GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Mechanisms of the Anti-Inflammatory and Antifibrotic Activity of a Sympatholytic Agent during Toxic Pulmonary Fibrosis

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The effect of a course treatment with a sympatholytic reserpine on the inflammatory response and connective tissue proliferation in the lungs of C57Bl/6 mice was studied on the model of toxic pulmonary fibrosis induced by intratracheal administration of bleomycin. This sympatholytic reduced infiltration of the alveolar interstitium and alveolar ducts with inflammatory cells (lymphocytes, macrophages, neutrophils, and plasma cells) and prevented connective tissue proliferation in the lungs. The anti-inflammatory effect of reserpine was associated with a decrease in activity of bone marrow granulocyte-erythroid-macrophage-megakaryocyte and granulocyte precursors (proliferation and mobilization). The antifibrotic effect of reserpine was due to a decrease in the number of committed precursors for mesenchymopoiesis.

Key Words: *bleomycin; pulmonary fibrosis; sympatholytic agent; granulocyte-erythroid-macrophage-megakaryocyte precursors; stromal precursor cells*

Idiopathic pulmonary fibrosis (IPF) is a chronic progressive disease of unknown etiology with a poor prognosis. Patient's condition is rapidly deteriorating. The life-span of IPF patients varies from 2 to 4 years. Therapeutic manipulations for pulmonary fibrosis are limited and ineffective. Clinical treatment is mainly based on the therapy for complications and maintenance therapy [9].

Much recent attention was paid to the involvement of mesenchymal stem cells (SC) in reparative regeneration (*e.g.*, during IPF) [12,13]. Previous experiments showed that deposition of collagen fibers in the lungs depends on pluripotent and multipotent hemopoietic SC. Due to the proliferation, differentiation into neutrophils and monocytes/macrophages, and migration into the lung tissue [2], bone marrow hemopoietic SC maintain a bleomycin-induced production of collagen via the secretion of profibrotic cytokines by inflammatory cells [14].

According to modern notions, hemopoietic SC are regulated by the adrenergic system [2,3,5]. The relationship exists between collagen fiber synthesis in the lungs and activity of adrenoceptors [6,7]. We hypothesized that the substances suppressing functional activity of the adrenergic system can be used as potent anti-inflammatory and antifibrotic agents during pul-

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monary fibrosis. Hemopoietic SC serves as a potential target for pharmacological agents.

Here we studied the effect of a sympatholytic reserpine on the course of pulmonary fibrosis and activity of hemopoietic SC after intratracheal administration of bleomycin.

MATERIALS AND METHODS

Experiments were performed on 7-8-week-old C57Bl/6 mice weighing 20 g (n=155). The mice (class I conventional strain) were obtained from the nursery of the Institute of Pharmacology (certified animals).

Pulmonary fibrosis was induced by intratracheal administration of bleomycin (Bleomycetin, Lensfarm Company) in a single dose of 80 μg in 30 μl physiological saline. Sympatholytic reserpine (Sigma) in a daily dose of 1 mg/kg was injected intraperitoneally (in a volume of 100 μl) on days 1-7, 9, 11, and 13 after surgery. Each group consisted of 10 animals. Intact animals served as the control (intact control, n=15).

The content of neutrophilic leukocytes and lymphocytes in the peripheral blood was determined routinely on days 3, 7, 14, 21, 25, 40, and 60 after bleomycin administration. The mice were killed by CO_2 overdose. Morphological characteristics of the lungs were evaluated. We estimated the number of morphologically distinct cells of the granulocyte and lymphoid hemopoietic stems [1]. For histological study, lung tissue samples were fixed in 10% formalin, processed using standard histological methods, and embedded into paraffin. Histological sections (5 μ) were prepared. Connective tissue was visualized after differential staining by the van Gieson method (raspberry pink-colored collagen fibers with picrofuchsin) [2,4]. The area of collagen fibers was measured by a computerized graphic analysis. The ratio of collagen fibers (relative to the area of lung tissue) was calculated.

