоєння рани, цитокіни, циркулюючі імунні комплекси.

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In cases of generalized periodontitis, immunological reactions develop both in the system of local immunity of the oral cavity and in the body as a whole [1, 3, 4]. It has been found out that patients with chronic periodontitis have impaired peripheral tolerance mechanisms, manifested by a decrease in the proportion of Treg regulatory cells in the peripheral blood. This can lead to hypersensitivity of the host immune system and the development of chronic inflammation in response to biofilm antigens, which is not resolved spontaneously after the elimination of the irritant [7]. Different clinical variants of periodontitis are characterized by ambiguous disorders of immune status. The condition of systemic immunity depending on the severity of generalized periodontitis is described. Many authors believe that dysfunction of the immune system in patients with generalized periodontitis is either a consequence of genetic predisposition, or it develops in connection with somatic pathology of internal organs, endocrine disorders, chronic viral, bacterial and fungal infections [2]. Recently, there have been studies in which it has been shown that prolonged chronic inflammatory process in periodontal tissues itself can also provoke the development of immunodeficiency [11, 15]. In addition, there are atypical, "aggressive" forms of periodontitis, when the failure of immune mechanisms is the main cause of the disease. Thus, genetically determined disorders of the immune system, mainly associated with DR-locus antigens of the HLA system (Human Leukocyte Antigen) class II have been revealed in patients with juvenile periodontitis accompanied by the specific microflora of periodontal pockets [6].

Complex pathochemical reactions are interconnected by specific mediators of intercellular interactions − cytokines, which to perform their function bind to specific receptors that are normal or they may appear during activation of target cells on their membrane. The term "cytokines" combines "growth" factors that regulate the proliferation, differentiation and function of blood cells, including cells of the immune system [5]. Cytokines are products of immunocompetent cells, and at the same time,

immunocompetent cells are targets of cytokines. Cytokines are secreted mainly by blood and immune system cells (polymorphonuclear leukocytes, macrophages, lymphocytes) and have autocrine (on the cells that produce them), paracrine (on cells in the microenvironment) and endocrine (on distant cells) effects. In this case, they interact with each other on antagonistic and agonistic principles and form a cytokine network in the body. To perform their function, cytokines bind to specific receptors that are normal or appear during the activation of target cells on their membrane [9]. According to the main mechanisms of action, cytokines are divided into: growth factors that control the production of immunocompetent cells (colony-stimulating factors – G-CSF, M-CSF, GM-CSF; transforming growth factor beta – TGFβ etc.); proinflammatory cytokines that provide mobilization and activation of cells involved in inflammation (interleukins − IL-1, IL-6, IL-8, IL-12; tumor necrosis factor alpha - TNFα; interferons - IFN and INFγ; factor that inhibits migration - MIF); anti-inflammatory cytokines with the alternative nature of action that limit the development of inflammation (IL-4, IL-10, IL-13, TGFβ); cytokines that regulate the cellular and humoral immune response (IL-1, IL-2, IL-4, IL-5, IL-6, IL-7 etc., IFN, TGFβ); cytokines with their own effector functions (antiviral, cytotoxic) [10]. Cytokines control the process of angiogenesis, regeneration processes, metabolic processes etc. The action of cytokines may be associated with morphofunctional disorganization of the endothelium and serious microcirculation disorders in the variety of pathological conditions, including inflammation. In recent decades, the role of cytokines in the development of immunological and inflammatory reactions in periodontitis has been found out. The maximum local release of proinflammatory cytokines turns the protective mechanisms into pathological, uncontrolled, which causes damage to periodontal tissues and bone resorption. A certain complex of cytokines, including IL-1, TNFα, IL-6 etc., determines the course of inflammation in the focus of tissue destruction that occurs in cases of generalized periodontitis [13, 14]. The formation of granulation tissue in the focus of chronic inflammation (periodontal pocket) is the result of the influence of growth factors of different genetic orientation, which are produced by macrophages, lymphocytes and fibroblasts on its cellular elements. The study of the level of various cytokines in the gingival fluid in patients with periodontitis showed significant changes in the composition and titer of cytokines compared with those of intact periodontitis. The content of pro-inflammatory cytokines IL-1β, which is the initiator of the cytokine cascade in periodontal tissues, in patients with periodontitis increases in the gingival fluid 3 times compared with healthy people. The level of anti-inflammatory cytokine IL-4 is significantly reduced, which is an unfavorable sign in the course of chronic periodontitis, as it leads to uncontrolled activation of macrophages and their production of IL-1, TNFα and other cytokines [8]. The expression of IL-6 and IFN is significantly higher in the tissues of patients with inflammatory periodontal disease compared to the tissues of healthy individuals. The content of IL-1 and TNFα in gingival fluid and peripheral blood serum has been studied. It is shown that the level of IL-1 in the gingival fluid correlates with the depth of periodontal pockets. In addition, the level of IL-1 in the serum was significantly lower than in gingival fluid, which indicates the presence of local mechanisms of production of this cytokine and its importance in the pathogenesis of periodontal disease [12].

