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XENOGENEIC LYMPHOCYTIC RNA STIMULATES SKELETAL MUSCLE REGENERATION

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Objective: to find evidence of the existence of distant lymphocytic RNA control of physiological myogenesis as a way to control the muscle tissue regeneration process. **Materials and methods.** The study was conducted on male Wistar rats, n=33. In the first part of the experiment, 12 rats were subjected to regular 40-day physical activity (swimming), half of them were intraperitoneally injected 4 times with total RNA isolated from pig spleen lymphocytes at 30 days of age; 6 rats made up the intact control group. In histological preparations of different skeletal muscle groups, the width and cross-sectional area of muscle fibers, the area of nuclei, and the number of myocytes and myosatellite cells were evaluated. In the second part of the experiment, 15 intact rats were injected with the studied xenogeneic RNA and the amounts of ribonucleic acids in peripheral blood lymphocytes, spleen lymphocytes, and skeletal muscles were determined 2 hours and 24 hours after injection. **Results.** After the 40-day physical activity, the width of the fibers and the area of myocyte nuclei in the skeletal muscles increased; the absolute number of myosatellite cells and the area of their nuclei did not change. After administration of xenogeneic RNA in the trained rats, in addition to an increase in the thickness and cross-sectional area of muscle fibers, the absolute number of myosatellite cells in *m. biceps femoris*, in *m. triceps brachii*, and in *m. pectoralis major* increased 1.4-fold, 1.3-fold, and 1.4-fold, respectively; the area of myosatellite nuclei increased on average by 7%. In intact rats, two hours after xenogeneic RNA injection, the amount of RNA in skeletal muscles remained unchanged, it increased by 19% in spleen lymphocytes, and by 16% in peripheral blood lymphocytes. At 24 hours, the RNA amount in the lymphocytes remained significantly higher than the control values, while in the muscle tissue, it didn't differ from the control. **Conclusion.** Xenogeneic lymphocytic RNA stimulates physiological myogenesis by activating myosatellite cell proliferation.

Key words: myogenesis, lymphocytes, RNA, regeneration.

Lymphoid cells are actively involved in regulation of regenerative processes [1, 2], thus providing body reactivity or resistance in a changing external or internal environment. This includes tissue adaptive responses to increased stress. Restoration of skeletal muscle integrity after acute injury and its physiological regeneration with moderately pronounced contractile activity occurs due to proliferation and differentiation of myosatellite cells, committed progenitor cells located between the basal membrane and sarcolemma of the muscle fiber. Hormones, growth factors, and neurotransmitters, many of which are synthesized and secreted by lymphoid cells, are involved in regulation of these processes. In the last 10 years, evidence has emerged that after acute muscle injury, T helpers of different phenotypes emerge in muscle tissue already at the initial stages of repair morphogenesis, whose presence significantly accelerates myosatellite emergence from dormancy and is accompanied by formation of complete muscle fibers both in vivo and in vitro conditions [3, 4]. However, the authors noted that severity of regenerative response does not correspond to quantitative composition of the T cells that have come into the muscle tissue. They suggested that T lymphocyte regulation of myogenesis probably occurs not by contact but remotely through soluble bioactive substances produced by T cells.

The lymphoid system is part of the neuroimmunoendocrine regulatory circuit; it is able to inform body tissues about modulation of its own function, as well as about changes occurring in the organ 'served' by it [5]. The intermediaries in this intercellular signaling system, besides hormones, cytokines, and growth factors, are RNA molecules (extremely diverse in their functional properties) capable of copying and transporting information, regulating gene expression, and catalyzing chemical bond formation. There is evidence that total RNA isolated from morphogenetically active cells has a more pronounced pro-regenerative effect than the cells themselves [6]. Earlier we have proved that lymphocytic total RNA has no allogeneic and xenogeneic limitations; this is particularly so with total RNA isolated from human peripheral blood lymphocytes, stimulated bone marrow

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erythropoiesis in rats both in vivo and in vitro. The aim of the present study was to find evidence for the existence of remote lymphocytic RNA control of physiological myogenesis as a way to control muscle tissue regeneration.

