GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Reactions of the Blood System and Stem Cells in Bleomycin-Induced Model of Lung Fibrosis

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On the model of toxic diffuse pulmonary fibrosis induced by intratracheal administration of bleomycin, we studied reactions of the blood system, content of stem cells, committed hemopoietic and stromal progenitor cells in the bone marrow, spleen and peripheral blood of C57Bl/6 mice. It was shown that the development of diffuse pulmonary fibrosis was accompanied by hyperplasia of bone marrow hemopoiesis and leukocytosis in the peripheral blood. Activation of the erythroid and granulocytic hemopoietic stems was related to stimulation of hemopoietic stem cells (polypotent cells, granulocyte/erythroid/macrophage/megakaryocyte precursor cells) and committed erythroid and myeloid progenitor cells in the bone marrow. At the same time, the number of stromal precursors increased. Bleomycin increased the count of hemopoietic stem cells the peripheral blood and spleen and reduced the content of mesenchymal stem cells in the spleen and bone marrow.

Key Words: *pulmonary fibrosis; hemopoiesis; stem cells; committed hemopoietic and stromal progenitor cells*

Idiopathic pulmonary fibrosis is a chronic progressive disease characterized by the development of inflammation, interstitial fibrosis, and increasing respiratory failure [4,6]. In most cases, idiopathic pulmonary fibrosis has a poor prognosis with life expectancy of about 5 years. The results of treatment with currently available drugs (glucocorticoids are used more frequently) are disappointing. Therefore, the development of new therapies is necessary [4].

The participation of hemopoietic and mesenchymal stem cells (MSC) in lung regeneration is now intensively studied [8,9,11]. It was demonstrated that MSC transplantation reduced fibrosis and increased the number of alveolar epithelial cells. However, the lack of the comprehensive picture of hemo- and mesenchymopoiesis during lung fibrosis complicates the use of stem cells in clinical practice.

Here we studied the reactions of the blood system, content of hemopoietic cells and MSC, committed hemopoietic and stromal stem cells in the bone marrow, spleen, and peripheral blood under experimental bleomycin-induced lung fibrosis.

MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old C57Bl/6 mice weighing 20 g (n=60), certified conventional mice obtained from Breeding Center of Institute of Pharmacology.

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Lung fibrosis was induced by a single intratracheal administration of 80 μg bleomycin (Bleomizetin, Lensfarm) dissolved in 30 μl saline. Control rats received equivalent volume of saline under similar conditions. Intact animals were used as background (intact control).

The total content and differential count of leukocytes in the peripheral blood were determined by standard hematological methods 3, 7, 14, 21, and 25 days after administration of bleomycin. Thereafter the mice were sacrificed by CO_2 overdose and lung morphology, cellularity of the bone marrow, content of morphologically recognizable granulocytic, lymphoid, and erythroid cells [2] were analyzed. For histological examination, lung specimens were fixed in 10% formalin, processed by standard histological technique, and embedded in paraffin. Sections (5 μ) were cut, deparaffinized, and stained with hematoxylin and eosin and after van Gieson for detection of the connective tissue [5]. The area of collagen fibers in the lung tissue was measured by computer graphic analysis and the ratio of this parameter to the standard area of histological section was calculated.

The formation of granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM), granulocyte (CFU-G) and erythroid (CFU-E) by non-adherent nuclears of the bone marrow, spleen, and peripheral blood was studied *in vitro* by cell culture methods. In the culture of adherent nuclears from the bone marrow, spleen, and peripheral blood, the intensity of growth of fibroblast colonies (CFU-F) was studied [2,3]. The content of polypotent hemopoietic precursors *in vitro* forming colonies consisting of undifferentiated hemopoietic stem cells (CFU-N) and MSC was determined in all examined tissues by the method of limiting dilutions [3].

