THE EFFICACY OF SYSTEMIC ENZYME THERAPY IN THE COMPLEX TREATMENT OF TROPHIC ULCERS OF VENOUS ETIOLOGY

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The current strategy for the treatment of venous trophic ulcers (VTU) suggests differentiated approach and a combination of conservative and surgical methods. This paper presents the results of the study of efficacy of systemic enzyme therapy (Phlogenzym by Mucos Pharma, Germany) in patients with varicose veins of lower extremities (CEAP class C6) and stage I, II and III VTU. The study included 38 patients aged 12 to 82 years. The patients were divided into the experimental (n = 20) and the control (n = 18) groups. The treatment lasted 1 month. Silcofix Professional wound dressings (Pharmaplast, Egypt) were used. All patients received Detralex (Les Laboratoires Servier, France) and wore class 2 and 3 knee-high compression socks. Patients of the experimental group also received Phlogenzym for 30 days (3 tablets 3 times a day). Total ulcer epithelization was observed in 8 (40 %) patients by week 3, and in 18 (90 %) patients by the end of treatment compared to 4 (22 %) and 9 (50 %) patients in the control group, respectively. In the control group, the regenerative process in the area of the ulcerous defect was less prominent compared to the experimental group. Immunoassays revealed a significant reduction in $CD4^+CD25^{Bright}$ cells and increased levels of $CD4^+CD45RO^+$ T-lymphocytes in the experimental group, corresponding with the observed positive clinical response. The use of immunomodulatory drug Phlogenzym contributed to a more rapid regression of clinical symptoms of chronic venous insufficiency and faster healing of stage I–III venous trophic ulcers.

Keywords: varicose veins of lower extremities, venous trophic ulcer, adaptive immunity, lymphocyte subpopulation

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ЭФФЕКТИВНОСТЬ СИСТЕМНОЙ ЭНЗИМОТЕРАПИИ В КОМПЛЕКСНОМ ЛЕЧЕНИИ ТРОФИЧЕСКИХ ЯЗВ ВЕНОЗНОГО ГЕНЕЗА

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Современная стратегия и тактика лечения венозных трофических язв (ВТЯ) предполагают дифференцированный подход и комбинацию консервативных и хирургических методов. В работе представлены результаты исследования эффективности системной энзимотерапии (препарат «Флогэнзим», Mucos Pharma, Германия) у пациентов с варикозным расширением вен нижних конечностей клинического класса С6 с ВТЯ в I–III стадии раневого процесса. В исследовании участвовали 38 пациентов в возрасте от 12 до 82 лет. Они были разделены на основную (n = 20) и контрольную (n = 18) группы. Лечение длилось 1 мес. Использовали раневые повязки линейки Silcofix Professional (Pharmaplast, Египет), также все пациенты принимали «Детралекс» (Les Laboratoires Servier, Франция) и носили гольфы 2–3 степени компрессии. В основной группе в протокол лечения включили «Флогэнзим»: курс 30 дней, по 3 таблетки 3 раза в день. В результате полная эпителизация язвы на 3 неделе лечения была отмечена у 8 (40 %) пациентов, к концу лечения — у 18 (90 %), тогда как в контрольной группе — у 4 (22 %) и 9 (50 %) соответственно. У пациентов контрольной группы регенеративные процессы в области язвенного дефекта были слабо выражены по сравнению с основной группой. Иммунологический анализ показал существенное снижение содержания $CD4^+CD25^{Bright}$-клеток и повышение содержания Т-лимфоцитов с фенотипом $CD4^+CD45RO^+$ в основной группе, что соответствовало наблюдаемым положительным изменениям в клинической картине. Включение в протокол лечения иммуномодулирующего препарата «Флогэнзим» способствовало более быстрому регрессу клинических симптомов хронической венозной недостаточности и ускорению регенерации ВТЯ в I–III стадии раневого процесса.

Ключевые слова: варикозное расширение вен нижних конечностей, венозная трофическая язва, адаптивный иммунитет, субпопуляция лимфоцитов

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Varicose veins of lower extremities (VV) are one of the most common pathologic conditions of the peripheral vessels of lower extremities leading to the development of venous trophic ulcers (VTU) in some cases [1, 2]. VTU of lower extremities are a serious problem in modern medicine: in 10 % of cases VTU are persistent and non-responsive to conservative therapy [3]. Relapse rates remain high and range from 4.8 to 31.6 % after surgery and from 15.0 to 100.0 % after complex conservative treatment [4]. It is clear that attempts to influence individual components involved in the pathogenesis of the disease are not effective.

