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Original papers

Effect of medicinal leeches' antigens on the proliferative response of human blood mononuclear cells and cytokine production in vitro

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ABSTRACT. The purpose of research is to study the influence of plant mitogens and antigens of the water-salt extract from the bodies of *Hirudo verbana*, *H. medicinalis*, *H. orientalis* on the reaction of lymphocyte blast-transformation and the synthesis of pro-inflammatory cytokines (IL-1β, IL-8, TNF-α) in patients' cell culture supernatants before and after hirudotherapy. Research methods: the reaction of lymphocyte blast-transformation; the determination of pro-inflammatory cytokines by ELISA. After hirudotherapy increased values of the reaction of lymphocyte blast-transformation have got bigger in all stimulated types of cell culture, but reactivity on medicinal leeches antigens proceeded to unproductive immunogenesis (apoptosis and necrosis). In supernatants of mitogen- and antigen-stimulated lymphocyte cultures induced high levels of pro-inflammatory cytokines after hirudotherapy have decreased adequately to apoptotic induction of lymphocytes by medicinal leeches' antigens.

Key words: lymphocyte blast-transformation reaction, hirudotherapy, medicinal leech, biologically active substances, mitogens, cytokines

Introduction

Currently hirudotherapy takes a significant place among effective natural therapeutic agents [1,2], especially in the treatment of chronic diseases [2–7], and in reconstructive surgery [3,4,7–10]. This is due to the fact that medicinal leech during feeding injects into the sucking lacuna with the salivary gland a secretion of more than 100 components of biologically active substances (BAS) [2,7] in the optimum dosage and consistency, and dose-bled contributes to the favorable redistribution of circulating blood and lymph circulation, which combine to provide general homeostatic action [2]. Among the BAS of the medicinal leech are revealed: hirudin, eglins, bdellins, enzymes (hyaluronidase, destabilase, collagenase, apyrase, elastase and others) [2–4,7,9,10], potent antibiotic (chloromycetin) [3], anesthetic-like substances [3,4,6,10,11], which in complex provide antithrombotic, thrombolytic, anti-hypertensive, anti-atherogenic, anti-hypoxic, regenerating, anti-inflammatory and analgesic effects [2,3,7]. In addition to the above, positive therapeutic effects of hirudotherapy are mediated by immunomodulatory effects on the immune system. However, the mechanism of immunotropic action is just beginning to be studied. Thus, the anti-inflammatory effect of hirudotherapy is observed at the organism level [2], and by methods of laboratory immunology is observed a stimulation of phagocytic reactions [12,13], and correction of helper-suppressor populations in blood of patients [14]. However, further specification of the influence of hirudotherapy on the immune system, will allow more objectively pathogenetically substantiate this type of treatment and come close to its standardization. It also remains unanswered an issue of antigen (Ag) identity of various medicinal leech species' BAS used in hirudotherapy. In this case, immunomodulatory therapeutic effects of saliva BAS of the medicinal leech on the level of the

organism may be mediated by a number of indirect mechanisms (e.g. by increased levels of pro-inflammatory cytokines, growth factors, regulation of maturation processes and cell migration, and others). The last ones are possible to be studied at modeling them in the culture of immunocompetent cells and cells of other tissues (muscle, nerve, and others). Therefore, the aim of our work is to study the influence of Ags of the water-salt extract from the bodies of three medicinal leeches species (*Hirudo verbana* Carena, 1820; *H. medicinalis* Linnaeus, 1758; *H. orientalis* S. Utevsky et Trontelj, 2005) on the reaction of lymphocyte blast-transformation (RLBT) and the production of basic pro-inflammatory cytokines (interleukin 1β – IL-1β, interleukin 8 – IL-8, tumor necrosis factor α – TNF-α) in the culture of peripheral blood mononuclear cells of patients with various chronic diseases during hirudotherapy. The choice by us of basic optimum of proinflammatory cytokines analysis is caused by the following immunological properties. Thus, IL-1β is synthesized by many immuno-competent cells and nonimmunocompetent cells with wide pleiotropic effect on immune cells [15,16] and other systems (e.g. nervous) [17]. The cytokine IL-8 is the basic neutrophil chemokine, the most numerous population of blood leukocytes [15,18]. TNF-α takes part in regulation of proliferative processes and regulation of other cytokines synthesis, including the pro-inflammatory ones [15,19].