The results were processed by standard methods of variation statistics. Significance of differences was

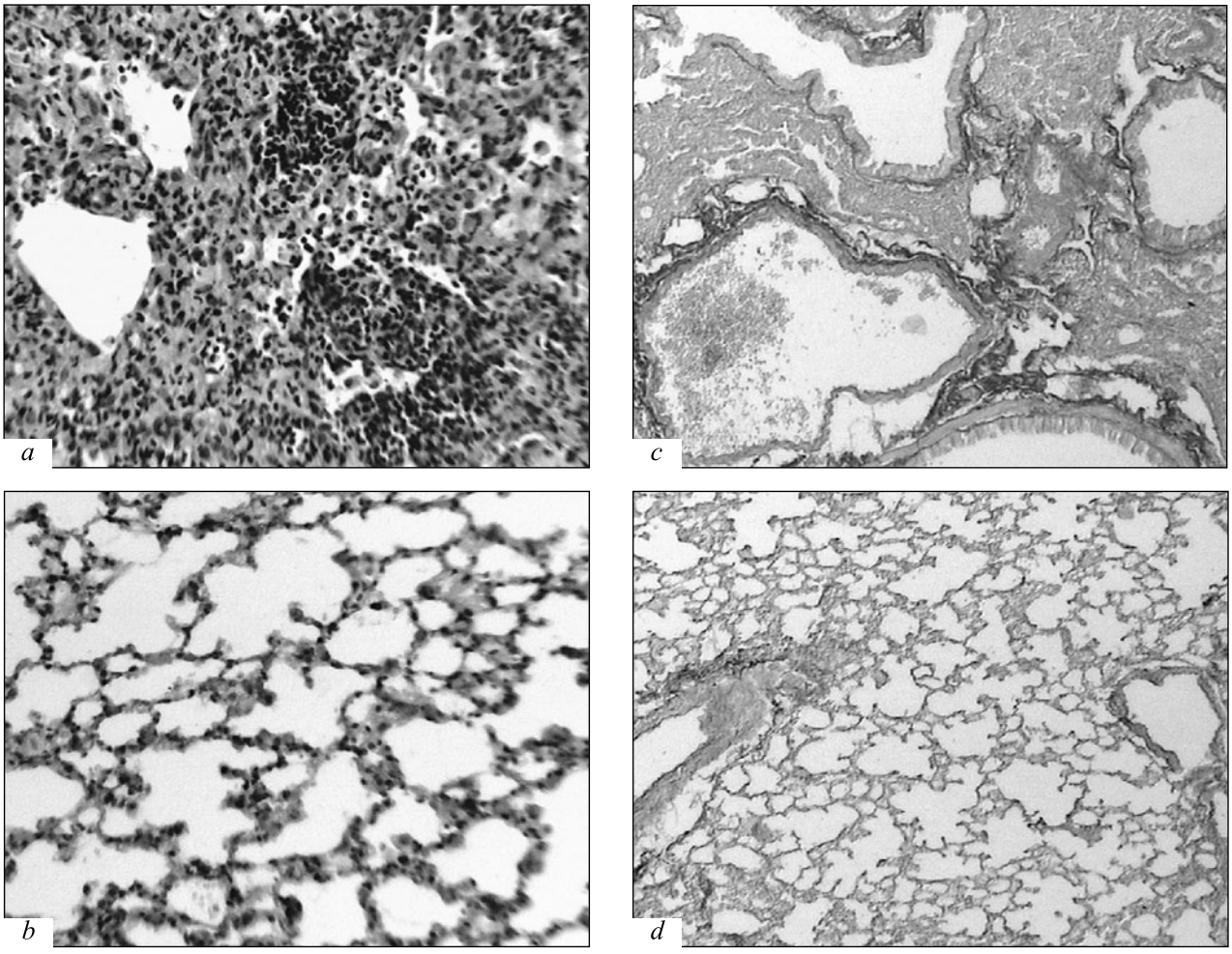

Fig. 1. Lung morphology in C57Bl/6 mice after intratracheal administration of bleomycin. *a*) diffuse infiltration of alveolar intersitium and alveolar ducts by neutrophils, lymphocytes, macrophages, and plasma cells; emphysematous enlargement of air spaces; *b*) lung of control mouse; *c*) peribronchial, perivascular, and interalveolar fibrosis (day 25); *d*) lung of control mouse. Hematoxylin and eosin staining, ×300 (*a*, *b*); staining with picrofuchsin after van Gieson, ×150 (*c*, *d*).

estimated using parametric Student's *t* test or non-parametric Mann–Whitney *U* test. The data expressed in fractions were analyzed using Fisher's exact test. The incidence of CFU-N and MSC was determined using the generalized linear model for Poisson distribution.

