BIOGERONTOLOGY

Peculiarities of Bone Marrow Hemopoiesis in Early Aging OXYS Rats

I. A. Orlovskaya, L. B. Toporkova, N. A. Feofanova, and N. G. Kolosova*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 7, pp. 96-99, July, 2007
Original article submitted February 1, 2007

Colony-forming activity of bone marrow cells in 3- and 12-month-old Wistar rats does not differ by the number of early and erythroid precursors and by the formation of granulocyte-macrophage colonies. In senescence-accelerated OXYS rats, the number of early and erythroid precursors significantly increases by the age of 12 months and surpasses the corresponding values in Wistar rats. The number of granulocyte-macrophage colonies in OXYS rats does not change with age, but the numbers of these colonies formed at the age of 3 and 12 months in these animals are higher than in Wistar rats. As a result, the total number of hemopoietic colonies in 12-month-old OXYS rats 2-fold surpassed that in 12-month-old Wistar rats. Activation of granulopoiesis and increased numbers of early and erythroid precursors indicate deep changes in the functional status of the hemopoietic stem cell in 1-year-old OXYS rats in the direction characteristic of aging animals.

Key Words: *hemopoietic precursors; hemopoietic stem cell; early aging; OXYS rats*

Disorders in functional activity of hemopoietic stem cells often precede or parallel immunopathological conditions associated with aging: immunodeficiency, autoimmune diseases, and cancer. It was assumed that baseline hemopoiesis did not change with age in humans and animals or changed negligibly [1]. Recent studies showed that proliferative activity of hemopoietic stem cells increases during aging, but their migration and repopulating activities decrease and differentiation processes are disordered: the erythroid direction predominates, while the content of myeloid precursors decreases [13,15]. Recent findings persuasively prove close relationship between genetically determined differences in functional reserves of hemopoietic stem cells and life span [7,8].

Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences; *Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences, Novosibirsk

We previously showed that cataract, retinal dystrophy [2], arterial hypertension [5], osteoporosis [4], deviations in the cognitive and emotional spheres [9,12] in OXYS rats developed against the background of early involution of the thymus and reduced activity of T-cellular component of the immune system [3]. The function of hemopoietic stem cells during the period of active formation of signs of early aging was never studied in OXYS rats. Here we compared colony forming activities of bone marrow cells of OXYS and Wistar rats.

MATERIALS AND METHODS

The study was carried out on 3- (12 animals per strain) and 12-month-old (6 Wistar and 5 OXYS rats) male Wistar and OXYS rats. The animals received standard granulated fodder and had free access to water. In order to evaluate the number of com-

0007-4888/07/1441-086

mitted precursors, bone marrow cells in a concentration of 5×10^4/ml were incubated in 24-well plates in methylcellulose medium Metho Cult GF M 3434 (Stem Cell Technology) with stem cell factor, erythropoietin, IL-3, IL-6. Granulocyte-macrophage (CFU-GM), erythroid (BFU-E+CFU-E), and mixed (CFU-GEMM) colonies were counted under an inverted microscope after 14-day incubation at 37°C in a humid atmosphere with 5% CO_2.

The data were processed using ANOVA/MANOVA (Statistica, 6.0 software). The genotype and age were regarded as independent factors. The results are presented as $M\pm m$.

RESULTS

The age was inessential for the formation of CFU-GM in the bone marrow: their number was the same in Wistar and OXYS rats at the age of 3 and 12 months (Fig. 1). On the other hand, genetic differences were detected. In OXYS rats, the number of CFU-GM formed in the bone marrow was higher than in Wistar rats ($F_{1.28}$=19.3, p=0.0002): by 1.4 times in 3-month-old animals (p<0.002) and by 1.7 times in 12-month-old animals (p<0.037).

The number of early hemopoietic precursors (CFU-GEMM) depended on the genotype ($F_{1.29}$=20.2, p=0.0001) and age of animals ($F_{1.29}$=17.0, p=0.0003). However, the number of CFU-GEMM was the same in the bone marrow of Wistar and OXYS rats aged 3 months (Fig. 2, *a*). This parameter changed with time only in OXYS rats: the number of CFU-GEMM increased 8-fold (p<0.0005) in these animals and surpassed the corresponding value in Wistar rats (p<0.023).

Similar results were obtained in evaluation of BFU-E+CFU-E: this parameter was the same in Wistar and OXYS rats at the age of 3 months. By the age of 12 months, the number of BFU-E+CFU-E increased 2-fold in OXYS rats (p<0.0005) and did not change in Wistar rats (Fig. 2, *b*).