MATERIALS AND METHODS

We used 33 male Wistar rats weighing 260–310 g. Experiment was performed in compliance with the principles of humanity set out in the European Community directive (86/609/EEC) and the Declaration of Helsinki, in accordance with Order No. 199H of the Russian Ministry of Health dated April 1, 2016 "On approval of rules of good laboratory practice". The animals were kept in standard cages (n = 6) with free access to water and food, at +24 ± 2 °C air temperature in a vivarium, in accordance with the SP 2.2.1.3218 rules. Daily ration of the animals consisted of specialized pelleted feed corresponding to the content of nutrients, vitamins and minerals of international standards and GOST R 50258-92 (Complete Feed for Laboratory Animals). All painful manipulations with the animals and their euthanasia by cervical dislocation were performed under ether anesthesia in a room separate from the vivarium.

The first part of the experiment used 18 animals, which were divided into 3 groups of 6 animals each. Group 1 consisted of intact rats, group 2 was the control rats participating in the training process for 6 weeks, and group 3 included rats participating in the training process for 6 weeks and receiving total RNA injections. The training process was a swimming load in a 200 L tank, the water layer was 0.5 m thick, and the water temperature was +22–23 °C. The rats were placed in the tank 3 times a week, and duration of exercise was increased every week by 5 minutes (from 30 to 55 minutes) [7]. The lymphocytes, the sources of morphogenetically active xenogeneic RNA, were isolated by suspending the spleen of a 30-day old piglet in a glass homogenizer, then filtering the suspension through a capron filter and centrifuging the cells 3 times in sterile 0.9% NaCl solution. Total RNA was isolated by guanidine thiocyanate-phenol-chloroform extraction. The concentration of isolated RNA was determined spectrophotometrically by the optical density of the preparation at 260 nm wavelength. The resulting total RNA was lyophilized and stored in sterile vials at +5 °C. Prior to injection, lyophilised RNA was dissolved in sterile 0.9% NaCl solution. RNA was injected intraperitoneally into rats through sterilizing filtration (sterile syringe nozzles with 0.22 μm pore diameter). A total of 4 injections were given to each group 3 rat once a week. The RNA dose at each injection was 30 μg/100 g body weight, and the volume of the injected solution was 0.5 ml. To assess the morphofunctional state of the skeletal muscle tissue, the preparations were fixed with 10% neutral formalin. After standard histological tracing, preparation of paraffin blocks and slicing, the preparations were stained with hematoxylin and eosin. The stained preparations were studied using a LEICA DMRXA microscope (Germany), a digital video camera LEICA DFC 290 (Germany), connected to a personal computer, obtaining images of micropreparations in *TIFF files in the RGB colour space. Licensed version of ImageScope M image analysis software (Russia) was used for morphometric studies. Myosatellite and myocyte counts were measured at 400× magnification (40× lens; 10× eyepiece). The area of cell nuclei was determined using the "manual selection" function at 1000× magnification.

In the second part of the experiment, 15 animals were divided into 3 groups. Five intact rats constituted the control group and were intraperitoneally injected with 0.5 ml of 0.9% NaCl solution. 10 rats were intraperitoneally injected with 30 μg/100 g weight of xenogeneic total RNA dissolved in 0.5 ml of 0.9% NaCl solution. Then five individuals were then withdrawn from the experiment 2 hours after RNA administration, the remaining 5 individuals were withdrawn after 24 hours. In all 15 rats, total RNA was isolated from peripheral blood lymphocytes (isolated by centrifugation in ficoll-verogran solution with density gradient 1.119 g/cm^3, PanEco, Russia), spleen lymphocytes and pectoralis major tissue by guanidine thiocyanate-phenol-chloroform extraction and its amount was measured spectrophotometrically by optical density of the preparation at 260 nm wavelength.

Statistical data processing was performed using a licensed software package: Excel 2020 and PAST version 4.03. Mann–Whitney non-parametric method was used to assess the significance of differences between the groups. Data are presented as arithmetic mean and its error (M ± m). Differences $p \leq 0.05$ were considered statistically significant.