Recent studies show that in the treatment of venous ulcers, elimination of venous hypertension and valvular incompetence seen as the main causes of chronic venous insufficiency (CVI) [5–7] does not provide a desired effect. It is necessary to treat microcirculatory disorders that lead to chronic inflammation in the ulcer, constant leukocyte infiltration and changes in the metabolism of endothelial cells [8]. A characteristic feature of chronic inflammation is an imbalance between the humoral and cell-mediated immunity; however, only limited data are currently available on the role of immune disorders in the pathogenesis of VV [9, 10]. The few and rather contradictory results of the studies of the immune status of patients with VTU indicate that the severity of trophic disorders depends not only on the anatomical characteristics of the vein, but also on the inadequate response of immune cells, which is a chronic damaging factor [11–14].

One of the understudied methods of VTU treatment is systemic enzyme therapy (SET). Several studies have shown its efficacy, in particular when using Wobenzym (Mucos Pharma, Germany) for the treatment of CVI of various etiology (post-thrombotic syndrome disease and varicose vein disease) [15, 16]. The aim of this study was to evaluate the efficacy of conservative therapy supported with systemic enzymes (Phlogenzym by Mucos Pharma).

METHODS

The study included 38 patients with VV (C6) who received treatment for 1 month. The inclusion criteria were stage I-III recurrent VTU with the area of the ulcerous defect up to 30 cm^2, and patients' age from 18 to 82 years. The exclusion criteria were the presence of a concomitant pathology: type 1 and 2 diabetes, atherosclerosis of lower extremities, systemic vasculitis, rheumatoid arthritis, hormone and immune therapy.

The patients were divided into two groups. The control group received conservative treatment: wound dressings, compression and phlebotropic drug therapy. It included 18 patients (mean age of 60.1 ± 10.5 years) and consisted of 12 (66.7 %) women and 6 (33.3 %) men; 10 (55.6 %) patients were >60 years of age. The experimental group received conservative treatment and systemic enzyme therapy with Phlogenzym for 30 days, 3 tablets 3 times a day. It included 20 patients (mean age of 61.2±12.6 years) and consisted of 13 (65.0 %) women and 7 (35.0 %) men; 13 (65.0 %) patients were >60 years of age. Each group included 4 patients with stage III VTU and 3 patients with stage I VTU; the rest of the patients had stage II VTU.

In the course of treatment, wound dressings Silcofix Professional (Pharmaplast, Egypt) were used. In patients with stage I VTU, absorbent dressings (Fibrosorb, Fibroclean Ag) were used. Atraumatic povidone-iodine dressings (Silkofix POVI) were applied on the defect with persistent fibrin deposits after exudation has decreased. In patients with stage II-III VTU, hydrocolloid dressings with silver ions (Fibrocold Ag) were used. At the first sign of epithelialization, hypoadhesive mesh coatings with a lipidocolloid complex (Fibrotul, Fibrotul Ag) were used. All patients received Detralex (Les Laboratoires Servier, France) for 1 month and wore knee-high compression stockings (classes 2 and 3).

Fasting venous blood samples were taken for immunological analysis in the morning using Vacuette blood collection systems (Greiner Bio One, Germany). Lymphocyte surface receptors were evaluated by multiparameter immunofluorescent staining with monoclonal antibodies (IQ Products, the Netherlands). Before and after treatment, absolute and relative counts of lymphocytes in the peripheral blood with the following surface antigens were determined using laser flow cytometry (FACSCalibur platform, Becton Dickinson, USA) and Simulset and CellQuest software (BD Biosciences, USA): $CD19^+$, $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+CD25^+$, $CD4^+CD25^{+Bright}$, $CD4^+CD45RA^+$, $CD4^+CD45RO^+$, CD45RA, CD45RO.

