GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Mechanisms of Hepatoprotective Effect of Immobilized Granulocyte Colony-Stimulating Factor

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The effect of immobilized granulocyte CSF on morphological characteristics and functional state of the liver was studied during chronic toxic hepatitis. The mechanisms of the therapeutic action of this agent were evaluated. The product had a strong hepatoprotective effect and exhibited the antiinflammatory and antisclerotic properties. The mechanism of activation of reserve systems for cell renewal (involved in restoration of the liver tissue) is probably related to an increase in proliferative activity of early precursor cells in the bone marrow, mobilization of these cells into the peripheral circulation, and directed homing into the liver tissue where they activate local regenerative mechanisms and prevent hepatocyte destruction. It should be emphasized that the concentration of SDF-1 increases in the liver tissue, but decreases in the bone marrow. These changes create the concentration gradient, which determines the migration of undifferentiated precursor cells to the liver.

Key Words: *chronic hepatitis; granulocyte colony-stimulating factor; cell therapy*

Granulocyte CSF (G-CSF) has a regulatory effect on the pool of regenerative and compensatory precursor cells [2,3,5]. Therefore, this agent can be used to recover functionally active tissue in damaged organs with stem cells. For example, it concerns the therapy for chronic hepatitis. Chronic hepatitis is one of the most common and severe chronic diseases of the liver. This state is characterized by insufficient regenerative capacity of the liver. Chronic hepatitis is accompanied complete substitution of the parenchyma with the connective tissue, structural reorganization of the liver, and development of cirrhosis. Liver cirrhosis cannot be treated by modern therapeutic methods [5].

The therapeutic use of G-CSF is limited by toxicity and high frequency of undesirable side effects. Hence, the product of immobilized G-CSF (immG-CSF) holds much promise for the therapy of chronic hepatitis. immG-CSF was developed in the Scientific Features Management Company (in collaboration with the Institute of Pharmacology). The molecules of recombinant human G-CSF are immobilized on low-molecular-weight polyethylene glycol by means of electron-beam treatment. Such procedure significantly increases the bioavailability of this product. Pegylated G-CSF is a nontoxic compound characteri-

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zed by high physical stability and long half-elimination period [6,9].

Studying the role of SDF-1 (major chemotactic factor for migration of progenitor cells) [8,10] is important for evaluation of fine mechanisms for therapeutic action of immG-CSF.

MATERIALS AND METHODS

Experiments were performed on 130 male CBA/CaLac mice and 59 male Wistar rats (aging 2 months). Chronic hepatitis was induced by intragastric administration of CCl_4 (0.2 ml of 50% olive oil solution per mouse; 2.5 ml of 20% solution per rat) for 3 weeks (twice a week). Experimental animals received a subcutaneous injection of immG-CSF in a daily dose of 100 μg/kg for 5 days (beginning from the next day after the last treatment with CCl_4). Control animals were similarly treated with an equivalent volume of physiological saline.

We evaluated mortality rate and body weight gain in rats. Activities of AST, ALT (De-Ritis quotient, AST/ALT ratio), and alkaline phosphatase (AP) in blood plasma were measured routinely on days 21 and 40. The measurements were conducted on a Cormay semiautomatic biochemical analyzer with Cormay and VectorBest kits.

Morphological study of the liver was performed on days 21 and 40 after the start of treatment with CCl_4. The weight index (ratio of the weight of the liver (mg) to body weight (g) served as an integral criterion for the hepatotropic effect of xenobiotics. The liver was weighted. Liver samples were fixed in 10% neutral formalin and embedded into paraffin. Deparaffinized sections were stained with hematoxylin and eosin and with picrofuchsin (for connective tissue). The study was performed by computerized methods for graphic data processing. The count of infiltrate cells was estimated on histological samples of the liver after staining with hematoxylin and eosin. The area of connective tissue was measured on histological samples of the liver after staining with picrofuchsin. Consecutive micrographs of 10 fields of view were obtained using a micro video camera (Digital micro) and image processing software (Elecard). We estimated the count of infiltration cells (per standard area of liver section) or measured the area of picrofuchsin-stained structures. The percentage area was calculated (relative to the standard area) [1].

The number of fibroblast CFU (CFU-F) in the bone marrow and peripheral blood and count of hepatocyte precursors in mice of all groups were evaluated on days 21, 23, and 26. Conditioned media of cultures from bone marrow cells and liver tissue were obtained in the same periods. The concentration of SDF-1 was measured by enzyme immunoassay [4]. The content of mesenchymal stem cells (MSC) in the bone marrow and peripheral blood was estimated by the method of limiting dilutions (day 21) [7].