The capacity of nonadherent nucleated cells from the bone marrow, peripheral blood, and spleen to form granulocyte-erythroid-macrophage-megakaryocyte (CFU-GEMM) and granulocyte colonies (CFU-G) was studied by the culture method. The growth of fibroblast colonies (CFU-F) was evaluated in a culture of adherent nucleated cells from the bone marrow, peripheral blood, and spleen [1,2].

The results were analyzed by standard methods of variation statistics. The significance of differences was evaluated by parametric Student's t test or nonparametric Mann–Whitney U test.

RESULTS

Administration of bleomycin to mice was followed by the development of toxic fibrosing alveolitis. Venous plethora of the alveolar wall and epithelial edema of the interalveolar septa were observed on day 3 of the study. Inflammatory infiltration of the lung tissue with lymphocytes, macrophages, and plasma cells was mainly found near large vessels and in the peribronchial space. The severity of hyperemia and edema increased on the 7th day. An increase in the degree of cell infiltration in the alveolar walls was followed their thickening. Plasma cells, neutrophilic and eosinophilic leukocytes, alveolar macrophages, and histiocytes were present in the alveolar lumen. Severe edema and progressive diffuse infiltration of the lung interstitium and alveolar ducts with inflammatory cells was accompanied by the impairment of lung tissue architectonics on day 14 of the study. The alveolar wall was significantly thickened (as compared to day 7). The alveolar lumen was filled with desquamated alveolar cells and infiltrate cells. The lung pattern was indefinable in some areas of the root of the lung due to massive inflammatory infiltration. Structural impairment of the alveolar wall was followed by the formation of alveolar cysts. These changes constitute the initial stage of the formation of the so-called honeycomb lung. The peak of inflammation was observed on day 21 (Fig. 1, *a*, *c*, *e*). Visualization of the connective tissue allowed us to reveal an increased deposition of collagen fibers in the lung tissue of animals on days 7-60 of the study (Table 1). The fibrogenic response to bleomycin was particularly significant on day 25 (Fig. 1, *b*, *d*, *f*).

Reserpine reduced the degree of bleomycin-induced destructive changes in the lungs of mice in all periods of the study. Epithelial edema and hyperemia of the interalveolar septa were less pronounced in animals with catecholamine depletion (as compared to

TABLE 1. Connective Tissue Content in the Lungs of C57Bl/6 Mice (% of Lung Tissue Area) after Intratracheal Administration of Bleomycin and Course Treatment with Sympatholytic Reserpine against the Background of Experimental Pulmonary Fibrosis ($M\pm m$)

Period, days	Bleomycin	Bleomycin+ reserpine
Intact control	1.03±0.20	
7	1.98±0.26*	1.33±0.06*+
14	3.09±0.23*	2.94±0.27*
21	3.02±0.44*	2.75±0.42*
25	5.45±0.74*	3.85±0.58*+
40	3.47±0.21*	2.58±0.30*
60	2.77±0.25*	1.59±0.39+

Note. p<0.05 in comparison with: *intact control; +bleomycin.