For the quantitative assessment of CVI symptoms ("heavy legs", pain, swelling, cramps) and the analysis of the wound healing dynamics (healing of the ulcer and the condition of the surrounding tissues), we used the Venous Clinical Severity Score (VCSS) and the ulcer and skin condition score. VCSS was used before and after treatment; ulcer assessment was performed before treatment and after 1 month of treatment. To assess the discomfort in daily activities, the Visual Analogue Scale (VAS) was used.

Bacteriological analysis of ulcer discharge was performed at the initial visit and after 2 weeks of treatment. The cultures were assessed using semiautomatic analyzers Sceptor and Crystal (Becton Dickinson).

Statistical analysis was performed using Statistica 6.0 software (StatSoft, USA). Statistical significance was determined using Student's t-test. The differences between mean values were considered statistically significant at $p < 0.05$. The data in the tables are presented as M ± m.

All patients provided written informed consent. The study protocol was approved by the Ethics Committee of Pirogov Russian National Research Medical University in 2013.

RESULTS

Complete healing of ulcers in the control group was observed in 4 (22.2 %) patients after 3 weeks of treatment and in 9 (50.0 %) patients after 5 weeks. In the experimental group regeneration was much faster: complete epithelialization of the ulcer was observed in 8 (40.0 %) patients after 3 weeks of treatment and in 18 (90.0 %) patients by the end of treatment (Table 1). Healing was confirmed by the evaluation of such parameters as wound pain, hyperpigmentation, maceration, hyperemia and eczematous dermatitis. In the experimental group, we observed faster ulcer surface clearing of purulent and necrotic tissues, reduction of induration and hyperemia, more active formation of granulation tissue, and marginal epithelialization (Table 2). The patients of the experimental group also reported more significant pain relief and reduction of discomfort in the area of the ulcer in the course of treatment compared to the control group (Table 3).

Microbiological tests conducted before treatment identified the presence of *Staphylococcus aureus* in 40 % of patients and its microbial associations in 10 % of patients. After 2 weeks of treatment, only non-pathogenic and opportunistic microbes below the critical level of contamination were found in all patients.

In both groups, the analysis of cell subpopulations showed no significant changes in the absolute and relative counts of

Table 1. The results of treatment with and without Phlogenzym in patients with stage I–III venous trophic ulcers

Healing	Experimental group (n = 20)	Control group (n = 18)
Week 1	0	0
Week 2	0	0
Week 3	8 (40.0 %)	4 (22.2 %)
Week 4	7 (35.0 %)	2 (11.1 %)
Week 5	3 (15.0 %)	3 (16.7 %)

peripheral B-lymphocytes ($CD19^+$-cells), T-lymphocytes ($CD3^+$-cells) and major subpopulations of T-lymphocytes ($CD3^+CD4^+$- and $CD3^+CD8^+$-cells (Table 4).

The analysis of Treg-lymphocytes showed a significant decrease in the absolute and relative counts of $CD4^+CD25^{Bright}$ cells after treatment in patients who received Phlogenzym ($p < 0.05$) (Table 4). Conversely, in the control group increased levels $CD4^+CD25^{+Bright}$ cells were detected. Also, a significant increase in the relative count of peripheral $CD4^+CD45RA^+$ T-lymphocytes was seen in the control group, while the experimental group demonstrated an increase in peripheral $CD4^+CD45RO^+$ T-lymphocyte count.

The analysis of CD45-T-lymphocytes revealed almost no changes in $CD4^+CD45RA^+/CD4^+CD45RO^+$ and $CD45RA^+/CD45RO^+$ ratios after treatment in the control group, while the experimental group demonstrated a significant decrease in these parameters (Table 4).

DISCUSSION

The results of the lymphocyte subpopulation analysis and lymphocyte functional activity in patients with VV (C6) showed beneficial clinical effects of systemic enzyme therapy with

Table 2. Assessment of ulcers and surrounding tissues before treatment and after 1 week of treatment

Parameter	Experimental group (n = 20)		Control group (n = 18)	
	Before treatment	After 1 week of treatment	Before treatment	After 1 week of treatment
Wound pain	**2.4 ± 2.1**	**1.3 ± 1.2***	**2.9 ± 1.9**	**2.4 ± 1.6***
Hyperpigmentation	1.0	1.0	1.0	1.0
Maceration	**0.4 ± 0.5**	**0.2 ± 0.3***	0.4 ± 0.5	0.3 ± 0.5
Hyperemia	**0.7 ± 0.5**	**0.2 ± 0.3***	**0.7 ± 0.5**	**0.4 ± 0.5***
Eczematous dermatitis	0	0	0	0

Note. The results are presented as M ± m. * — $p < 0.05$ when comparing two values within a group.