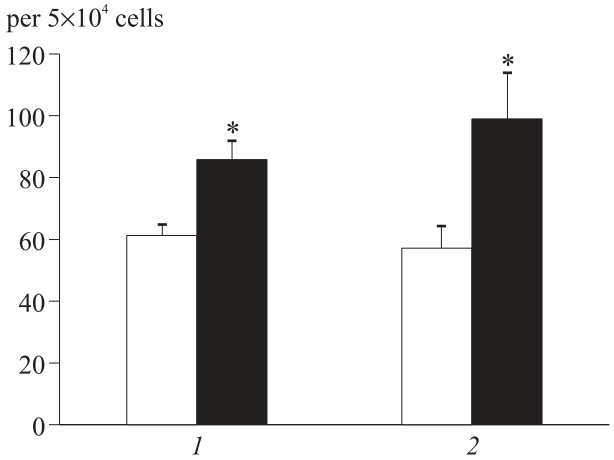

Fig. 1. Content of CFU-GM in the bone marrow of Wistar (light bars) and OXYS rats (dark bars) at the age of 3 (*1*) and 12 months (*2*). *p<0.05 compared to Wistar rats.

The detected changes led to shifts in the basal clonogenic activity (summary number of hemopoietic colonies; Fig. 3), which was higher in OXYS rats in comparison with Wistar rats at the age of 3 months (p<0.019) and 12 months (p<0.001).

Hence, the number of CFU-GM formed in OXYS rats is higher than in Wistars. Activation of the granulocyte-macrophagic stem, usually means compensatory replenishment of the granulocyte and macrophage pools during inflammatory processes and immune responses [9]. Phagocytic activity of neutrophils and monocyte/macrophages can increase with aging, but bactericidal activity decreases due to reduced capacity to "respiratory burst", production of proinflammatory cytokines increases,

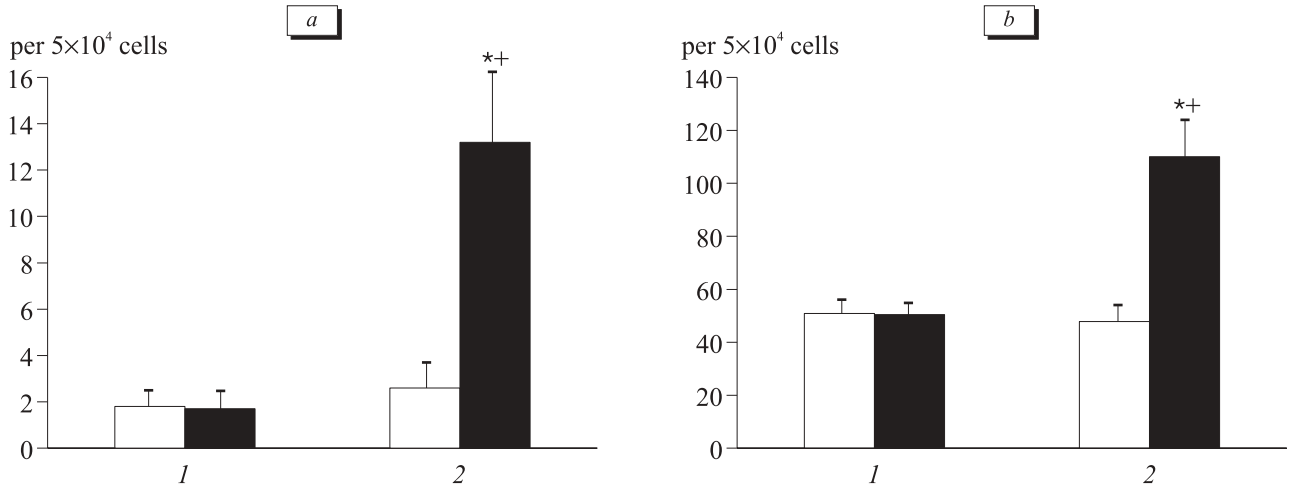

Fig. 2. Content of mixed (CFU-GEMM; *a*) and erythroid (BFU-E+CFU-E; *b*) precursors in the bone marrow of Wistar (light bars) and OXYS rats (dark bars) at the age of 3 (*1*) and 12 months (*2*). p<0.001 compared to: *Wistar rats, +3-month-old animals.

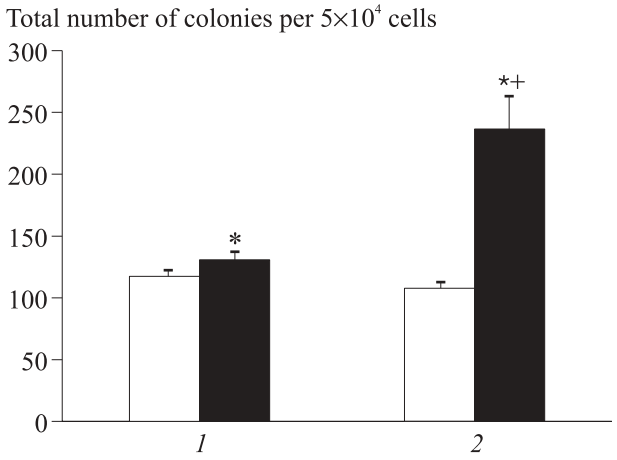

Fig. 3. Basal clonogenic activity of bone marrow hemopoietic precursors in 3- (*1*) and 12-month-old (*2*) Wistar (light bars) and OXYS rats (dark bars). $p<0.02$ compared to *Wistar rats, +3-month-old animals.

