IMMUNOLOGY AND MICROBIOLOGY

Effect of Regular Physical Training on Hemopoiesis in Experimental Animals

O. T. Kudaeva, O. P. Kolesnikova, T. G. Sukhenko, and V. A. Kozlov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 2, pp. 183-187, February, 2012
Original article submitted October 13, 2010

Regular physical activity of moderate intensity over a long period (swimming at water temperature of 33-35°C without load, 2 h per day, 5 days a week for 4 months) activated hemopoiesis in mice: stimulation of myelopoiesis in the bone marrow and increase in the percentage of erythropoietic elements in the spleen were observed. In the peripheral blood, the relative content of lymphocytes increased, that of granulocytes decreased, and plasma cells appeared. Stimulation of erythropoiesis in the spleen can be partially responsible for suppression of immune responses during physical exercise.

Key Words: *physical activity; hemopoiesis; spleen; plasma cells*

Long-term intense exercise can produce multidirectional effects on the immune system; in many cases, suppression of immune processes occurs [2,5,9]. In our previous animal experiments, regular physical exercise (swimming for 2 h without load at water temperature 33-35°C 5 days a week for 4 months) significantly modulated functional characteristics of the immune system: reduced spontaneous and mitogen-induced proliferation of splenocytes and inhibits cellular and humoral immune response to T-dependent antigen [3]. Increased physical activity is accompanied by increased oxygen demand in working muscles, which necessarily affects the state of the hemopoiesis in long-term regular training. A shift of balanced hemopoiesis processes towards preferential stimulation of erythropoiesis can be a possible mechanism of the inhibitory effect of physical exercise on the immune response.

Here we studied the status of hemopoiesis in experimental animals subjected to physical exercise inducing suppression of the immune responses.

Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** olga_kudaeva@mail.ru. O. T. Kudaeva

MATERIALS AND METHODS

Experiments were performed on 2-month-old male and female (CBA×C57Bl/6)F_1 hybrid mice (Rassvet nursery, Tomsk). The animals were kept in accordance with rules adopted by the European Convention for the Protection of Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Swimming was chosen as a model of physical training in regimen leading to suppression of immune reactivity in experimental animals (swimming without load in a tank 60×30×20 cm^3 at water temperature of 33-35°C, 2 h per day, 5 days a week for 4 months) [3]. To minimize the stress effect, swimming duration was gradually increased over 2 weeks. Water temperature did not allow overcooling of animals, which was controlled by thermometry. As mice are capable to swim at the specified water temperature for more than 6 hours, the exercise intensity was moderate. Long duration of the experiments (4 months) allowed animal adaptation to the procedure. Under the specified experimental conditions, blood level of corticosterone in experimental animals did not significantly increase,

0007-4888/12/1532-0217

which attested to the absence of significant stress factors [3].

The control group comprised intact sex- and age-matched animals of the same genotype. Cell counts in the femoral bone marrow (BM), spleen, and peripheral blood (PB) were determined. BM smears, impression smears from spleen, and PB smears were made to determine differential cell counts. Organs, tissues, and cells were isolated by standard methods. Blood smears were stained after Romanowsky–Giemsa and analyzed routinely. BM smears and impression smears of the spleen were stained with azure II-eosin by the Pappenheim–Kryukov method and analyzed routinely.

Hemoglobin content in BP was determined by hemichrome method using Hemoglobin-Novo test system (Vector-Best). The results were expressed in mg/ml.

The data were processed by methods of nonparametric statistics; differences were significant at $p<0.05$.

RESULTS

Hemoglobin content in PB increased in both male and female mice: to 163.5 mg/ml (*vs.* 143.3 mg/ml in the control, $p<0.05$) in males and to 168.6 mg/ml (*vs.* 144.8 mg/ml in the control, $p<0.05$) in females. Elevated hemoglobin content reflects appropriate response of the organism to regular training and attests to activation of erythropoiesis under conditions of increased oxygen demand in actively working muscles.

Cell composition of BM, spleen, and PB were determined in intact animals and after 4-month course of regular physical exercises. Long-term regular physical exercises induced no significant changes in cell counts in the BM, spleen, and PB. A minor increase in cell count in the spleen and PB observed in animals of both sexes did not attain the level of significance.