Table 3. Assessment of CVI symptoms severity and the degree of patient's discomfort before and after treatment

Parameter	Experimental group (n = 20)		Control group (n = 18)	
	Before treatment	After treatment	Before treatment	After treatment
Severity of clinical manifestations of CVI, the average score (VCSS scale)	5.6 ± 0.5	2.4 ± 0.7*	5.7 ± 0.5	3.9 ± 0.7*
The degree of patient's discomfort, the average score in cm (VAS scale)	4.9 ± 2.1	0.3 ± 0.8*	5.5 ± 1.1	1.2 ± 1.4*

Note. * — $p < 0.05$ when comparing two values within a group.

Table 4. Peripheral lymphocyte subpopulations in patients with VV (C6) who received treatment with and without Phlogenzym

Parameter		Experimental group (n = 20)		Control group (n = 18)	
		Before treatment	After treatment	Before treatment	After treatment
$CD19^+$	%	7.7 ± 0.79	8.8 ± 0.9	9.0 ± 0.9	10.2 ± 1.3
$CD3^+$		73.1 ± 1.76	74.6 ± 1.9	69.1 ± 1.7	72.9 ± 1.6
$CD3^+CD4^+$		47.0 ± 2.2	48.4 ± 2.2	44.3 ± 2.4	47.0 ± 1.9
$CD3^+CD8^+$		24.7 ± 2.8	23.9 ± 1.7	22.2 ± 1.7	24.8 ± 2.1
$CD3^+CD25^+$		4.1 ± 0.3	3.4 ± 0.3	7.6 ± 1.2	5.4 ± 0.4
$CD4^+CD25^{+Bright}$		**3.2 ± 0.3**	**2.4 ± 0.2***	**2.4 ± 0.3**	**3.5 ± 0.3***
$CD4^+CD45RA$		16.8 ± 2.0	16.3 ± 2.1	15.8 ± 1.7;	20.4 ± 2.0*
CD45RA		**57.4 ± 1.8**	**54.0 ± 1.8***	**53.3 ± 2.5;**	**63.0 ± 2.3***
$CD4^+CD45RO^+$		**26.7 ± 1.6**	**30.9 ± 1.5***	25.9 ± 1.4	28.4 ± 1.1
$CD45RO^+$		**36.5 ± 1.9**	**44.8 ± 2.2***	38.6 ± 2.5	41.6 ± 2.0
$CD4^+CD45RA^+/ CD4^+CD45RO^+$		**0.7 ± 0.1**	**0.5 ± 0.1***	0.6 ± 0.1	0.7 ± 0.1
$CD45RA^+/CD45RO^+$		**1.6 ± 0.1**	**1.3 ± 0.1***	1.4 ± 0.1	1.5 ± 0.1

Note. The results are presented as M ± m. * — $p < 0.05$ when comparing two values within a group.

Phlogenzym, a statistically significant reduction in the number of regulatory $CD4^+CD25^{+Bright}$ T-cells and an increase in $CD4^+CD45RO^+$ memory cells.

The described lymphocyte subpopulations are associated with the inhibition of the synthesis of proinflammatory cytokines and the antigen-presenting function of dendritic cells and macrophages, cell apoptosis induction, decreased generation of type 1 and 2 T-helpers (Th1, Th2) and reduced cytokine production by the latter. This leads to a less effective immune response and contributes to the development of chronic inflammation [17]. An important advantage of memory T-cells that constantly circulate in all organs and tissues of the body even in the absence of any inflammation over naive T-lymphocytes is their ability to detect antigens and eliminate them long before they reach secondary lymphoid structures [18].

After treatment completion, multidirectional dynamics of Treg and memory T-cells content in blood were revealed in the experimental group that corresponded with the observed positive clinical changes. This indicates a more effective immune response in patients who received Phlogenzym.

CONCLUSIONS

The present study demonstrated positive effects of systemic enzyme therapy on the regenerative processes in damaged tissues and on the function of T-cell mediated immunity in patients with VV (C6). The results of this study validate the inclusion of immunomodulatory drugs into the treatment protocol. Immunomodulatory drugs contribute to the regression of clinical symptoms of CVI and accelerate the healing process of stage I-III VTU.