RESULTS

After intratracheal bleomycin administration, mouse lungs showed morphologic changes specific for toxic fibrosing alveolitis. Thus, starting from experimental day 3, edema of interalveolar septa and infiltration of the alveolar interstitium by neutrophils, lymphocytes, macrophages, and plasma cells were observed; on days 14 and 21, increasing edema and diffuse infiltration of the alveolar interstitium and alveolar lumens resulted in considerable distortion of lung architectonics. On experimental day 25, infiltration of lung tissue was most pronounced. In central regions of the lungs, the lung pattern was absent, some alveoli were emphysematous and expanded (Fig. 1). Bleomycin-induced activation of cell proliferation led to thickening of the alveolar interstitium, compression of capillaries, hypoxia, and disorganization of alveolar structures. In alveolar interstitium, enhanced formation of collagen fibers and progressive proliferation of the connective tissue were detected. On experimental day 7, connective tissue was found mainly in the perivascular and peribronchial areas. By the day 14, diffuse fibrosis was observed; on day 25, pulmonary fibrosis was most pronounced.

Bleomycin-induced damage to the lung tissue was accompanied by lymphocytosis (days 3 and 14) and leukocytosis (days 3-25) in mouse peripheral blood. Increased count of both immature and mature neutrophils in the bone marrow was observed throughout the observation period; the content of lymphocytes and erythrokaryocytes was increased on days 7, 21 and 14, respectively, compared to the corresponding parameters in intact animals (Fig. 2).

Thus, intratracheal administration of bleomycin to C57Bl/6 mice led to not only diffuse fibrosis, but also the hyperplasia of bone marrow hemopoiesis (predominantly the granulocytic lineage), and leukocytosis in the peripheral blood.

Under the influence of hemopoiesis-stimulating and inhibitory factors, activity of hemopoiesis is determined by the state of primitive and mature hemopoietic precursors [1]. In our experiments, bleomycin increased the content of CFU-GEMM and CFU-E in the bone marrow by 4 and 5.2 times, respectively, on experimental day 3; the content of granulocytic precursors was increased throughout observation period (Fig. 2). Increased numbers of polypotent hemopoietic precursors were detected on experimental days 3 and 14.

Thus, under conditions of experimental bleomycin-induced pulmonary fibrosis, stimulation of functional activity of hemopoietic stem cells and committed precursors is a mechanisms underlying the development of hyperplasia of hemopoiesis.

Under conditions of exposure to various factors (irradation, immobilization, hypoxia, cytotoxic and neurotic effects), the role of hemopoiesis-inducing microenvironment in the regulation of hemopoiesis dramatically increases [1]. Stromal mechanocytes, adherent cells of the bone marrow stroma (fibroblasts, reticular cells), forming mechanical framework for he-