Analysis of cell composition of BM revealed changes in the relative content of cells of different hemopoietic lineages despite unchanged total cell number. Physical exercise led to activation of myelopoiesis in BM, while the content of erythroid cells decreased (Fig. 1). In contrast, in the spleen the relative content of erythropoietic elements increased (Fig. 2). Thus, increased oxygen demand in muscle tissue led to a shift of erythropoiesis from BM to the spleen, an active site of hemopoiesis in adult rodents, especially under the influence of negative factors on erythropoiesis [12,14].

Regular physical exercise also induced changes in the hemogram. The percentage of granulocytes

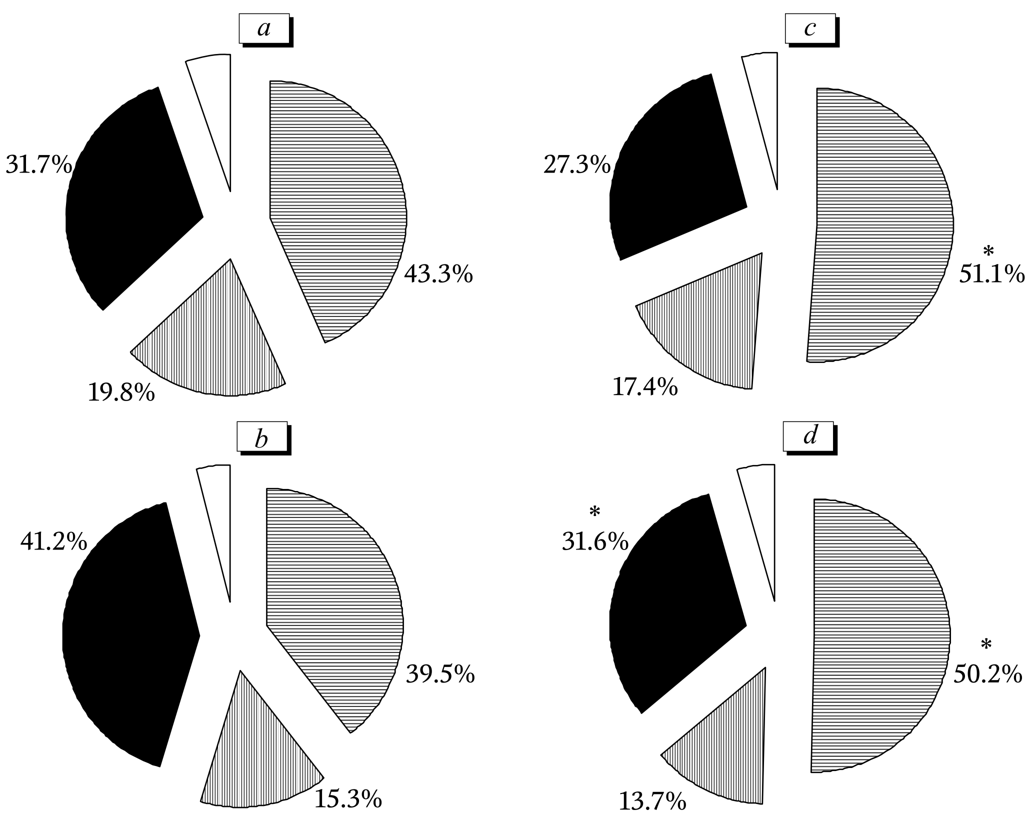

Fig. 1. Differential BM cell count in mice after long-term regular physical exercises (*M*; *n*=11). Here and in Fig. 2, 3: *a*, *b*) control group, males and females respectively; *c*, *d*) males and females after 4-month course of swimming, respectively. Dark sector: erythroid lineage cells; horizontal hatching: myeloid lineage cells; vertical hatching: lymphoid lineage cells; light sector: other cells. *$p<0.05$ in comparison with the control.