References

1. Lipnitskiy EM. Lechenie troficheskikh yazv nizhnikh konechnostey. Moscow: Meditsina; 2001. 160 p. Russian.
2. Saveliev VS, Kirienko AI, Bogachev VYu. [Chronic venous diseases in the Russian Federation. Results of VEIN CONSULT international research program]. Flebologiya. 2010. 4 (3): 9–12. Russian.
3. Bradbury AW. Epidemiology and aetiology of C4-6 disease. Phlebology. 2010 Oct; 25 Suppl 1: 2–8. DOI: 10.1258/phleb.2010.010s01.
4. Khanevich MD, Khrupkin VI, Shchelokov AL, Fominykh EM. Oslozhnennye formy khronicheskoy venoznoy nedostatochnosti nizhnikh konechnostey. Moscow: MedEkspertPress; Petrozavodsk: IntelTek; 2003. P. 53–8. Russian.
5. Kirienko AI. Flebologicheskie problemy klinicheskoy praktiki. Rossiyskie meditsinskie vesti. 2007; 12 (4): 53–6.
6. Assotsiatsiya flebologov Rossii, Vserossiyskoe obshchestvo khirurgov. Klinicheskie rekomendatsii po diagnostiki i lecheniyu khronicheskikh zabolevaniy ven. Flebologiya. 2009; 3 (3): 75.
7. Shval'b PG, Gryaznov SV, Shval'b AP. Nekotorye voprosy patogeneza troficheskikh yazv venoznogo proiskhozhdeniya. Angiologiya i sosudistaya khirurgiya. 2005; 11 (1): 61–4. Russian.
8. Korthuis RJ, Gute DC. Adhesion molecule expression in postischemic microvascular dysfunction: activity of a micronized purified flavonoid fraction. J Vasc Res. 1999; 36 Suppl 1: 15–23.
9. Lebedev KA, Ponyakina ID, Kozachenko NV. Physiology of chronic inflammatory processes and their treatment. Human Physiology. 2005; 31 (1): 86–97. DOI: 10.1007/s10747-005-0012-5.
10. Bergan JJ, Pascarella L, Schmid-Schönbein GW. Pathogenesis of primary chronic venous disease: Insights from animal models of venous hypertension. J Vasc Surg. 2008 Jan; 47 (1): 183–92. DOI: 10.1016/j.jvs.2007.09.028.
11. Kirienko AI, Ataullakhanov RI, Bogachev VYu, Bogdanets LI, Pichugin AV, Zhuravleva OV. Troficheskie yazvy venoznoy etiologii i ikh svyaz' s immunnym statusom. Angiologiya i sosudistaya khirurgiya. 2007; 13 (1): 76–86. Russian.
12. Powell CC, Rohrer MJ, Barnard MR, Peyton BD, Furman M, Michelson AD. Chronic venous insufficiency is associated with increased platelet and monocyte activation and aggregation. J Vasc Surg. 1999 Nov; 30 (5): 844–51.
13. Štvrtinova V, Jahnova E, Weissova S, Horvathova M, Ferenčik M. Expression of adhesion molecules on leukocytes of patients with chronic venous insufficiency. Phlebologie. 2002; 31 (3): 63–8.
14. Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Ann Vasc Surg. 2000 Sep; 14 (5): 427–35.
15. Kirienko AI, Koshkin VM, Bogachev VYu. Sistemnaya enzimoterapiya v angiologii. In: Materialy Vserossiyskoy nauchno-prakticheskoy konferentsii khirurgov. Vserossiyskaya nauchno-prakticheskaya konferentsiya khirurgov; Kaluga, 1997 Sep 26–27. Kaluga; 1996. P. 91. Russian.
16. Kirienko AI, Koshkin VM, Agafonov VF. Sistemnaya enzimoterapiya posstromboflebeticheskoy bolezni. In: Kriticheskaya ishemiya. Itogi XX veka. Materialy 3-go Mezhdunarodnogo kongressa severnykh stran i regionov; Petrozavodsk–Parizh, 1999. Petrozavodsk; 1999. P. 151. Russian.
17. ZHeleznikova GF. [Regulatory t cells in immune response to infection]. Journal Infectology. 2011; 3 (1): 6–13. Russian.
18. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. Science. 2001 Mar 23; 291 (5512): 2413–7.