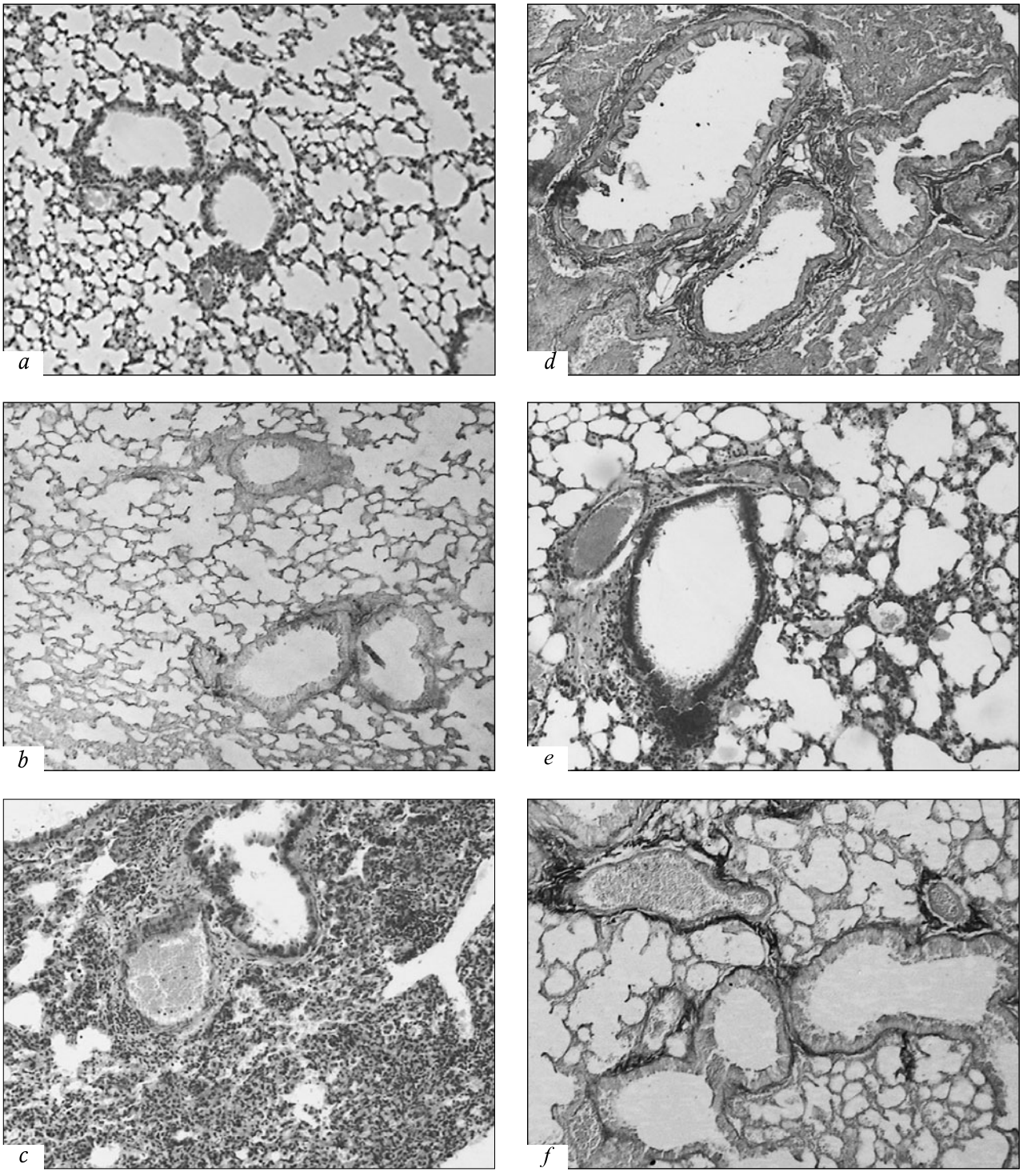

Fig. 1. Morphological signs of the lungs in C57Bl/6 mice after intratracheal administration of bleomycin and sympatholytic treatment. Staining with hematoxylin and eosin (day 21, ×300; *a*, *c*, *e*) and picrofuchsin by the van Gieson's method (day 25, ×150; *b*, *d*, *f*). Lung of a control mouse (*a*, *b*); lung of a bleomycin-treated mouse (*c*, *d*); lung of a mouse treated with sympatholytic against the background of bleomycin administration (*e*, *f*).

untreated mice of the bleomycin group; bleomycin control). Lung tissue airiness was preserved under these conditions. The animals were characterized by peribronchial and perivascular cell infiltration. As differentiated from animals of the bleomycin control group, combined treatment of mice with the cytostatic and sympatholytic was accompanied by a slight release of inflammatory cells into the alveolar lumen.