Materials and Methods

Ethics statement. The study was carried out according to the ethical standards of the Ministry of Health of Ukraine with the personal consent of the patient with the preparation of relevant agreement, in which have been specified research purposes and objectives.

Examined groups. Investigated the venous blood of 23 patients (11 men and 12 women, mean age 50.3±2.47 years) with various chronic diseases (hypertension, varicose expansion of veins, thrombosis, diabetes, osteochondrosis, etc.) in remission, stabilized by crystalline heparin (0.2 mg/ml, Spofa) before and after the standard course of ambulatory hirudotherapy duration of 3.5–4 weeks, in which there were applied 25–40 hungry *H. verbana*. The similarity of the hirudotherapy courses in patients enabled to consider it as a basic factor, affecting the condition of immune system. Blood samples before and after hirudotherapy were analyzed from the same individuals.

Lymphocyte cultures. Lymphocytes from venous blood were isolated by Ficoll-verografin density gradient (ρ=1,077) [20], were suspended in a nutritious mixture (199 medium, supplemented with 20% fetal calf serum, 20 mM HEPES, 0.3 mg/ml glutamine, 0.15 mg/ml asparagine, 100 μg/ml gentamicin, 10 μM 2-mercaptoethanol) at a final cell concentration of 2 million/ml the culture were added to 0.25 ml in eppendorf tubes in volume of 2 ml. Set the following types of the RLBT: spontaneous (control), mitogen-stimulated – adding of 20 μg/ml phytohaemagglutinin (PHA, Bulgaria), of 20 μg/ml concanavalin A (ConA, Germany), the Ag-stimulated – adding of 125 μg/ml Ags in recalculation at protein from water-salt extract of three medicinal leeches species (*H. verbana*, *H. medicinalis*, *H. orientalis*). Water-salt extract of Ags from the bodies of medicinal leeches was prepared according to the method [21]. This Ag dose of the medicinal leech we selected after calibration as the sufficient to induce an immune response without previous initial cytotoxic effect. Lymphocytes were cultured for 24 h at 37°C in a nutritious mixture, and then the test samples were centrifuged and the supernatant was used to determine the cytokine production and smears were prepared from the cell pellet, that were stained by Pappenheim.

Evaluation of the reaction of lymphocyte blast-transformation. The RLBT evaluated by morphological method, taking into account 500–600 lymphocytes. As activated ones were considered small, medium, large blasts taking into account the morphology changes and tinctorial signs of nucleus and cytoplasm according to recommendations [22], and also lymphocytes with visible signs of apoptosis (vacuolization of nucleus and cytoplasm, karyopyknosis, karyorrhexis, ceiosis (lacyness) of cell membrane, protrusion of the nucleus and cytoplasm and other) and necrosis (oxyphilic stains) [23].

Determination of interleukins by ELISA. In the supernatants culture of mononuclear cells was determined the production of cytokines (IL-1β, IL-8, TNF-α) using the certified reagent kit for the quantitative determination of blood plasma cytokines by ELISA produced by LLC "UkrmedDon", Donetsk (Ukraine), according to standardized procedure presented by the manufacturer, on automatic analyzer "Chemwell-2910" (Awarenes Tech., USA).

Statistical analysis. Statistical processing of the experimental data was performed by application package IBM SPSS v. 20.0, using parametric and nonparametric statistical methods. Checking of the normality of the distribution was carried out using one-sample Kolmogorov-Smirnov test, at the normal distribution statistical significance of differences between examined groups were evaluated by Student's criterion (t-test for paired samples), with the value in the tables are presented as M ± m, where M – the mean value of the sign, m – mean error of the arithmetic mean. In case of absence of the data consent to the normal distribution to evaluate differences between dependent samples was used nonparametric Wilcoxon test, and the data are represented as Me (Q1; Q3), where Me – median, Q1 and Q3 – the 1 and 3 quartile. The differences of the results were considered valid if P>95%, $p<0.05$ [24].