Литература

1. Липницкий Е. М. Лечение трофических язв нижних конечностей. М.: Медицина; 2001. 160 с.
2. Савельев В. С., Кириенко А. И., Богачев В. Ю. Хронические заболевания вен в Российской Федерации. Результаты международной исследовательской программы VEIN CONSULT. Флебология. 2010. 4 (3): 9–12.
3. Bradbury AW. Epidemiology and aetiology of C4-6 disease. Phlebology. 2010 Oct; 25 Suppl 1: 2–8. DOI: 10.1258/phleb.2010.010s01.
4. Ханевич М. Д., Хрупкин В. И., Щелоков А. Л., Фоминых Е. М. Осложненные формы хронической венозной недостаточности нижних конечностей. М.: МедЭкспертПресс; Петрозаводск: ИнтелТек; 2003. С. 53–8.
5. Кириенко А. И. Флебологические проблемы клинической практики. Рос. мед. вести. 2007; 12 (4): 53–6.
6. Ассоциация флебологов России, Всероссийское общество хирургов. Клинические рекомендации по диагностики и лечению хронических заболеваний вен. Флебология. 2009; 3 (3): 75.
7. Швальб П. Г., Грязнов С. В., Швальб А. П. Некоторые вопросы патогенеза трофических язв венозного происхождения. Ангиол. и сосуд. хирургия. 2005; 11 (1): 61–4.
8. Korthuis RJ, Gute DC. Adhesion molecule expression in postischemic microvascular dysfunction: activity of a micronized purified flavonoid fraction. J Vasc Res. 1999; 36 Suppl 1: 15–23.
9. Лебедев К. А., Понякина И. Д., Козаченко Н. В. Физиология хронических воспалительных процессов и их лечение. Физиология человека. 2005; 31 (1): 100–13.
10. Bergan JJ, Pascarella L, Schmid-Schönbein GW. Pathogenesis of primary chronic venous disease: Insights from animal models of venous hypertension. J Vasc Surg. 2008 Jan; 47 (1): 183–92. DOI: 10.1016/j.jvs.2007.09.028.

11. Кириенко А. И., Атауллаханов Р. И., Богачев В. Ю., Богданец Л. И., Пичугин А. В., Журавлева О. В. Трофические язвы венозной этиологии и их связь с иммунным статусом. Ангиол. и сосуд. хирургия. 2007; 13 (1): 76–86.
12. Powell CC, Rohrer MJ, Barnard MR, Peyton BD, Furman M, Michelson AD. Chronic venous insufficiency is associated with increased platelet and monocyte activation and aggregation. J Vasc Surg. 1999 Nov; 30 (5): 844–51.
13. Štvrtinova V, Jahnova E, Weissova S, Horvathova M, Ferenčik M. Expression of adhesion molecules on leukocytes of patients with chronic venous insufficiency. Phlebologie. 2002; 31 (3): 63–8.
14. Takase S, Bergan JJ, Schmid-Schönbein G. Expression of adhesion molecules and cytokines on saphenous veins in chronic venous insufficiency. Ann Vasc Surg. 2000 Sep; 14 (5): 427–35.
15. Кириенко А. И., Кошкин В. М., Богачев В. Ю. Системная энзимотерапия в ангиологии. В сб.: Материалы Всероссийской научно-практической конференции хирургов. Всероссийская научно-практическая конференция хирургов; Калуга, 26–27 сентября 1996 г. Калуга; 1996. С. 91.
16. Кириенко А. И., Кошкин В. М., Агафонов В. Ф. Системная энзимотерапия посстромбофлебетической болезни. В сб.: Критическая ишемия. Итоги XX века. Материалы 3-го Международного конгресса северных стран и регионов; Петрозаводск–Париж, 1999 г. Петрозаводск; 1999. С. 151.
17. Железникова Г. Ф. Регуляторные Т-лимфоциты в иммунном ответе на инфекцию. Журнал инфектологии. 2011; 3 (1): 6–13.
18. Masopust D, Vezys V, Marzo AL, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. Science. 2001 Mar 23; 291 (5512): 2413–7.