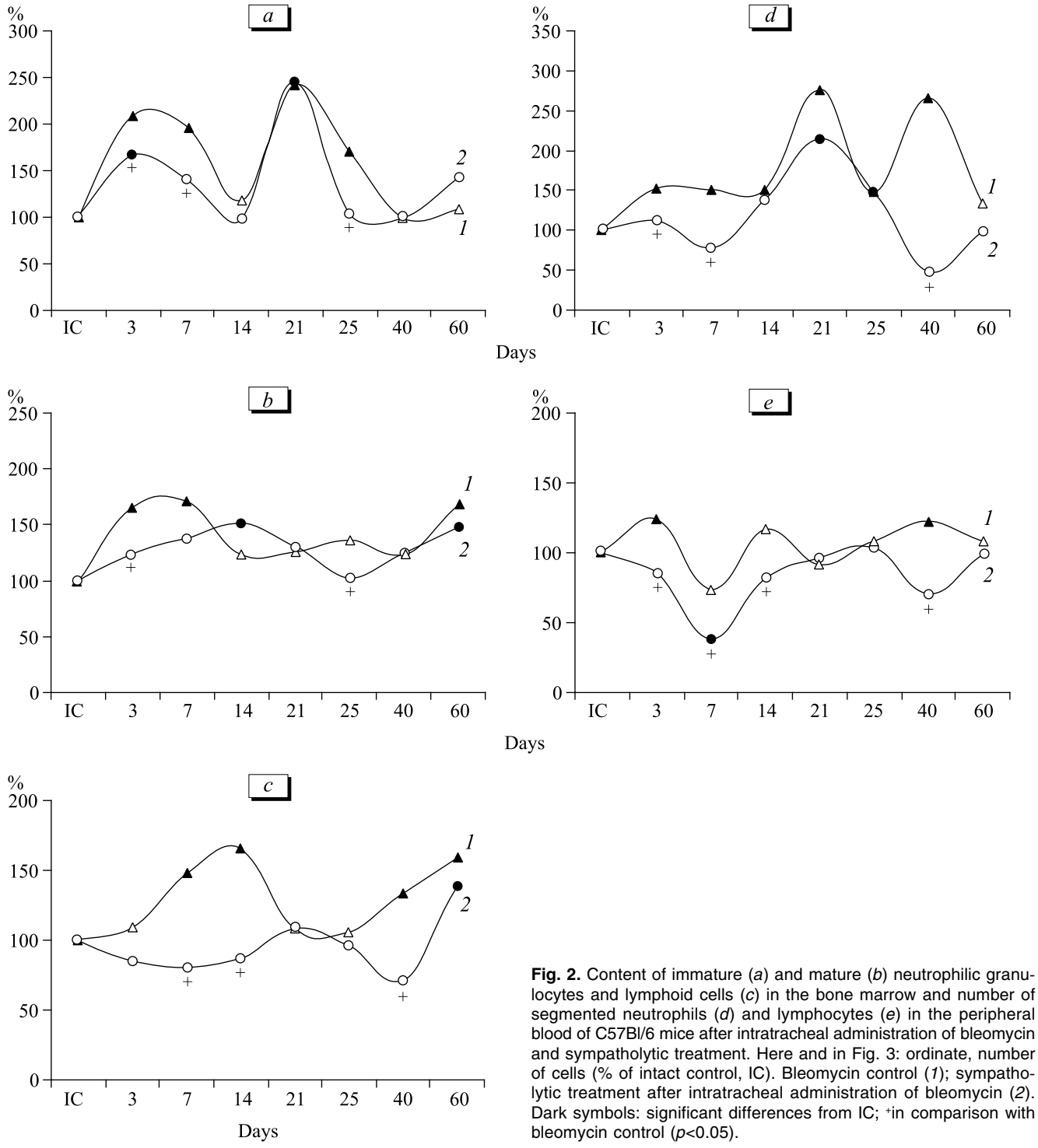

Fig. 2. Content of immature (*a*) and mature (*b*) neutrophilic granulocytes and lymphoid cells (*c*) in the bone marrow and number of segmented neutrophils (*d*) and lymphocytes (*e*) in the peripheral blood of C57Bl/6 mice after intratracheal administration of bleomycin and sympatholytic treatment. Here and in Fig. 3: ordinate, number of cells (% of intact control, IC). Bleomycin control (*1*); sympatholytic treatment after intratracheal administration of bleomycin (*2*). Dark symbols: significant differences from IC; +in comparison with bleomycin control ($p<0.05$).

These data indicate that sympatholytic treatment delays the development of alveolitis and fibrosis of the lung interstitium in mice after intratracheal administration of bleomycin.

Analysis of the mechanisms for anti-inflammatory and antifibrotic activity of reserpine revealed the following regulations. Neutrophilic leukocytosis (days 3, 7, and 40) and lymphocytosis of the peripheral blood (days 3, 7, 14, and 40) were not observed in sympatholytic-treated animals (as compared to mice of the bleomycin control group; Fig. 2). Reserpine had a similar effect on bone marrow cells. This agent decreased the number of neutrophilic granulocytes (days 3, 7, and 25) and lymphocytes (days 7, 14, and 40) in mice, which did not differ from the intact control. These changes in the content of mor-