Summarizing the above-mentioned facts, we can assume that the intercellular and intersystem interaction involving different populations of leukocytes and substances produced by them determines the nature and speed of the healing process in periodontal tissues.

The purpose of the work was to study the dynamics of immunological parameters (pro- and anti-inflammatory cytokines, circulating immune complexes of different sizes) after surgical treatment of spontaneous periodontitis which is accompanied by normo-, hyper- and hyporeactivity of the organism.

Material and methods. The experimental studies take into consideration the recommendations suggested in the Regulation “General Ethical Principles in Animal Experimentation”, approved by the First National Congress on Bioethics on September 20, 2001 (Kyiv, Ukraine) and the Regulation of “the European Convention for the Protection of Vertebrate Animals which are used for experimental and other scientific purposes” (Strasbourg, 1985).

The experiments were performed on 24 adult not purebred dogs weighing 8-12 kg with spontaneous periodontitis. The animals were divided into three equal groups. In the first group, drugs that disrupt the reactivity of the organism were not used (normoreactivity of the organism). In this case, “placebo” was injected with saline solution to the animals. In the second group of animals according to the scheme the immunostimulant of lipopolysaccharide nature – pyrogenal at a dose of 10 μg/kg/day was administered intramuscularly. Thus, the condition of hyperreactivity of the organism was modeled. According to the same scheme animals of the third group received immunosuppressant – azathioprine at a dose of 1.5 mg/kg/day enterally. Thus, the condition of hyporeactivity of the organism was modeled. All the animals with spontaneous periodontitis underwent careful instrumental removal of soft and hard supragingival and subgingival dental plaque, medical treatment by irrigating the oral cavity with “Parodontax” rinse. Then all the animals underwent periodontal surgery – the flap operation according to Cieszyn-Widman-Neumann was performed.

In the period after surgery, blood sampling was performed on the 1st, 2nd, 6th and 9th day of the experiment. In the morning venous blood sampling was performed on an empty stomach from the ulnar vein with a volume of 10 ml. Immunological status was determined by the dynamics in the blood of cytokines (IL-1β, IL-6, TNF, IL-4) and circulating immune complexes (CIC) of different sizes. The state of cytokine regulation was determined by the content of IL-1β, IL-6, TNF, IL-4 by solid-phase enzyme-linked immunosorbent assay (ELISA) using commercial reagent kits ProCon IL-1β, ProCon IL-6, ProCon TNF, ProCon IL-4. With the help of the spectrophotometer, the optical density at the given wavelength was measured. Based on the obtained data, calibration curves were constructed for the corresponding cytokines and the results were obtained using the ELx800 Universal Microplate Reader (BIO-TEK INSTRUMENTS.INC). The content of the CIC was determined by radioimmunoassay. Using a spectrophotometer, the optical density was measured over a given wavelength. Based on the obtained data, calibration curves were constructed for the corresponding cytokines and the results were read using an ELx800 Universal Microplate Reader (BIO-TEK INSTRUMENTS.INC).

Statistical processing of the obtained digital data was performed using the computer program Statistica 8.0 (STA862D175437Q). For each sample set of observations (n), the arithmetic mean (M), the standard error of the mean (SE), the standard deviation (SD), and the 95 % confidence interval of the true mean were calculated using Student's t-test. The level of significance of differences in the average values of the indicator in independent samples (p) was calculated by the distribution function of Student's t-test: at $p<0,05$ – the difference is significant; at $p>0.05$ – the difference is insignificant.

Results of the study and their discussion. The nature of the course of mucosal wound healing is determined by cytokines, which are produced mainly by activated macrophages, neutrophils and lymphocytes. The content of some pro-inflammatory (IL-1β, IL-6, TNF) and anti-inflammatory (IL-4) cytokines directly characterizes the quantitative composition and functional activity of the cells that produce them. The results of determination of cytokines IL-1β, IL-6, TNF, IL-4 in the serum of animals accompanied by different conditions of different reactivity of the organism are demonstrated in table 1.