RESULTS

The dynamic characteristics of swimming movements in land rodents are such that the greatest paddling efforts are provided by the biceps femoris, pectoralis major and triceps brachii muscles. Histomorphological examination of these muscles revealed that regular moderate-intensity physical activity leads to hypertrophy of muscle fibres but is not accompanied by regenerative hyperplasia. After a 40-day training process, muscle fibre width and myocyte nuclear area increased in all skeletal muscles studied (Table). At the same time, the number of mature muscle cells decreased significantly in the biceps femoris, despite pronounced contractile activity and tension during swimming. These results are consistent with those obtained by other researchers, who found partial fragmentation and focal globular necrosis of myocytes in the muscle tissue of rodents subjected to regular swimming exercise [8]. The cellular form of muscle tissue regeneration under regular moderate-intensity physical activity was absent: absolute number of myosatellite cells and the size of their nuclei in all the studied muscles did not differ from those of intact animals.

Injection of xenogeneic total RNA was accompanied by both a significant increase in indicators characterizing the development of a hypertrophic muscle tissue regeneration, and appearance of signs of cellular hyperplasia in the muscle tissue experiencing regular physical activity (Table). In experimental rats injected with RNA, the cross-sectional area of muscle fibres increased 1.2-fold on average, and the thickness of the fibres increased by 8–10%. Visually, the fibres were more densely and compactly arranged (Fig. 1). In the biceps femoris, RNA injection allowed the number of actively functioning mature muscle cells to remain at the physiological level (myocyte count in group 3 rats did not differ from that registered in intact animals).

Obviously, under the influence of exogenous lymphocytic RNA, the mature myocyte pool started replenishing due to proliferation of myosatellite cells: in all the studied striated muscles, the count of these committed progenitor cells and their nuclei sizes increased as compared to those of the control group rats that received no lymphocytic RNA. Myosatellite cells were easily visualized in histological preparations (Fig. 1): they had rounded large light nuclei with evenly distributed chromatin in the form of large clumps and small dust-like inclusions.

In healthy young rodents living under laboratory conditions, the proportion of myosatellite cells in striated muscles does not exceed 25%. After regular forced swimming, the proportion of young proliferating muscle cells in the fore and hind limb muscles in trained animals was significantly higher in percentage terms than in intact rats (Fig. 2), although the absolute count of myosatellite cells in the skeletal muscles did not change (Table). This relative increase in the proportion of myogenic progenitor cells in the trained animals was associated with decrease proportion of mature myocytes resulting from the regular damaging effects of physical exercise. Against the background of xenogeneic lymphocytic RNA administration, the proportion of muscle progenitor cells increased more markedly, and its increase was observed not only in the limb muscles, but also in the pectoralis major. An increase in the percentage of myosatellite cells in trained animals receiving lymphocytic RNA was coupled with an absolute increase in the number of these cells. Thus, compared with group 2, in group 3 rats, the myosatellite cell count in biceps femoris increased 1.4-fold, in triceps brachii – 1.25-fold, in the pectoralis major – 1.43-fold (Table). The increased proliferative activity of myosatellite cells in group 3 rats was also evidenced by a marked increase in the size of the nuclei of these cells.

The radar chart demonstrating the severity of signs of hypertrophic and hyperplastic regenerative processes in specific skeletal muscles (Fig. 3) after administration of lymphocytic RNA shows that both types of regenerati-

Table

Effect of moderate-intensity physical activity and xenogeneic lymphocytic RNA on physiological myogenesis