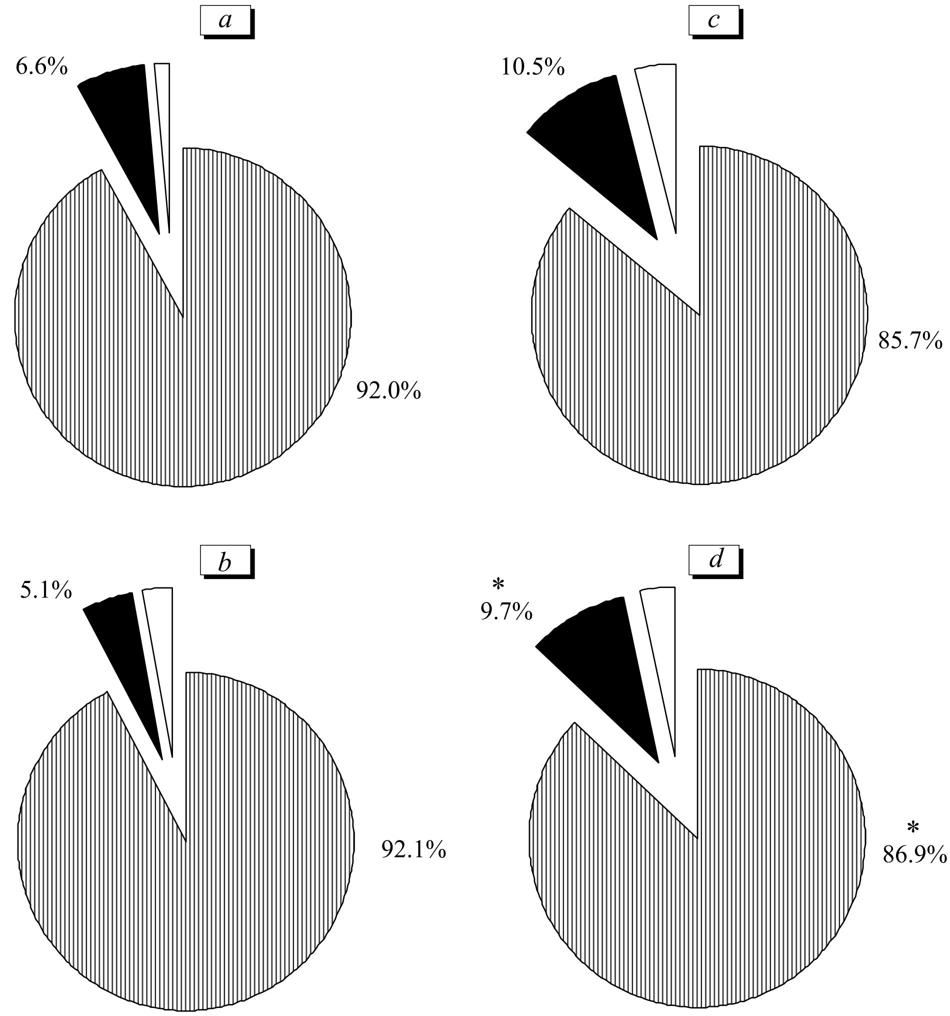

Fig. 2. Spleen cell count after long-term regular physical exercises (*M*; *n*=11). Dark sector: erythroid lineage cells; vertical hatching: lymphoid lineage cells; light sector: other cells.

decreased, the percentage of lymphocytes increased, plasma cells appeared in PB (Fig. 3).

The response of hemopoietic organs and PB to physical training demonstrated unidirectional changes in hemopoiesis in male and female mice, but in females they were more pronounced.

Reduced relative content of granulocytes in PB and, probably, activation of myelopoiesis in BM, can be associated with the development of inflammation in muscle tissue characteristic of actively working muscles, migration of granulocytic cells towards the site of inflammation and their rapid death in these areas [6]. Elevated levels of hemopoietic growth factors G-CSF and GM-CSF occurred at physical training support enhanced myelopoiesis processes [15].

The data on the effect of physical activity on the immune system of humans, as well as experimental animals are contradictory. The effect depends on the type, duration, and intensity of loads and immune parameters studied. In many cases, suppression of immune processes was observed [2,3,5,9]. The mechanisms of immunosuppression involving various elements of the immune system depend on various factors and often remain unclear.