Results

Mitogen- and antigen-stimulated reaction of lymphocytes blast-transformation

RLBT data of examined groups are presented in Table 1. The levels of RLBT in mitogen- and Ag-stimulated cultures were much higher than their spontaneous values (7.1±0.69%), which indicates on conventional cultivation conditions according to the natural characteristics of lymphocytes stimulators. So, before hirudotherapy RLBT to PHA and ConA, respectively equaled 64.9±1.79% and 37.0±1.75% and coincided with those of other authors [25]. Features of the RLBT were mainly associated with the specificity of lymphocytes' response to Ags of medicinal leeches. Thus, in intact patients before hirudotherapy the levels of the Ag-stimulated RLBT were more than twofold higher of their spontaneous values without their preliminary contact with medicinal leech. Herewith discovered a tendency for increasing of the RLBT levels in cultures with Ags of *H. orientalis* (15.4±1.14%), reaching a statistically significant level compared to *H. verbana* (11.8±1.04%) and *H. medicinalis* (13.2±0.78%) ones ($p<0.05$).

Morphological differences of the RLBT were observed when lymphocytes were stimulated with plant mitogens and Ags of medicinal leeches. In mitogen-stimulated lymphocytes cultures were observed typical blasts with synthetically active nucleus and developed basophilic cytoplasm that indicates productive proliferative response. Under Ags of medicinal leeches stimulation blasts had an underdeveloped basophilic cytoplasm, and most of them were with signs of apoptosis (see Fig. 1). In such cultures were also observed necrotic lymphocytes as diffuse eosinophilic stains.

After hirudotherapy its stimulating effect revealed itself in all cultures. Had increased spontaneous (18.3±1.01%) and mitogen-stimulated RLBT levels (70.4±1.46% and 41.8±1.58% in cultures with PHA and ConA, respectively) as an indicator of increasing potential proliferative activity of lymphocytes. After hirudotherapy also had increased RLBT in the Ag-stimulated cultures in 2–2.5 times from the initial level with preservation of orientation to increasing in cultures with Ags of *H. orientalis* (35.4±1.66%), whereas hirudotherapy was carried out with *H. verbana*. In this case specific Ags of medicinal leeches initiated unproductive stage of immunogenesis, which proceeded in apoptosis and necrosis.

The synthesis of cytokines in supernatants of lymphocytes cultures

Changing of lymphocytes activational ability under the influence of mitogens and Ags of the medicinal leech was accompanied by corresponding differences in production of pro-inflammatory cytokines (Table 2). Their wide range of dispersion associated with the individual differences that depend on the variation of the original ratio of populations and subpopulations of mononuclear cells and their functional state in vivo, and also from variations in the microenvironment of the cells in vitro, which is consistent with literature data [15]. There were observed three general regularities in the given synthesis. Firstly, the levels of cytokines at mitogen- and Ag-stimulated cultures significantly exceeded their spontaneous values (without stimulation). Secondly, in cultures of mononuclear cells after hirudotherapy there was a tendency to lowering levels of pro-inflammatory cytokines. Thirdly, the oscillation amplitudes of cytokines levels at Ag-stimulated cultures were higher than those in cultures of mononuclear cells with plant mitogens. But, the degree of changes in the levels of cytokine synthesis were dependent on the type of the corresponding stimulator of mononuclear cells and the nature of the particular cytokine. Thus, the levels of IL-1β synthesis before and after hirudotherapy were much lower than IL-8 and TNF-α production by mononuclear cells. At the same time the synthesis of IL-1β and IL-8 under the influence of Ags of

Table 1. The mitogen- and Ag-stimulated RLBT of patients (n = 23) before and after hirudotherapy

Examined groups	Levels of lymphocytes stimulation (M ± m), %					
	Spontaneous	PHA	ConA	Ags of *H. verbana*	Ags of *H. medicinalis*	Ags of *H. orientalis*
Before hirudotherapy	7.1 ± 0.69	64.9 ± 1.79 (b)	37.0 ± 1.75 (b)	11.8 ± 1.04 (b, c, d) / 2.35 ± 0.142	13.2 ± 0.78 (b, c, d) / 2.72 ± 0.201 (e)	15.4 ± 1.14 (b, c, d, e, f) / 2.89 ± 0.186 (e)
After hirudotherapy	18.3 ± 1.01 (a)	70.4 ± 1.46 (a, b)	41.8 ± 1.58 (a, b)	24.6 ± 0.77 (a, b, c, d) / 4.67 ± 0.139 (a)	27.3 ± 1.41 (a, b, c, d) / 5.22 ± 0.185 (a, e)	35.4 ± 1.66 (a, b, c, d, e, f) / 5.61 ± 0.121 (a, e)