Indicators	Intact rats (group 1), n = 6	Physical activity (swimming)	
		Control (group 2), n = 6	RNA injection (group 3), n = 6
M. biceps femoris			
Muscle fiber width (μm)	26.1 ± 1.4	31.1 ± 0.7*	34.6 ± 2.5*▲
Muscle fiber cross-sectional area (μm^2)	1450.0 ± 58.6	1514.3 ± 41.7	2009.0 ± 127.9*▲
Myosatellite cell count (cells/mm^2)	275.9 ± 22.7	302.2 ± 14.9	423.1 ± 28.6*▲
Area of myosatellite nuclei (μm^2)	30.7 ± 0.6	31.0 ± 0.9	32.2 ± 1.7
Myocyte count (cells/mm^2)	1058.4 ± 46.5	877.2 ± 36.9*	1001.2 ± 64.4
Area of myocyte nuclei (μm^2)	14.7 ± 0.9	20.9 ± 0.8*	19.6 ± 1.1*
M. pectoralis major			
Muscle fiber width (μm)	25.0 ± 1.0	30.4 ± 0.9*	32.6 ± 1.9*▲
Muscle fiber cross-sectional area (μm^2)	1404.6 ± 77.2	1470 ± 27.5	1786.2 ± 92.3*▲
Myosatellite cell count (cells/mm^2)	265.8 ± 14.9	280.7 ± 11.3	399.3 ± 23.2*▲
Area of myosatellite nuclei (μm^2)	29.9 ± 0.9	30.5 ± 1.3	32.0 ± 1.6*▲
Myocyte count (cells/mm^2)	791.4 ± 40.5	785.5 ± 32.2	965.4 ± 51.3*▲
Area of myocyte nuclei (μm^2)	14.2 ± 0.6	17.7 ± 0.7*	17.3 ± 0.5*
M. triceps brachii			
Muscle fiber width (μm)	26.7 ± 0.6	30.8 ± 0.8*	33.5 ± 2.0*▲
Muscle fiber cross-sectional area (μm^2)	1632.7 ± 54.1	1745.8 ± 82.9	1815.0 ± 65.9
Myosatellite cell count (cells/mm^2)	292.6 ± 20.3	327.8 ± 13.7	411.1 ± 31.6*▲
Area of myosatellite nuclei (μm^2)	31.11 ± 1.05	32.24 ± 0.83	33.99 ± 1.47*▲
Myocyte count (cells/mm^2)	910.6 ± 33.4	923.7 ± 33.4	977.4 ± 50.1
Area of myocyte nuclei (μm^2)	17.1 ± 0.6	20.8 ± 0.6*	17.4 ± 0.7▲

Notes. * – differences between groups 2, 3 and group 1 ($p < 0.05$); ▲ – differences between group 3 and group 2 ($p < 0.05$).

ve activity are almost equally evident in the hind limb muscles. In the forelimb muscles, regenerative response to RNA injection during exercise was weaker than in the other muscles studied, but hyperplastic processes associated with activation of satellite cell proliferation predominated. The greatest response to the lymphoid RNA signal was registered in the pectoralis major: under the influence of xenogeneic lymphocytic RNA, muscle fibers increased their size by 21%, and the myosatellite cell count in these muscles increased by 42%. Probably, this phenomenon was associated with both the initial relatively higher proportion of myosatellite cells in *m. pectoralis major* compared to the limb muscles (Fig. 2) and with increased load on the pectoral muscles when swimming.

When studying the in vivo properties of any exogenous bioactive substances, the question always arises as to how exactly these substances perform their regulatory functions. It is currently unknown which cells in the body are the target for exogenous morphogenetically active lymphocyte RNA, i.e. whether it acts directly on the tissue or transmits an information signal to recipient T lymphocytes, which, in turn, activate or inhibit cell proliferation and differentiation. Within the existing concept of lymphoid RNA regulation of repair processes [5, 9], we suggest that morphogenetically active lymphocytic RNA acts on cells of other, non-lymphoid organs indirectly, through T lymphocytes committed to a particular tissue or organ. Therefore, in the second part of the experiment, we evaluated the dynamics of total RNA content in peripheral blood lymphocytes, spleen lymphocytes and muscle tissue of intact animals after administration of xenogeneic lymphocytic RNA (Fig. 4). Even a day the introduction of exogenous RNA into the animals, the content of total RNA in the striated muscle tissue did not change, i.e. exogenous lymphocytic RNA did not penetrate into myocytes. In contrast to muscle cells, peripheral blood lymphocytes and spleen lymphocytes