Physical activity modulating the physiological condition of the body reduces or increases the content of many biologically active substances. Some of them have a direct effect on hemopoiesis and/or immune processes. It was shown that intensive physical activity is accompanied by increased levels of IL-6, a pleiotropic cytokine with a wide range of effects including regulation of immune processes, hemopoiesis, and inflammatory responses [1,4]. The influence of sex hormones on many parameters of the immune and hemopoietic systems has been clearly demonstrated in previous studies. They can mediate the effects of physical activity on the hemopoiesis and immune processes. Physical exercises are shown to increase the production of testosterone suppressing immune reactivity and stimulating erythropoiesis. Estrogens have a

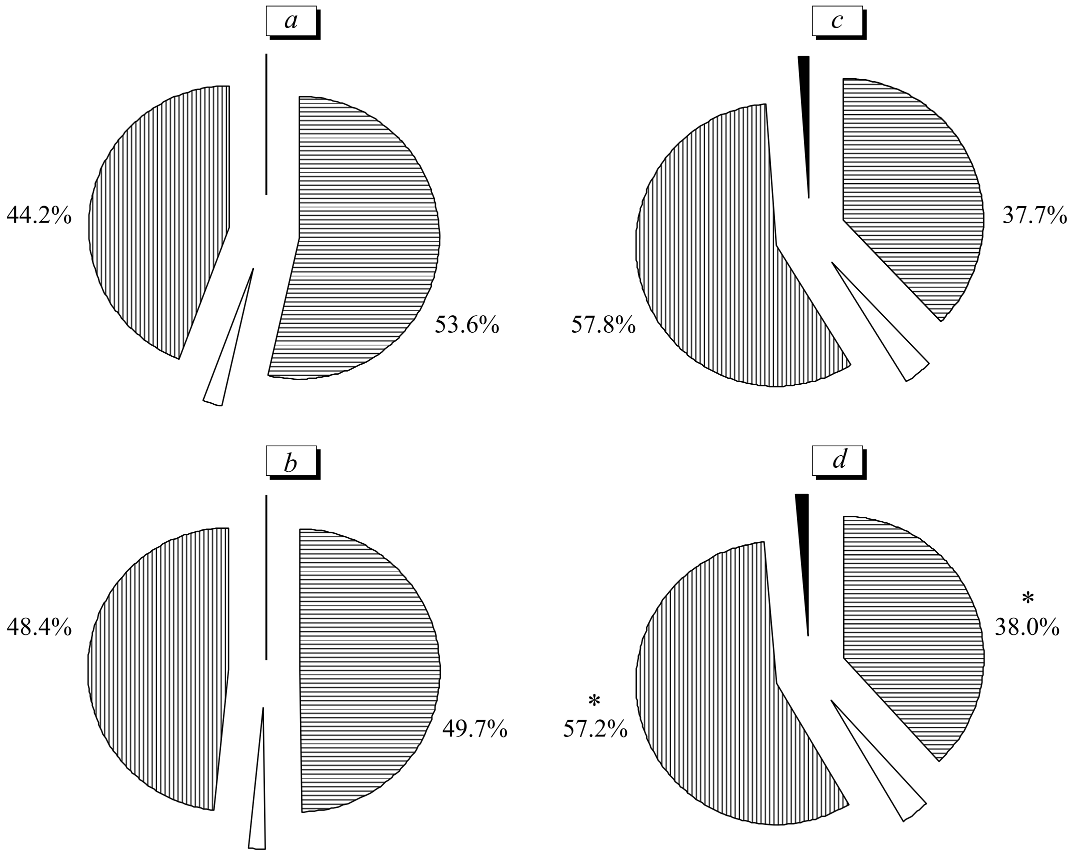

Fig. 3. Differential blood cell count in animals after long-term regular physical exercises (*M*; *n*=11). Dark sector: plasma cells; light sector: other cells; horizontal hatching: granulocytes; vertical hatching: lymphocytes.

stimulating effect on B-lymphocytes [7,11]; estrogen content is known to correlates with physical activity not only in females, but also in males.