Table 1

The content of cytokines IL-1β, IL-6, TNF, IL-4 in the blood in cases of normo- (1), hyper- (2) and hyporeactivity (3) of the body (M±SE)

Indices	Reference value	Study groups	Terms of Observation (24-hours)			
			1-a	2-a	6-a	9-a
IL-1β (pcg/ml)	72.4±25.7	1	212.9±46.6 *	149.1±42.6 *	142.3±48.0	78.8±23.0
		2	346.2±52.2 *	265.6±45.0 *	130.1±41.2	64.2±19.8
		3	158.2±28.4 *	184.1±32.0 *	146.3±24.8 *	68.3±20.2
IL-6 (pcg/ml)	83.6±28.3	1	173.4±40.1 *	165.4±34.6 *	98.3±29.3	85.6±27.3
		2	244.8±40.4 *	163.4±33.4 *	97.0±24.3	75.0±18.5
		3	142.8±36.9 *	154.2±30.1 *	128.4±21.6	70.6±15.8
TNFα (pcg/ml)	75.1±25.4	1	168.3±34.2 *	149.4±28.8 *	86.9±26.6	74.9±23.8
		2	277.6±47.2 *	145.4±26.8 *	79.6±252	78.0±23.2
		3	138.4±32.2 *	140.7±24.3 *	71.2±196	68.3±18.4
IL-4 (pcg/ml)	41.2±10.8	1	62.4±278	70.4±28.3	82.4±26.6 *	58.2±12.8
		2	93.2±27.1 *	52.9±13.9	35.2±8.8	36.4±9.2
		3	58.1±12.4	48.8±10.5	32.1±8.6	34.2±9.1

Note: * – $p<0.05$ against control values

As can be seen from this table, the dynamics of these cytokines accompanied by normoreactivity was unidirectional towards increasing their concentration. On the 1st day after surgery, the largest increase in proinflammatory cytokines was observed: IL-1β – by 2.94 times, IL-6 – by 2.07 times, TNF – by 2.24 times ($p<0.05$). The content of these cytokines significantly exceeded the control values on the 2nd day, but to a lesser extent than on the 1st. On the 6th day, the decrease in the content of all proinflammatory cytokines in the serum was registered. On the 9th day, the content of all the proinflammatory cytokines returned to normal. The dynamics of the anti-inflammatory cytokine IL-4 differed from that of IL-1β, IL-6, TNF. The content of IL-4, gradually increased on the 1st and 6th day, reaching a peak on the 6th day (the content was 2 times higher than in the control, $p<0.05$). On the 9th day, the value of IL-4 remained 1.41 times higher in comparison with the control ($p>0.05$). The nature of changes in the content of pro- and anti-inflammatory cytokines in the serum after surgery in animals with spontaneous periodontitis accompanied by normoreaction − increased levels of IL-1β, IL-6, TNF at the time of increasing necrosis of periodontal tissues, as well as their reduction and increase of IL-4 with the appearance of markers of reparative processes−confirm their participation in the healing processes of the mucosa and bone wound.

Immunological factors − circulating immune complexes (CICs) play a significant role in the regenerative processes in periodontitis. The content of CIC of different sizes in the blood plasma of animals with different reactivity of the organism is shown in table 2.

Table 2

The content of circulating immune complexes: large, medium and small in blood at normo- (1), hyper- (2) and hyporeactivity (3) of the organism (M±SE)

Indices	Reference value	Study groups	Terms of Observation (24 hours)			
			1-a	2-a	6-a	9-a
вCIC (mcg/ml)	2016 ±250	1 2 3	1855±260 1884±270 * 1988±140	2722±144* 1830±150 1988±93	3204±308* 2841±321 1898±310	2088±263 3240±315 * 3154±360 *
SCIC (mcg/ml)	421±62	1 2 3	417±51 264±42 * 172±22 *	475±80 784±82 * 480±62	815±91 * 892±94 * 218±43 *	576±50 * 1930±125 * 690±60 *
MCIC (mcg/ml)	339±72	1 2 3	317±82 396±73 295±45	436±71 654± 63 * 319±53	256±86 470±92 * 187±23	188±58 * 1306±125 * 615±55 *

Note: * – $p<0.05$ against control values

As can be seen from this table, the dynamics of the content of CIC of different masses with normoreactivity of the organism had a qualitatively identical picture and was characterized by their increase on the day after the initial fall on the 1st day. Moreover, the earlier (on the 2nd day) and marked (by 1.35 times compared to the control level, $p<0.05$) rise in concentration was observed for вCIC with subsequent delayed involution. The peak concentration of SCIC was observed on the 6th day – by 1.93 times higher than in the control ($p<0.05$) after their gradual increase on the 1st and 2nd day. On the 9th day, the level of SCIC was kept at a significantly higher level compared to the control. Changes in MCIC were less distinct: after a slight rise in the level on the 2nd day (1.28 times higher compared with the control, $p>0.05$), further the decrease was recorded.