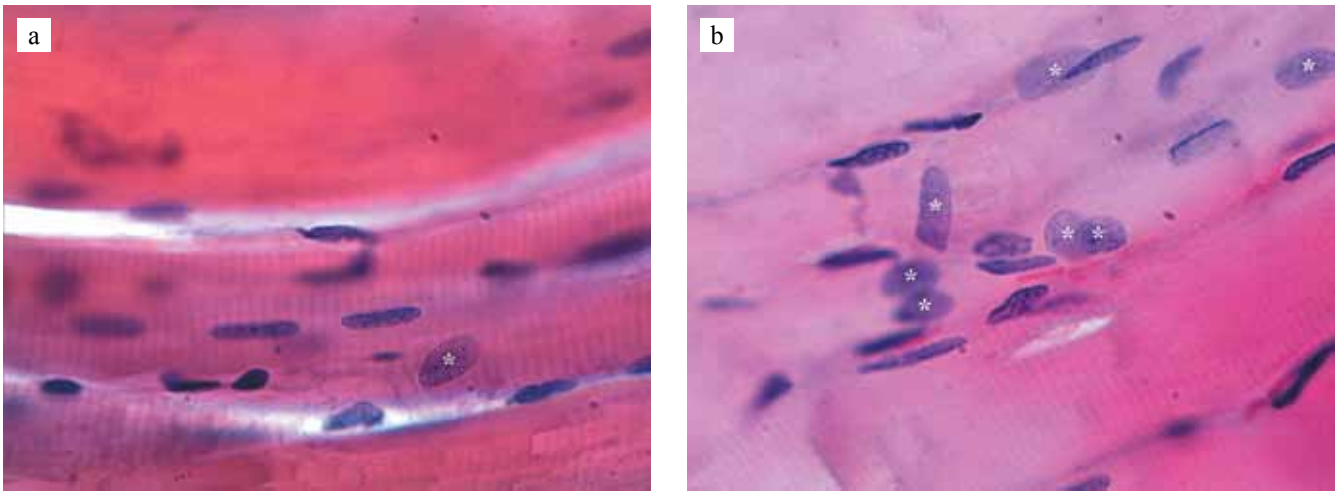

Fig. 1. Myosatellite cells (nuclei*) in the transverse striated muscle fiber of the *m. biceps femoris*: (a) intact muscle (group 1), (b) muscle after injection of lymphocytic RNA against the background of regular load of moderate intensity (group 3). H&E staining, 1000× magnification, oil immersion

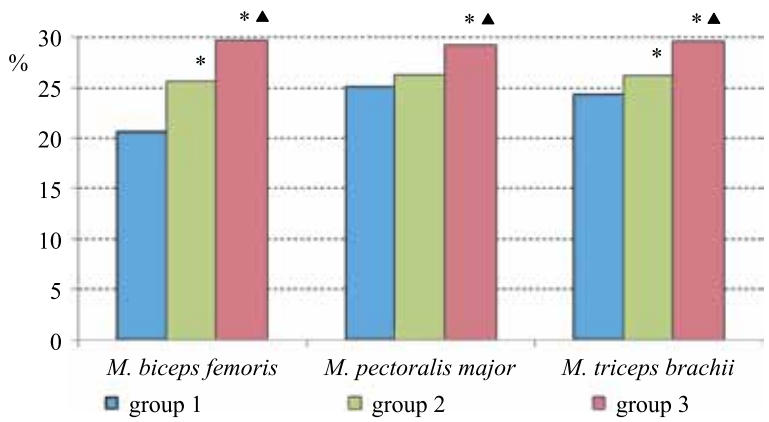

Fig. 2. Percentage of myosatellite cells in the muscles of intact rats (group 1), in the muscles of rats after regular exercise (group 2), in the muscles of rats after regular physical activity and injection of xenogeneic lymphocytic RNA (group 3). * – differences between groups 2, 3 and group 1 ($p < 0.05$), ▲ – differences between groups 2 and 3 ($p < 0.05$)

appeared to actively absorb exogenous RNA for at least 2 hours after injection. By 24 hours after lymphocyte RNA injection, RNA content in peripheral blood and spleen lymphocytes decreased, but remained significantly higher than the control level.