Explanations: Numerator, the total number of blast transforming lymphocytes in cell culture; denominator, including with the signs of apoptosis, the number of necrotic cells in the table are not separately indicated;(a) the differences between the group before and after hirudotherapy are significant at $p<0.05$;(b) the differences between spontaneous and mitogen-stimulated (PHA, ConA) or Ag-stimulated (Ags of *H. verbana, H. medicinalis, H. orientalis*) RLBT are significant at $p<0.05$;(c) the differences between mitogen- (PHA) and Ag-stimulated (Ags of *H. verbana, H. medicinalis, H. orientalis*) RLBT are significant at $p<0.05$;(d) the differences between mitogen- (ConA) and Ag-stimulated (Ags of *H. verbana, H. medicinalis, H. orientalis*) RLBT are significant at $p<0.05$;(e) the differences between Ags of *H. verbana* stimulated RLBT and Ags of *H. medicinalis*, *H. orientalis* stimulated RLBT are significant at $p<0.05$;(f) the differences between Ags of *H. medicinalis* stimulated RLBT and Ags of *H. orientalis* stimulated RLBT are significant at $p<0.05$.Comparison of results between groups using the t-test for paired samples

Table 2. The levels of spontaneous and mitogen/Ag-stimulated production of IL-1β, IL-8 and TNF-α in the culture mononuclear cells of patients (n = 23) before and after hirudotherapy

	Examined groups	Levels of cytokines [Me (Q1; Q3)] in the test samples, pg/ml					
		Spontaneous	PHA	ConA	Ags of *H. verbana*	Ags of *H. medicinalis*	Ags of *H. orientalis*
IL-1β	Before hirudotherapy	31.45 (24.15; 39.43)	66.21 (44.12; 88.75)(b)	80.16 (56.14; 102.24)(b)	86.74 (59.18; 121.67)(b, c)	87.08 (64.48; 108.10)(b, c)	80.45 (54.14; 98.53)(b, c, e, f)
	After hirudotherapy	26.19 (18.93; 36.05)	41.30 (34.34; 55.18)(a, b)	51.70 (40.02; 79.48) (a, b)	69.89 (44.54; 97.52) (b, c, d)	77.25 (52.70; 102.10) (b, c, d)	69.03 (44.63; 86.28)(b, c, d, f)
IL-8	Before hirudotherapy	601.20 (410.50; 848.20)	5350.10 (1016.30; 9722.50)(b)	1552.80 (895.40; 2217.90)(b)	10150.40 (4972.50; 19676.30)(b, c, d)	10920.10 (2642.60; 14965.20)(b, c, d)	11569.00 (1963.90; 15119.80)(b, c, d)
	After hirudotherapy	247.60 (143.80; 423.20)(a)	1967.50 (834.40; 3919.80)(a, b)	863.20 (577.40; 1653.80)(a, b)	5696.80 (1421.50; 9154.50)(a, b, d)	4819.60 (1682.20; 7831.60)(a, b, c, d)	5249.90 (715.60; 10080.30)(a, b, d)
TNF-α	Before hirudotherapy	352.30 (221.20; 507.50)	1927.60 (1182.30; 4602.40)(b)	962.80 (758.50; 1377.80)(b)	3314.80 (1536.80; 4797.00)(b, d)	2352.70 (1043.00; 4126.20)(b, d)	2027.60 (765.70; 3726.00)(b, d, e)
	After hirudotherapy	144.00 (86.40; 246.10)(a)	814.50 (562.80; 2301.30)(a, b)	451.90 (339.00; 630.10)(a, b)	1669.60 (714.70; 2828.40)(a, b, d)	1955.50 (745.20; 3034.40)(a, b, c, d)	816.50 (357.20; 2509.60)(a, b, d, e, f)

Explanations: see Table 1. Comparison of results between groups using the Wilcoxon test.