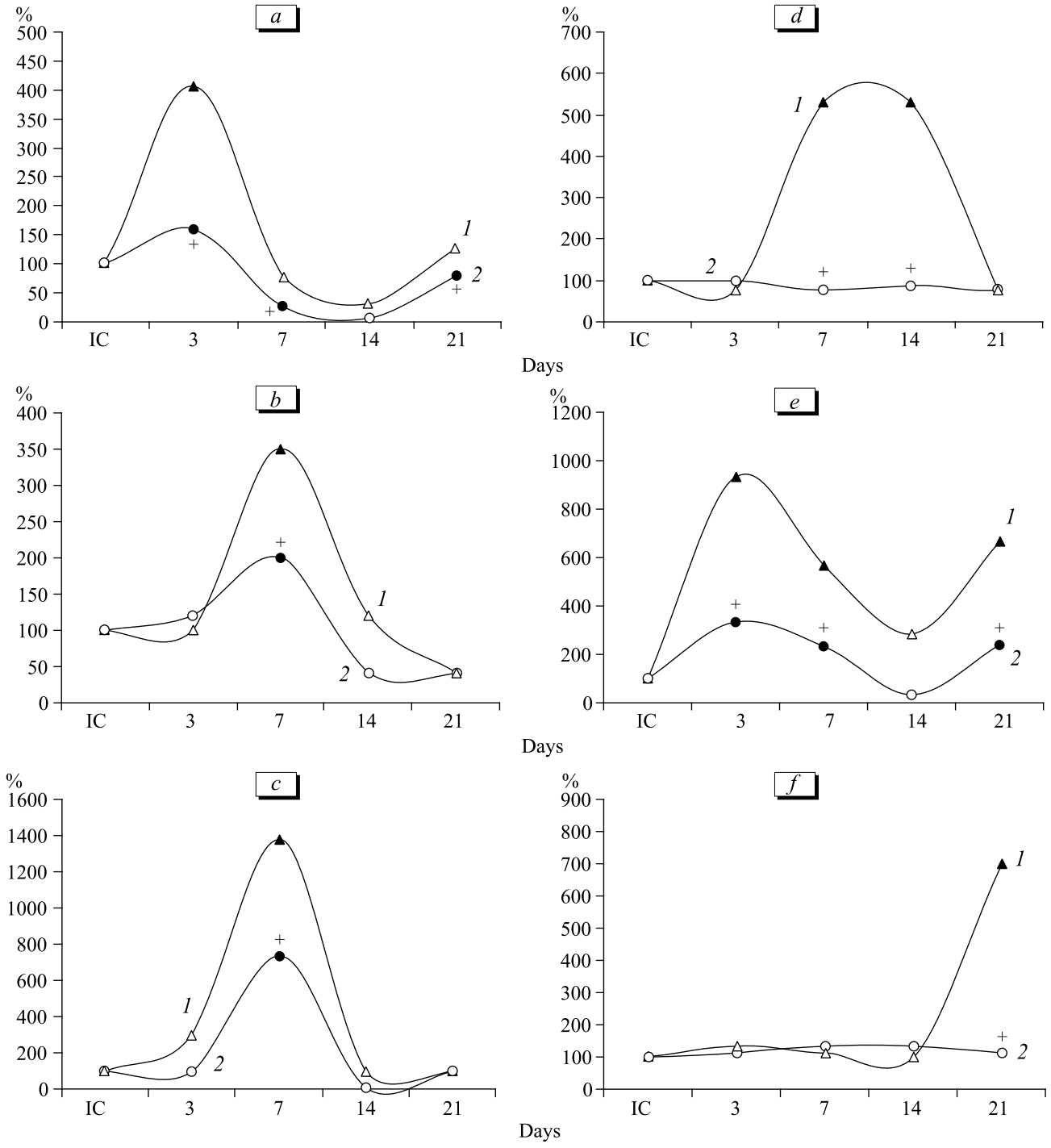

Fig. 3. Content of CFU-GEMM (*a*-*c*) and CFU-G (*d*-*f*) in the bone marrow (*a*, *d*), spleen (*b*, *e*), and peripheral blood (*c*, *f*) of C57Bl/6 mice after intratracheal administration of bleomycin and sympatholytic treatment.

phologically distinct white blood cells after sympatholytic treatment were associated with a decrease in the activity of CFU-GEMM (days 3, 7, and 21) and CFU-G (days 3 and 7) precursor cells in the bone marrow (Fig. 3).

Bleomycin-induced fibrosis of the lung accompanies mobilization of CFU-GEMM and CFU-G [2]. Some authors reported that hemopoietic cells migrating over the vascular system into various organs, including the lungs, are involved in reparation and regeneration [10]. We showed that reserpine decreases the content of CFU-GEMM and CFU-G in the peripheral blood and spleen. These changes illustrate a decrease in the number of mobile multipotent hemopoietic SC and

granulocyte precursor cells under conditions of experimental pulmonary fibrosis. Besides the reduction of bone marrow hemopoiesis, catecholamine depletion is probably followed by the impairment of migration of myeloid hemopoietic cells into the bleomycin-damaged lung tissue.

According to the modern notions, circulating fibroblasts are accumulated in the inflammatory site and become involved in the development of fibrotic changes in the lungs [8,15]. Culture studies showed that reserpine decreases the content of CFU-F in the bone marrow and peripheral blood on days 7, 14, and 21 (by 40% compared to mice of the bleomycin control group; Fig. 3). The antifibrotic effect of this sympatholytic is primarily related to a decrease in the number of mobile fibroblast cells.

Our experiments on the model of bleomycin-induced pulmonary fibrosis showed that reserpine prevents the development of inflammation and outgrowth of the connective tissue in the lungs. These effects of a sympatholytic agent are associated with the inhibition of multipotent hemopoietic SC, granulocyte precursor cells, and stromal precursor cells.

Our results extend the knowledge of bleomycin-induced fibrotic changes in the lungs. We believe that the adrenergic regulation of collagen fiber synthesis is realized not only via β-adrenoceptors of the bronchopulmonary system [6,7]. Hemopoietic SC and committed precursor cells of hemopoiesis and mesenchymopoiesis are strongly involved in the adrenergic mechanism of pulmonary fibrosis.

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