DISCUSSION

The fact that allogeneic and xenogeneic RNA can rapidly penetrate into lymphoid cells has long been known: 3% of RNA, isolated from the peripheral blood lymphocytes of healthy subjects or patients with chronic lymphoblastic leukemia, was found inside allogeneic lymphocytes and lymphoid cells of mice spleen after 3 minutes of incubation, and after 15 minutes, 8% of exogenous RNA was already inside these cells [10]. A slight decrease in specific radioactivity of the cells after repeated washing and treatment with pancreatic ribonuclease indicated that exogenous RNA was indeed absorbed by lymphocytes instead of being adsorbed on their membrane. Homologous total RNA penetrated into lymphoid cells of the spleen just as quickly, stimulating their synthetic activity [11]. Current reports suggest that the main players in lymphoid regulation of myogenesis are T helper cells, namely regulatory T cells (Treg), which are found in large numbers around regenerating muscle fibers [12, 13]. It is assumed that this is a special population of Treg that proliferates not in the lymph nodes and spleen, but at the muscle tissue damage site. A special feature of these "muscle-type" Treg cells is their ability to synthesize growth factor amphiregulin. Since amphiregulin-producing T cells have been found among T lymphocytes in the spleen, it is possible that

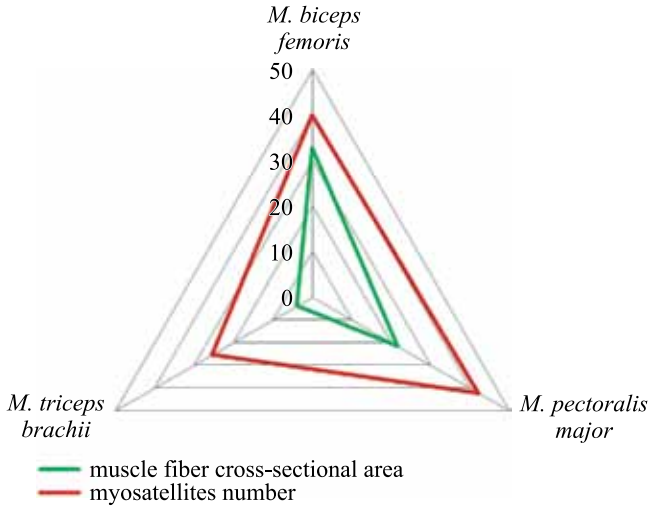

Fig. 3 Severity of hypertrophy and hyperplasia in different skeletal muscles after injection of xenogeneic lymphocytic RNA under conditions of regular physical activity (as a percentage of control values)

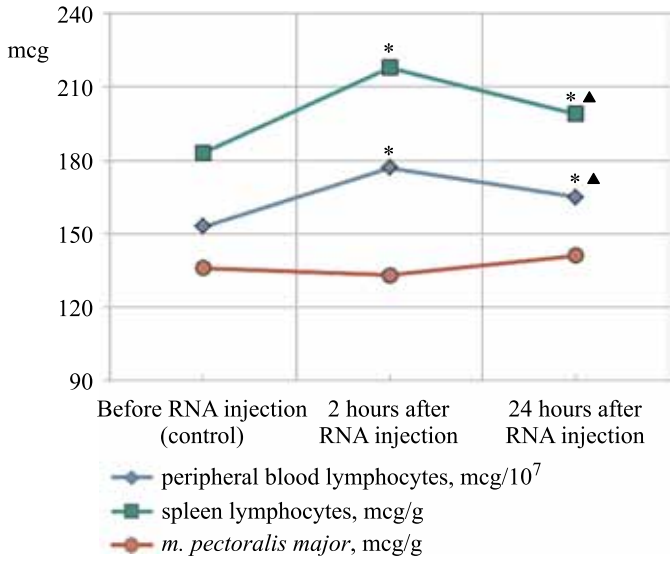

Fig. 4 Dynamics of total RNA content in tissues of intact rats after injection of xenogeneic lymphocytic RNA. * – differences from control ($p < 0.05$), ▲ – differences between the indicators obtained 2 hours and 24 hours after RNA injection ($p < 0.05$)

these already "trained" T cells migrate from the spleen to the injured muscle. To date, it has been proven that Tregs "serving" a particular muscle through amphiregulin directly enhance proliferation and differentiation of myosatellite cells in vivo and in vitro, while the regenerative potential of myosatellite cells is significantly reduced in Treg-deprived mice [12]. In addition, memory T cells, which start expressing specific myoregulatory proteins when repeated muscle damage occurs, have been found in laboratory rodents [14].