RESULTS

Before the start of drug treatment, the mortality rate of animals due to toxic dystrophy was 11.9%. Body weight gain in rats of experimental groups was delayed after administration of CCl_4. By the end of observations, body weight of immG-CSF-receiving animals practically did not differ from that in intact rats.

Biochemical study of blood plasma showed that the animals with CCl_4-induced hepatitis were characterized by severe metabolic dysfunction of the liver. On days 21 and 40 of the experiment, we revealed an increase in activity of a cytolysis marker ALT (by 28 and 15%, respectively) and decrease in the De-Ritis quotient in saline-receiving rats. Administration of immG-CSF to experimental animals had a normalizing effect on study parameters. The elevated liver weight index returned to normal, which serves as a favorable prognostic criterion for chronic hepatitis. AP activity in control rats was increased to 308 and 240% on days 21 and 40, respectively. The increase in AP activity was less pronounced in immG-CSF-receiving animals (183 and 146%, respectively).

Histological study of liver samples from all rats revealed the development of fatty degeneration, hepatocyte necrosis, and infiltration of the liver parenchyma with macrophages and lymphocytes (of different severity) on day 21 of observations.

The lobular structure of the liver was impaired in control animals. The hepatocytes were characterized by monocellular and focal necrosis. Dead hepatocytes were substituted with the granulation tissue. Small-and large-drop fatty degeneration was found in preserved hepatocytes. Fusion of some cells was followed by the formation of fatty cysts. Regenerative hypertrophy of hepatocytes was observed under these conditions. The infiltrative process was diffuse. The number of infiltrate cells and relative area of the connective tissue were much higher compared to the baseline (by 2 and 5 times, respectively; Fig. 1).

The lobular structure of the liver remained unchanged in immG-CSF-treated rats. We found single Councilman bodies (necrotic hepatocytes). The hepatocytes were characterized by fatty degeneration (diffuse small-drop degeneration or, more rarely, large-drop degeneration). Regenerative hypertrophy of hepatocytes was well pronounced. Portal tracts were infiltrated. The infiltrate did not spread in the lobule. The number of infiltrate cells and relative area of the connective tissue in treated animals were much lower than in control specimens.

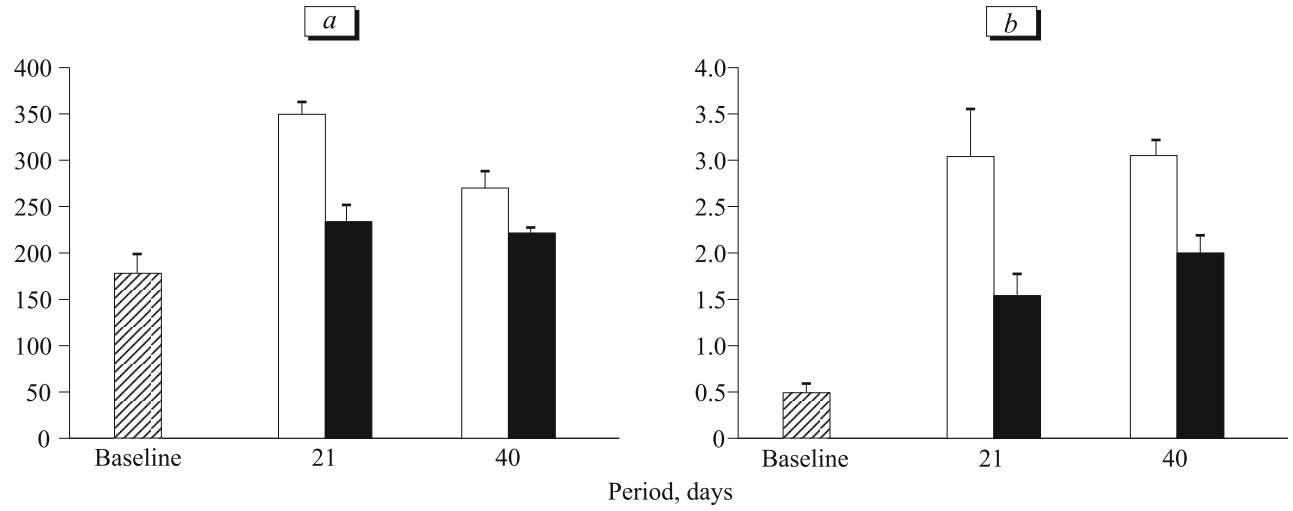

Fig. 1. Effect of the immG-CSF on morphological characteristics of the liver in rats with chronic hepatitis. Number of inflammatory infiltrate cells (*a*); amount of connective tissue in histological preparations of the liver (*b*). Here and in Figs. 2 and 3: light bars, control; dark bars, treatment.