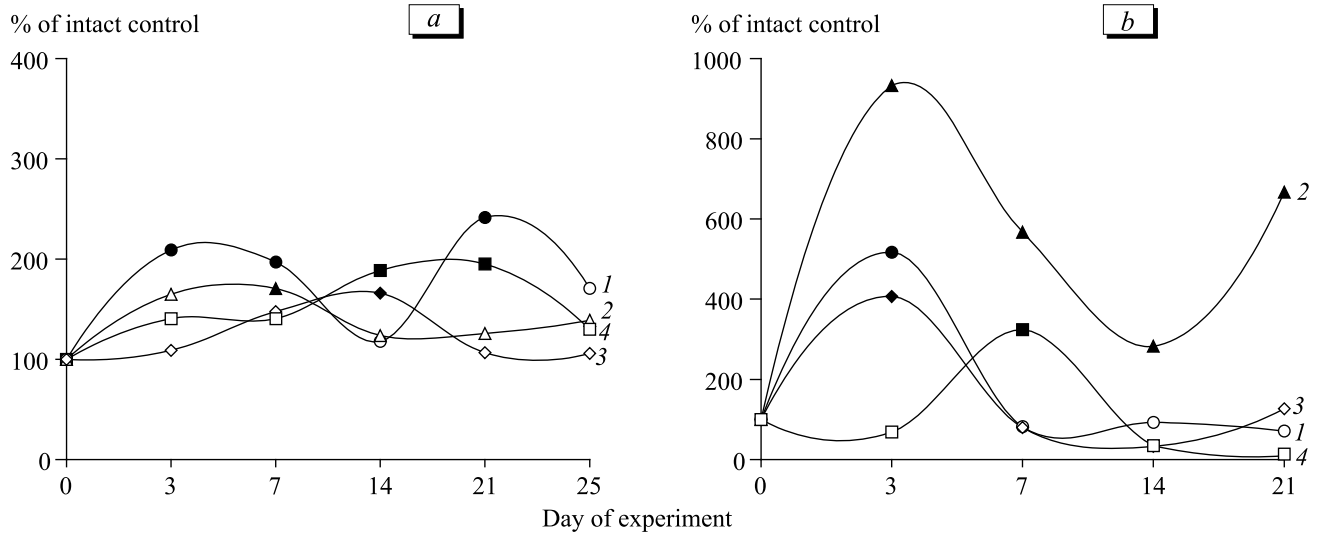

Fig. 2. Parameters of bone marrow hemopoiesis in C57Bl/6 mice after intratracheal administration of bleomycin. *a*) content of immature (*1*) and mature neutrophil granulocytes (*2*), lymphocytes (*3*) and erythrocaryocytes (*4*) in the bone marrow; *b*) content of CFU-E (*1*), CFU-G (*2*), CFU-GEMM (*3*), and CFU-F (*4*) in bone marrow cell culture. Here and in Fig. 3: dark signs, significant differences between intact the control and treatment groups, $p<0.05$.

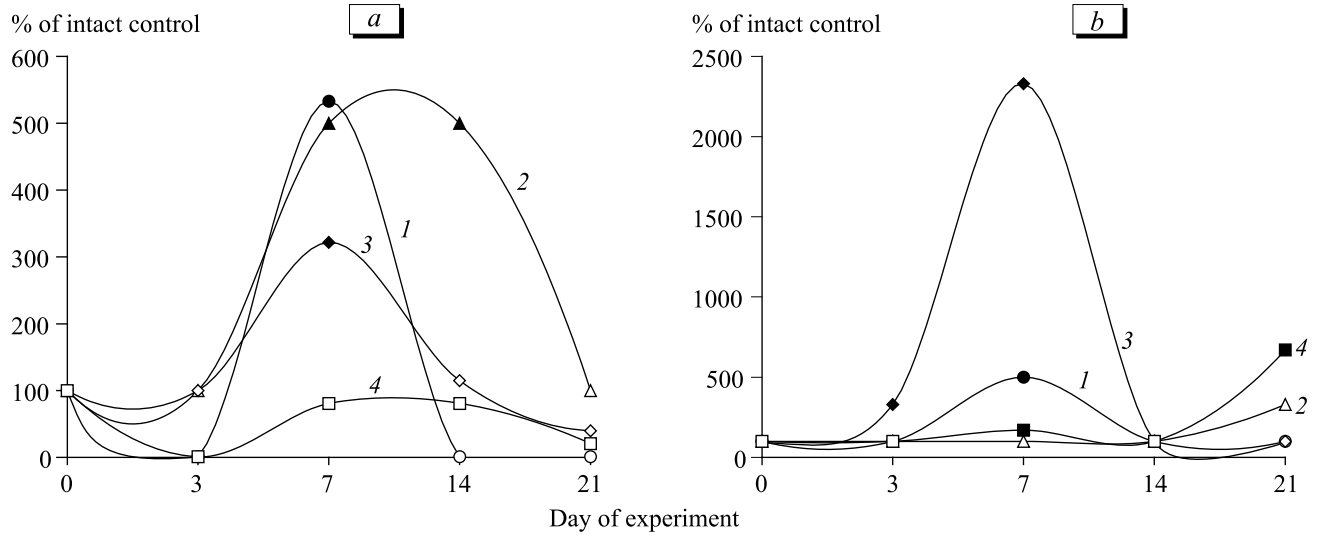

Fig. 3. Content of CFU-E (*1*), CFU-G (*2*), CFU-GEMM (*3*), and CFU-F (*4*) in spleen cell culture (*a*) and peripheral blood (*b*) of C57Bl/6 mice after intratracheal bleomycin administration.