while that of antiinflammatory cytokines decreases [11]. These functions are normalized as a result of injection of recombinant granulocytic CSF, which is paralleled by a significant increase in the number of peripheral granulocytes [15]. By some parameters, the status and reactivity of the mononuclear phagocyte system in 3-month-old OXYS rats correspond to parameters in old animals: basal activity of inflammatory effector cells increases, while the capacity to respond to an extra stimulus decreases [3]. These results are in line with the fact of chronically hyperactive myelopoiesis in OXYS rats detected in our study.

Proliferation, differentiation, and maturation of hemopoietic precursors are closely related to their migration activity. Migration activity decreases with age, which presumably leads to an increase in the number of immature hemopoietic cells in the bone marrow of old animals. The excess of cells in the bone marrow can become a cause of "ineffective" hemopoiesis, when some early hemopoietic precursors are destroyed [12]. An appreciable increase in the number of early hemopoietic precursors (CFU-GEMM) and erythroid precursors were detected in the bone marrow of 12-month-old OXYS rats, while in Wistar rats their content was the same at the age of 3 and 12 months. This attests to increased proliferative activity of hemopoietic stem cells, which, according to some authors, is observed in aging animals and in diseases associated with aging [12]. Increased proliferative activity of hemopoietic stem cells promotes their erythroid differentiation and accumulation of erythroblasts in hemopoietic organs. It seems that accumulation of early erythroid precursors in the bone marrow and spleen can be a compensatory reaction limiting activity of immunocompetent cells. The expression of the majority of proinflammatory cytokines (IL-1, IL-3, EPO, IL-6) virtually does not change or even increases during aging [11]. This dysregulation can be responsible for senile phenotype of hemopoiesis with enhanced erythropoiesis. Presumably, the population of early hemopoietic precursors is involved in the formation of pathological phenotype in OXYS rats: the detected changes in the differentiation potentialities of hemopoietic stem cells (active granulopoiesis, increased content of early and erythroid precursors with age) indicate deep disorders in their functional activity, characteristic of aging animals.

The study was supported by the Russian Foundation for Basic Research (grant No. 05-04-48483).

REFERENCES

1. V. N. Anisimov, *Molecular and Physiological Mechanisms of Aging* [in Russian], St. Petersburg (2003).
2. N. G. Kolosova, P. A. Lebedev, A. Zh. Fursova, *et al.*, *Usp. Gerontol.*, No. 12, 143-148 (2003).
3. E. B. Markova, L. A. Obukhova, and N. G. Kolosova, *Byull. Eksp. Biol. Med.*, **136**, No. 10, 427-429 (2003).
4. O. V. Falameyeva, M. A. Sadovoi, Yu. V. Khrapova, and N. G. Kolosova, *Khir. Pozvon.*, No. 1, 88-94 (2006).
5. A. A. Bobko, S. V. Sergeeva, E. G. Bagryanskaya, *et al.*, *Biochem. Biophys. Res. Commun.*, **330**, No. 2, 367-370 (2005).
6. W. B. Ershler, *J. Am. Geriatr. Soc.*, **41**, No. 2, 176-181 (1993).
7. H. Geiger, G. Rennebeck, and G. Van Zant, *Proc. Natl. Acad. Sci. USA*, **102**, No. 14, 5102-5107 (2005).
8. E. Henckaerts, J. C. Langer, and H. W. Snoeck, *Blood*, **104**, No. 2, 374-379 (2004).
9. G. Jovcic, N. Stojanovic, M. Kataranovski, *et al.*, *Ann. Hematol.*, **66**, No. 4, 195-201 (1993).
10. N. G. Kolosova, T. V. Shcheglova, S. V. Sergeeva, and L. V. Loskutova, *Neurobiol. Aging*, **27**, No. 9, 1289-1297 (2006).
11. P. L. McGeer and E. G. McGeer, *Ann. N. Y. Acad. Sci.*, **1035**, 104-116 (2004).
12. I. A. Orlovskaya and V. A. Kozlov, *Rus. J. Immunol.*, **6**, No. 2, 168-176 (2001).
13. S. Sergeeva, E. Bagryanskaya, E. Korbolina, and N. Kolosova, *Exp. Gerontol.*, **41**, No. 2, 141-150 (2006).
14. Z. Xing, M. A. Ryan, D. Daria, *et al.*, *Blood*, **108**, No. 7, 2190-2197 (2006).
15. T. Yoshino, M. Tamura, M. Kawabe, *et al.*, *Bri. J. Haematol.*, **82**, No. 2, 664-670 (1992).