Increased levels of estrogen and IL-6 during exercise can lead to polyclonal activation of B-lymphocytes, which is confirmed by the appearance of plasma cells in PB of experimental animals observed by us as well as by previously demonstrated increase in the spontaneous production of immunoglobulins by B-cells in the spleen and BM and elevated concentrations of immunoglobulins in PB against the background of regular physical activity over a long period [3]. It is known that polyclonal activation of B cells is accompanied by reduced intensity of the immune response [8,10]. Hence, phenomenon of polyclonal activation of B-lymphocytes during exercise can be a cause of immunosuppression under these conditions.

Another possible factor leading to suppression of the immune response during exercise can be stimulation of erythroid cells, which is confirmed by elevated hemoglobin concentration in PB of experimental animals. Immunosuppression can develop as a result of competitive relationships between cells of the erythroid and lymphoid lineages. In addition, activated erythroid cells can directly influence the immune response: bearing in mind the suppressive effect of erythroblasts on the development of humoral immune response, stimulation of erythropoiesis in the spleen can inhibit proliferation and differentiation of antigen-specific B cells [13].

Thus, long-term exercises of moderate intensity activate hemopoietic processes in experimental animals: stimulation of myelopoiesis in the bone marrow and increase in the content of erythropoietic elements in the spleen. In PB, the percentage of lymphocytes increased, that of granulocytes decreased, and plasma cells appeared. The suppression of immune reactivity observed during physical exercise can be explained by polyclonal activation of B-lymphocytes. In parallel, immunosupression during physical exercise can be caused by stimulation of erythropoiesis due to, first, direct suppressive effect of erythroblasts on B cells due to a shift of erythropoiesis from BM to the spleen, and secondly, by indirect effects of competitive interactions between cells of the erythroid, myeloid, and lymphoid lineages.

REFERENCES

1. A. E. Donnikov, M. Yu. Shkurnikov, E. B. Akimov, *et al.*, *Byull. Eksp. Biol. Med.*, **147**, No. 11, 573-576 (2009).

2. V. A. Kozlov and O. T. Kudaeva, *Med. Immunol.*, **4**, 427-438 (2002).
3. O. T. Kudaeva, O. P. Kolesnikova, I. N. Os'kina, *et al.*, *Immunologiya*, No. 2, 102-105 (2007).
4. C. P. Fischer, *Exerc. Immunol. Rev.*, **12**, 6-33 (2006).
5. M. W. Kakanis, J. Peake, E. W. Brenu, *et al.*, *Exerc. Immunol. Rev.*, **16**, 119-137 (2010).
6. P. Liao, J. Zhou, L. L. Ji, and Y. Zhang, *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **298**, No. 3, R599-R607 (2010).
7. J. T. Lightfoot, *Int. J. Biol. Sci.*, **4**, No. 3, 126-132 (2008).
8. R. Marques, I. Antunes, U. Eksmond, *et al.*, *Immunol.*, **181**, No. 5, 3432-3440 (2008).
9. H. Moir, M. Hughes, S. Potter, *et al.*, *J. Appl. Physiol.*, **108**, No. 5, 1284-1292 (2010).
10. C. L. Montes, E. V. Acosta-Rodriguez, M. C. Merino, *et al.*, *J. Leukoc. Biol.*, **82**, No. 5, 1027-1032 (2007).
11. M. P. Muehlenbein and R. G. Bribiescas, *Am. J. Hum. Biol.*, **17**, No. 5, 527-558 (2005).
12. J. M. Perry, O. F. Harandi, P. Porayette, *et al.*, *Blood*, **113**, No. 4, 911-918 (2009).
13. G. V. Seledtsova, V. I. Seledtsov, D. M. Samarin, *et al.*, *Immunol. Lett.*, **93**, Nos. 2-3, 171-178 (2004).
14. A. W. Suttie, *Toxicol. Pathol.*, **34**, No. 5, 466-503 (2006).
15. K. Suzuki, S. Nakaji, M. Yamada, *et al.*, *Exerc. Immunol. Rev.*, **8**, 6-48 (2002).