mopoietic elements and producing extracellular matrix components, are important components of hemopoiesis-inducing microenvironment. We found that in bleomycin-induced model of pulmonary fibrosis, the growth rate of fibroblast colonies derived from adherent bone marrow nuclears significantly exceeded intact values (day 7; Fig. 2). It can be hypothesized that in pulmonary fibrosis increased content of stromal precursors is necessary for the maintenance of self-renewal of hemopoietic stem cells (KOE-N, CFU-GEMM) and production of more differentiated cells (CFU-E, CFU-G).

According to modern concepts, hemopoietic cells can migrate through the bloodstream to various peripheral organs (spleen, lungs, liver, *etc.*), participate in reparation and regeneration and return to the blood via lymphatic system [7]. Evaluation of colony-forming capacity of nonadherent spleen cells revealed increased content of CFU-E (day 7), CFU-G (days 7 and 14), and CFU-GEMM (day 7) in tissue culture (Fig. 3). The formation of GEMM-colonies by peripheral blood cells was also observed (day 7). These findings suggest mobilization of CFU-E, CFU-G, and CFU-GEMM under conditions of experimental pulmonary fibrosis. We do not exclude the fact that hemopoietic stem cells as well as erythroid and myeloid progenitor cells can migrate into the bleomycin-damaged lung tissue and then be involved in the inflammatory response.

In recent reports, the possibility of replenishment of lung alveolar cells due to MSC under the influence of hepatocyte growth factor, so-called mesenchymal-epithelial transition, was discussed [11]. In the group of animals treated with bleomycin intratracheally, decreased number of MSC in the bone marrow and spleen was revealed compared with intact control (day 3). This effect can be explained by migration of mesenchymal stem cells from the hemopoietic tissue into the lungs followed by their transformation.

In general our results suggest that stem cells and committed hemopoietic and mesenchymal precursors participate in the development of toxic diffuse fibrosis. At the same time, hemopoietic cells replenish neutrophils, lymphocytes, and macrophages and thus maintain inflammation in the lung tissue. In turn, MSC from bone marrow and spleen probably support epithelial cell replenishment after bleomycin administration (initiation of the epithelial-mesenchymal transition).

REFERENCES

1. E. D. Goldberg, A. M. Dygai, V. V. Zhdanov, *et al*., *Pharmacological Regulation of Blood System in Experimental Neurotic Influences* [in Russian], Tomsk (2007).
2. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
3. A. M. Dygai, E. G. Skurikhin, O. V. Pershina, *et al*., *Byull. Eksp. Biol. Med.*, **148**, No. 4, 400-404 (2010).
4. M. M. Ilkovich and L. N. Novikova, *Interstitial Lung Disease: A Guide for Physicians,* [in Russian]. Eds. M. M. Ilkovich, A. N. Kokosova, St.-Petersburg (1969), pp.127-183.
5. G. A. Merkulov, *The Course of Histopathological Techniques* [in Russian], St.-Petersburg (1969).
6. L. N. Novikova, E. S. Lebedeva, and I. V. Dvorakovskaya, *Pulmonologiya*, No. 2, 82-85 (2008).
7. S. Massberg, P. Schaerli, I. Knezevic-Maramica, *et al*., *Cell*, **131**, No. 5, 994-1008 (2007).
8. Y. Moodley, D. Atienza, U. Manuelpillai, *et al*., *Am. J. Pathol*., **175**, No. 1, 303-313 (2009).
9. L. A. Ortiz, F. Gambelli, C. McBride, *et al*., *Proc. Natl. Acad. Sci. USA*, **100**, No. 14, 8407-8411 (2003).
10. M. Rojas, J. Xu, C. R. Woods, *et al*., *Am. J. Respir. Cell Mol. Biol*., **33**, No. 2, 145-152 (2005).
11. M. N. Shukla, J. L. Rose, R. Ray, *et al*., *Am. J. Respir. Cell Mol. Biol*., **40**, No, 6, 643-653 (2009).