Thus, in spontaneous periodontitis accompanied by normoreactivity of the body after surgery, there were a number of changes in immunological parameters in the blood. Increase in the level of IL-1β, IL-6, TNF at the time of increasing necrosis of periodontal tissues, as well as their decrease and increase in the level of IL-4 with the appearance of markers of reparative processes. The dynamics of the content of CIC of different sizes in normoreactivity of the organism had a qualitatively identical picture and was characterized by their increase with the maximum value on the 6th and 9th day after the initial fall on the 1st day, which confirms their participation in mucosal wound healing.

The results of determination of cytokines IL-1β, IL-6, TNF, IL-4 in the blood of animals with spontaneous periodontitis after surgery accompanied by hyperreactivity are presented in table 1. On the 1st day there was a significant increase in all pro- and anti-inflammatory cytokines ($p<0.05$). The maximum level of IL-1β was 4.78 times higher in comparison with the control, the minimum of IL-4 was by 2.26 times higher ($p<0.05$). On the 1st day this increase in the content of cytokines in cases of hyperreaction of the body was, on average, by 1.55 times more significant in cases of normoreaction. Subsequently, the level of all studied indicators decreased sharply, on the 6th day reaching values below those of normoreactivity. Normalization of reduced cytokine levels was not observed until the end of the experiment. Thus, during the development of young connective tissue, the content of cytokines was lower than that of normoreaction, which slows down the healing process of mucosa and bone wound during hyperreaction of the body.

Immunological changes in the blood of animals with increased reactivity of the organism were more pronounced than with normoreactivity (table 2). As can be seen from this table, on the 1st day in the blood of animals there was a sharp drop in the level of vCIC and SCIC, as a consequence of the pronounced stress response. Then the content of circulating immune complexes increased, reaching the maximum by the end of observations. The content of all the studied CIC was significantly higher compared with the control, and with such values at the rate of reactivity of the organism ($p<0.05$).

Thus, in cases of spontaneous periodontitis accompanied by hyperreactivity of the body after surgery the following dynamics of immunological parameters in the blood was typical: significantly higher level of all the studied CIC with the maximum by the end of observations; marked early rise of all cytokines (IL-1β, IL-6, TNFα, IL-4).

The dynamics of pro- and anti-inflammatory cytokines (IL-1β, IL-6, TNFα, IL-4) with reduced reactivity of the organism is described – in table 1. On the 1st day, the level of all studied parameters increased. The peak of IL-4 was observed on the 1st day ($p>0.05$), IL-1β, IL-6 and TNFI on the 2nd ($p<0.05$). However, the amplitude of growth of all the indicators was the smallest in comparison with that of normal and hyperreactivity of the organism. The low content of pro-inflammatory cytokines in comparison with that of normoreactivity, which contributes to the slowing down of reparative processes, should be taken into consideration. As a result, the low content of IL-4 was observed during the whole period of observation in cases accompanied by hyporeactivity of the body.

As can be seen from the table, the level of circulating immune complexes during the first week decreased with a subsequent sharp increase until the end of observations. On the 9th day, the content of CIC in the blood of animals of this group was intermediate in value: higher than in animals with normoreactivity of the body and lower than in animals with hyperreactivity. That is, the level of CIC during the development of young connective tissue was higher than that of normoreaction.

It should be noted that the amplitude of increase of all investigated pro- and anti-inflammatory cytokines at the time of increase of necrosis of periodontal tissues on the 1st day after surgery was the smallest in comparison with that at normo-, and at hyperreactivity of an organism, and the CEC level during development of young connective tissue was probably higher than that of normoreaction, which leads to a delay in the development of reparative processes in the wound, which is narrowed with data from other researchers [10, 12, 14].

Thus, in spontaneous periodontitis accompanied by hyporeactivity of the body after surgery the following dynamics of immunological parameters in the blood was typical: delayed involution of elevated values of circulating immune complexes; slight late rise in the concentration of all cytokines (IL-1β, IL-6, TNFα, IL-4) [5, 8].

Conclusion

Thus, adequate time-balanced changes in immunological parameters (pro- and anti-inflammatory cytokines, circulating immune complexes) form a normoreactive response with synchronization of necrotic and reparative processes. In cases of hyperreactivity of the body after surgical treatment of spontaneous periodontitis, changes in the studied immunological parameters contribute to the strengthening of necrotic and delayed reparative processes in the healing of mucosal wounds. In cases of hyporeactivity of the body after surgical treatment of spontaneous periodontitis, changes in pro- and anti-inflammatory cytokines and circulating immune complexes of different sizes contribute to the slowing of necrotic and delayed reparative processes in the healing of mucosal wounds. The revealed varying dynamics of immunological indicators depending on the condition of reactivity of the organism preconditions the necessity of their directed medical correction with reduction of values to those which are typical for normoreaction. This is considered to be a condition for optimizing the healing process after surgical treatment of the inflammatory-destructive process in periodontal tissues, which has become the subject of further research.

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