The results we arrived at provide evidence that the signal for enhanced proliferation of a tissue-specific clone of regulatory T lymphocytes located in muscles can be transmitted remotely via RNA molecules. First, we used animal lymphocytes during the active histogenesis period (weight of piglets increases 8-fold during the first month of life) as a source of morphogenetically active RNA.

Secondly, injection of xenogeneic morphogenetically active lymphocytic RNA into experimental rats not only promoted sarcoplasmic and myofibrillar hypertrophy of muscle fibers, but also stimulated cell regeneration, accompanied by increased myosatellite cell count in the striated muscle tissue. Thirdly, after RNA injection in the muscle tissue itself, the amount of ribonucleic acids did not change, but significantly increased in both spleen and peripheral blood lymphocytes. Most likely, microRNA molecules, which are not degraded by plasma and tissue RNAases, mediate this remote RNA signaling from lymphoid cells to skeletal muscle.

Both regenerative functions of different miRNAs and miRNA spectra secreted by different types of T lymphocytes are being actively studied worldwide, and there is every reason to believe that the RNA mechanisms of regenerative lymphocyte information transmission will be deciphered in the near future. The first step on this path has already been taken. In 2011, the microRNA-223 family was discovered in T lymphocytes [15], and in 2020, microRNA-223-3p was shown to be activated at an early stage of skeletal muscle regeneration after damage, and knockout of the gene encoding this microRNA synthesis leads to increased inflammation, inhibition of regeneration and development of interstitial fibrosis in the striated muscle tissue [16].

It was also found that microRNA-27 intensively expressed in T-lymphocytes [17] suppresses myostatin synthesis, a protein inhibiting myosatellite proliferation [18]. Therefore, increased microRNA-27 expression in T cells in muscle tissue injury may be one of the mechanisms by which T lymphocytes stimulate myogenesis.

The efficacy of specifically xenogeneic lymphocytic RNA suggests that T-lymphocytic control of the effector link of the motor system is a universal mechanism of regulation of the functional state of muscle tissue in all mammals, because the ability to move in space is a vital function necessary for realization of the motivation to find food or a mate. Further study on lymphocyte regulation of myogenesis is a top priority for medicine, since creation of xenogeneic RNA preparations suitable for use in regenerative medicine may become one of the advanced methods of subcellular therapy in muscular dystrophies, as well as other diseases associated with skeletal muscle conditions.

The authors declare no conflict of interest.