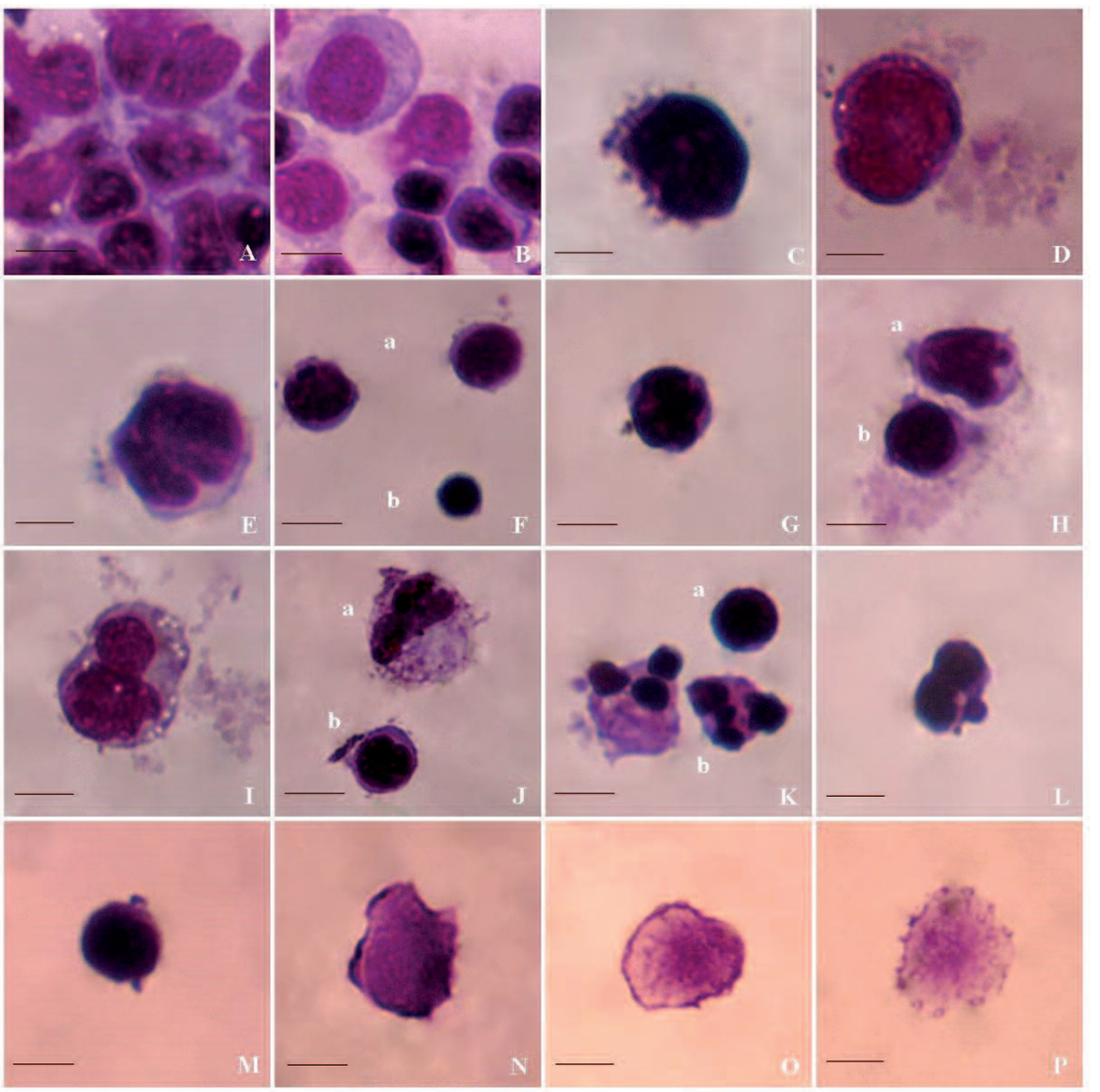

Fig. 1. Morphological forms of activated lymphocytes under the influence of plant mitogens and antigens of the different medicinal leech species

Typical blasttransformed lymphocytes (decondensation and increasing of nucleus, presence of nucleoli, developed basophilic cytoplasm) under the influence of: A – PHA; B – ConA.

Blasttransformed lymphocytes under the influence of antigens of different medicinal leeches species with signs of apoptosis and necrosis: C – heterochromatization of nucleus, absence of nucleoli, underdevelopment of cytoplasm, weak basophilia, ceiosis (lacyness) of cell membrane; D – heterochromatization of nucleus, absence of nucleoli, karyorrhexical invagination of nucleus, vacuolization of undeveloped cytoplasm; E – heterochromatization of nucleus, absence of nucleoli, karyorrhexical invagination of nucleus, undeveloped cytoplasm, weak basophilia; F – heterochromatization of nucleus, absence of nucleoli, karyorrhexical invagination of nucleus, undeveloped cytoplasm with weak basophilia, ceiosis (lacyness) of cell membrane (a); pyknosis of nucleus and cytoplasm, ceiosis (lacyness) of cell membrane (b); G – heterochromatization of nucleus, absence of nucleoli, karyorrhexical invagination of nucleus, undeveloped cytoplasm with weak basophilia, ceiosis (lacyness) of cell membrane; H – heterochromatization of nucleus, absence of nucleoli, karyorrhexis; ceiosis (lacyness) of cell membrane, weak basophilia of cytoplasm (a); heterochromatization of nucleus, absence of nucleoli, destruction of cytoplasm, weak basophilia (b); I – heterochromatization of nucleus, absence of nucleoli, karyorrhexis, vacuolization of nucleus, weak basophilia of cytoplasm with vacuolization, ceiosis (lacyness) of cell membrane; J – heterochromatization of nucleus, absence of nucleoli, multiple protrusions of nucleus, weak basophilia of cytoplasm with vacuolization, ceiosis (lacyness) of cell membrane (a); heterochromatization of nucleus, absence of nucleoli, karyopyknosis, undeveloped cytoplasm with weak basophilia, protrusion of cytoplasm (b); K – heterochromatization of nucleus, absence of nucleoli, karyopyknosis, undeveloped cytoplasm (a); karyorrhexis, weak basophilia of cytoplasm with vacuolization, ceiosis (lacyness) of cell membrane (b); L – protrusion of nucleus and cytoplasm with vacuolization of cytoplasm, undeveloped basophilia of cytoplasm; M – karyopyknosis, undeveloped cytoplasm, ceiosis (lacyness) of cell membrane; N – beginning of necrosis of blasttransformed lymphocyte; O – karyolysis passing in necrosis; P – eosinophilic necrotic stains.

Light microscope (objective lens 100×, eyepiece K7×). Bars in A, B, F, I–P: 7 μm; in C, D, E, G, H: 5 μm.

different medicinal leech species did not significantly differ. Identified a statistically significant inhibition of TNF-α synthesis in the cultures with Ags of *H. orientalis* in comparison with Ags other species of medicinal leech ($p<0.05$).

Discussion

Significant differences of RLBT levels in spontaneous, mitogen- and Ag-stimulated cultures of mononuclear cells make it possible to conclude on the adequacy of the chosen method and its sufficient informativity in assessing of potential proliferative cells activity in vitro. Thus, in mitogen-stimulated cultures of mononuclear cells with PHA the levels of RLBT and cytokines were always higher than with ConA, reflecting their different lectin properties: PHA activates all T-lymphocytes [26], while ConA – predominantly their killer/suppressor fraction with the phenotype of $CD8^+$ [27].

Significant levels of RLBT in the cultures stimulated by Ags of medicinal leech, compared with spontaneous RLBT in patients before and after hirudotherapy may be explained by the presence of common patterns in protein organization of all types. The immune response starts with the patterns interactions on pattern-recognizing receptors of the innate immune cells, which through cell contacts and by cytokines involve lymphocytes to immunogenesis [28]. From these positions follows a logical explanation also the fact of RLBT increasing to Ags of three medicinal leeches species (*H. verbana*, *H. medicinalis*, *H. orientalis*) after hirudotherapy as a result of increasing in recirculation of lymphocytes, which are sensibilized to Ags of *H. verbana* that have common patterns with other species. At the same time different levels of the RLBT confirm their specific differences. Earlier established the presence of the similarity of proteins and peptides from salivary gland secretion of *H. verbana*, *H. medicinalis* and *H. orientalis* by 30–40%, with the highest affinity existed between *H. medicinalis* and *H. orientalis* [29].

For the first time it has been observed the activation of immunogenetic lymphocyte response to Ags of the medicinal leech by unproductive pathways (apoptosis and the following necrosis) in the culture of mononuclear cells which is new not previously described property of the medicinal leech, which is probably one of the forms of their immunological defense reaction from the immune cells of the host-breadwinner blood cells. Otherwise the medicinal leech, as obligate haematophagous animal, with the volume of eaten blood at 3–5 times more than their initial weight after feeding would have developed immunological response by the type of graft versus host reaction.