REFERENCES

1. *Tishevskaya NV, Gevorkyan NM, Kozlova NI*. The role of T-lymphocytes in the hormonal regulation of morphogenetic processes. *Uspekhi sovremennoj biologii*. 2015; 135 (2): 189–202.
2. *D'Alessio FR, Kurzhagen JT, Rabb H*. Reparative T lymphocytes in organ injury. *J Clin Invest*. 2019; 129: 2608–2618. doi: 10.1172/JCI124614.
3. *Fu X, Xiao J, Wei Y, Li S, Liu Y, Yin J et al*. Combination of inflammation-related cytokines promotes long-term muscle stem cell expansion. *Cell research*. 2015; 25 (6): 655. doi: 10.1038/cr.2015.58.
4. *Kwee BJ, Budina E, Najibi AJ, Mooney DJ*. CD4 T-cells regulate angiogenesis and myogenesis. *Biomaterials*. 2018; 178: 109–121. doi: 10.1016/j.biomaterials.2018.06.003.
5. *Tishevskaya NV, Babaeva AG, Gevorkyan NM*. The role of lymphocytic RNA in intercellular information exchange and regulation of regenerative processes. Rossiyskiy fiziologicheskiy *Zhurnal im. I.M. Sechenova*. 2016; 102 (11): 1280–1301.
6. *Gonikova ZZ, Nikolskaya AO, Kirsanova LA, Shagidulin MYu, Onishchenko NA, Sevastyanov VI*. The comparative analysis of the effectiveness of stimulation of liver regeneration by bone marrow cells and total RNA of these cells. *Vestnik transplantologii i iskusstvennykh organov*. 2019; 21 (1): 113–121. doi: 10.15825/1995-1191-2019-1-113-121.
7. *Gallyamutdinov RV, Astahova LV, Golovneva ES, Serysheva OYu*. Vliyanie lazernogo infrakrasnogo izlucheniya na nekotorye morfofunkcional'nye pokazateli regeneriruyushchej skeletnoj myshcy v vozrastnom aspekte. *Lazernaya medicina*. 2020; 24 (2–3): 90–94. doi: 10.37895/2071-8004-2020-24-2-3-90-94.
8. *Khabibulin RM, Bakirova AU, Khabibulin ГМ, Akhmadullina ET*. Blood biochemical parameters and morphological changes of muscle tissue in mice after exercise on the application of rhaponticum carthamoides. *Uchenyye zapiski Kazanskoy gosudarstvennoy akademii veterinarnoy meditsiny im. N.E. Baumana*. 2019; 238 (2): 215–219. doi: 10.31588/2413-4201-1883-238-2-215-220.
9. *Tishevskaya NV, Gevorkyan NM, Kozlova NI*. A modern view of the role of T-lymphocytes in regulation of erythropoiesis. *Uspekhi sovremennoy biologii*. 2016; 136 (1): 83–96.
10. *Blinov MN, Luganova IS, Vladimirova AD*. Vklyucheniye ekzogennoy RNK v leykotsity cheloveka. *Problemy gematologii i perelivaniya krovi*. 1981; 26 (1): 38–40.
11. *Wang SR, Giacomoni D, Dray S*. Physical and chemical characterization of RNA incorporated by rabbit

spleen cells. *Exp Cell Res*. 1973; 78 (1): 15–24. doi: 10.1016/0014-4827(73)90032-3.

12. *Burzyn D, Kuswanto W, Kolodin D, Shadrach JL, Cerletti M, Jang Y et al*. A special population of regulatory T-cells potentiates muscle repair. *Cell*. 2013; 155 (6): 1282–1295. doi: 10.1016/j.cell.2013.10.054.
13. *Li J, Tan J, Martino MM, Lui KO*. Regulatory T-Cells: Potential Regulator of Tissue Repair and Regeneration. *Front Immunol*. 2018; 9: 585. doi: 10.3389/fimmu.2018.00585.
14. *Tidball JG*. Regulation of muscle growth and regeneration by the immune system. *Nat Rev Immunol*. 2017; 17 (3): 165–178. doi: 10.1038/nri.2016.150.
15. *Grigoryev YA, Kurian SM, Hart T, Nakorchevsky AA, Chen C, Campbell D et al*. MicroRNA regulation of molecular networks mapped by global microRNA, mRNA, and protein expression in activated T lymphocytes. *J Immunol*. 2011; 187 (5): 2233–2243. doi: 10.4049/jimmunol.1101233.
16. *Cheng N, Liu C, Li Y, Gao S, Han YC, Wang X et al*. MicroRNA-223-3p promotes skeletal muscle regeneration by regulating inflammation in mice. *J Biol Chem*. 2020; 295 (30): 10212–10223. doi: 10.1074/jbc.RA119.012263.
17. *Kuchen S, Resch W, Yamane A, Kuo N, Li Z, Chakraborty T et al*. Regulation of microRNA expression and abundance during lymphopoiesis. *Immunity*. 2010; 32 (6): 828–839. doi: 10.1016/j.immuni.2010.05.009.
18. *Wang XH*. MicroRNA in myogenesis and muscle atrophy. *Curr Opin Clin Nutr Metab Care*. 2013; 16 (3): 258–266. doi: 10.1097/MCO.0b013e32835f81b9.

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