Induction of apoptosis and necrosis by medicinal leech's Ags, aggravated after hirudotherapy, may explain its general dynamics of decrease in the synthesis of studied cytokines. So, according to the principle of secondary immune response after hirudotherapy increases the number of sensibilized lymphocytes to medicinal leech's Ags, registered by us at the level of the RLBT. At the same time, according to our data [14], after hirudotherapy takes place homeostatic reorganization of lymphocyte subpopulations, which reduces excessive increase in the number of lymphocyte subpopulations with upregulation of the immune response ($CD4^+$, $CD25^+$), at adequate increasing of subpopulations with negative regulation of immunogenesis ($CD8^+$, $CD16^+$). Against this background population shifts of blood lymphocytes after hirudotherapy natural properties of medicinal leech's BAS to for inclusion (induction) apoptosis in immunocompetent cells is accompanied by a decrease in synthesis of studied pro-inflammatory cytokines in cell culture. Revealed dynamics of the RLBT and synthesis of pro-inflammatory cytokines in vitro under the influence of Ags medicinal leech after hirudotherapy confirmed by clinical observations: 1) the healing of significant bite (1.5–2 mm in depth, 4–5 mm in diameter) occurs rapidly (within 2–3 days) with no signs of inflammation; 2) anti-inflammatory effect of hirudotherapy on the level of the organism is the leading among its clinical mechanisms [2].

The levels of IL-1β are significantly lower than other pro-inflammatory cytokines (IL-8, TNF-α) in the supernatants culture of mononuclear cells, and probably are a result of well-known dynamics of the synthetic sequence and relative cross-regulation of a set of pro- and anti-inflammatory cytokines. Synthesis of cytokines by immunocompetent cells and auxiliary cells occurs in the first minutes and hours after Ag/mitogenic stimulation [15]. Thus IL-1β, by virtue of its functional pleiotropy is synthesized by one of the first and mobilizing organism to activate cellular system. Therefore, it is the first to be in negative regulation as the one that has fulfilled its function from the other cytokines and in this case the TNF-α [15].

The fact of exceeding the synthesis of pro-inflammatory cytokines (IL-8, TNF-α) under the influence of the medicinal leech Ags compared to cultures stimulated with plant mitogens needs a further study. The levels of RLBT to the latter were significantly higher and RLBT passed on productive lymphopoiesis, whereas reactivity to Ags of the medicinal leech was lower, mainly in abortive form. Probable reasons of such cross-values are in difference of receptors that are responsive to plant lectins and Ags (patterns) of the medicinal leech, and therefore differences in the activation and regulation of transcription factors. It is known that ConA and PHA react with corresponding carbohydrate residues of lymphocytes' glycocalyx [30] mainly by stimulating the synthesis of cytokines which induce cell proliferation (such as IL-2). Receptors on immune cells to various Ags of the medicinal leech still need to be studied, but as follows from the results of this work, the spectrum of cytokines induced by them more directed to a protection mobilization of immune cells by the synthesis a wide range of cytokines, including the pro-inflammatory. Whereas it is known that the immunogenetic step of activation and synthesis of transient receptors and structures, including cytokines, is preceded by blasttransformation and further proliferation of lymphocytes. But considering the phylogenetically adapted ability of medicinal leech Ags to inhibit immunogenesis of host-feeder blood cells by blocking further blasttransformation and proliferative response of lymphocytes by apoptosis explains the lower levels RLBT to Ags of medicinal leech, compared with plant mitogens. While processes of the synthesis of various cytokines on lymphocyte activation stage were preceded apoptotic suppression of Ag-stimulated RLBT.

Thus, it is first observed a variety of RLBT and the specific dynamics of the pro-inflammatory cytokines production in cultures stimulated with Ags from different species of medicinal leeches is the basis for further experimental study of medicinal leeches BAS immunotropic action and clinical study of hirudotherapy with an individual approach to its purpose and evaluation of the effectiveness.

Conclusions

1. As the result, it is diagnosed that the RLBT of patients after hirudotherapy was increased, while in mitogen-stimulated cultures it has evolved through a typical stereotype immunogenesis, whereas in cultures with Ags of medicinal leeches — by unproductive type, passing into apoptosis and necrosis.

2. In the supernatants of mitogen- and Ag-stimulated lymphocyte cultures induced high levels of pro-inflammatory cytokines (IL-1β, IL-8, TNF-α) after hirudotherapy were reduced adequately to apoptotic induction of lymphocytes by Ags of medicinal leeches.

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