Morphological signs of hepatitis in rats of both groups on day 40 were less pronounced than on day 21. Small-drop fatty degeneration of hepatocytes was shown to persist under these conditions. The relative area of infiltration in the liver parenchyma and relative area of connective tissue in immG-CSF-receiving animals were much lower than in untreated specimens with hepatitis (Fig. 1). The infiltrate was mainly re-

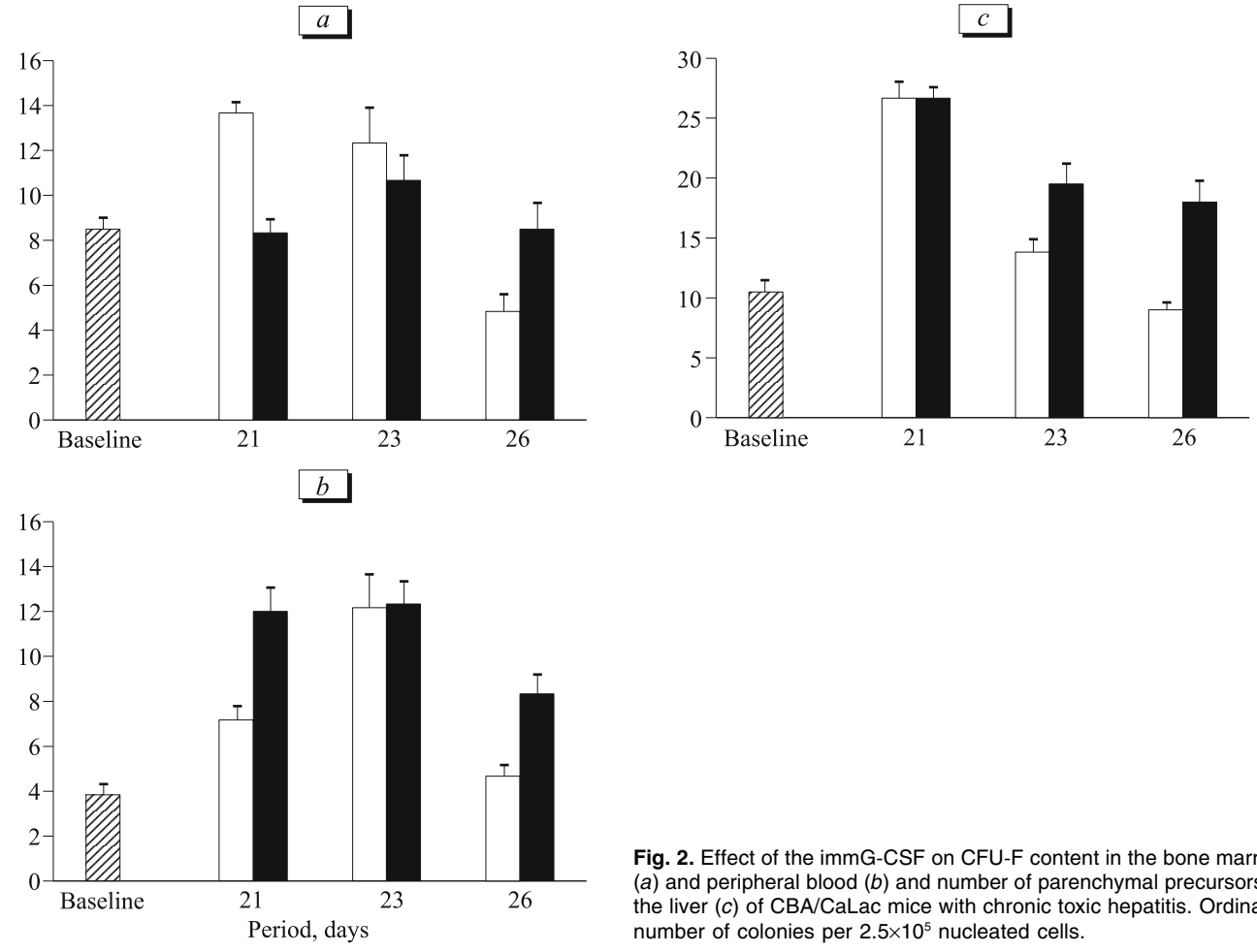

Fig. 2. Effect of the immG-CSF on CFU-F content in the bone marrow (*a*) and peripheral blood (*b*) and number of parenchymal precursors in the liver (*c*) of CBA/CaLac mice with chronic toxic hepatitis. Ordinate: number of colonies per 2.5×10^5 nucleated cells.

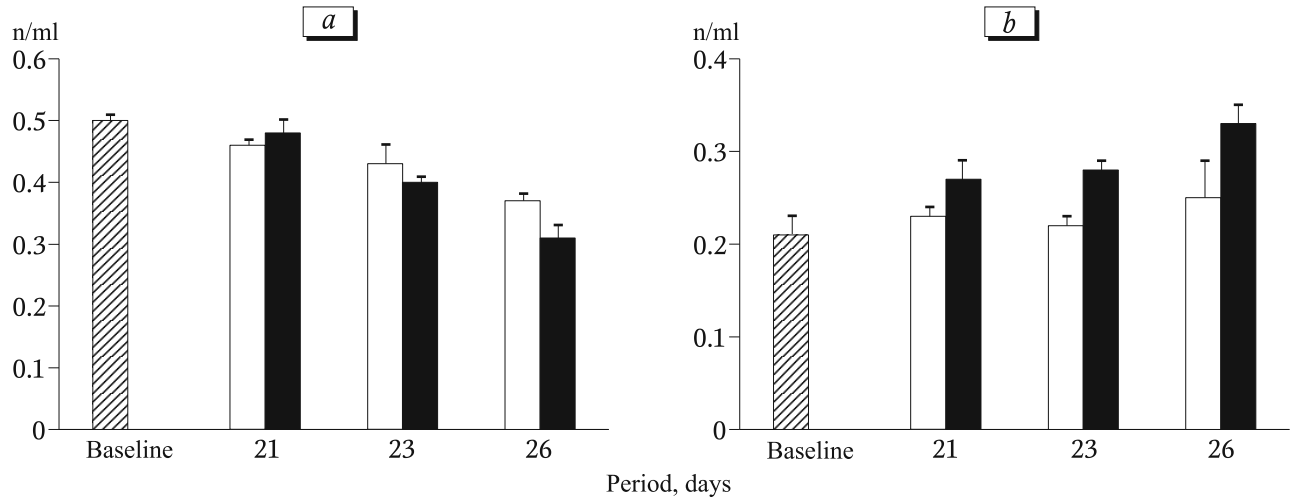

Fig. 3. Effect of the immG-CSF on SDF-1 content in conditioned media from adherent myelokaryocytes (*a*) and liver cells (*b*) of CBA/CaLac mice with chronic toxic hepatitis.

vealed in portal regions. Focal infiltration was found in hepatic lobules.

Our results indicate that immG-CSF has a strong hepatoprotective effect and exhibits the antiinflammatory and antisclerotic properties on the model of chronic toxic hepatitis.

A special series on mice was performed to evaluate the mechanisms of the hepatoprotective effect of immG-CSF. The toxic action of CCl_4 increased the number of CFU-F in the bone marrow, peripheral blood, and liver tissue on days 21 and 23 after treatment (Fig. 2). This parameter returned to normal in the follow-up period. Administration of immG-CSF abolished the accumulation of stromal precursor cells in the bone marrow. We revealed only a transient increase in the number of these cells on day 25 after treatment. The count of CFU-F in the peripheral blood, as well as the content of parenchymal precursors (CFUliv) in liver samples from treated animals surpassed the baseline and control level. Administration of the product prevented the decrease in the content of CFUliv in cultured liver cells (as observed in untreated specimens; Fig. 2).

These data show that study product has a stimulatory effect on reserve systems for cell renewal, which are involved in the restoration of liver tissue. The action of these systems is probably related to an increase in proliferative activity of early precursor cells in the bone marrow, mobilization of these cells to the peripheral circulation, and directional homing into the liver tissue. These cells activate the local regenerative mechanisms and prevent hepatocyte destruction.

On day 21, the count of bone marrow MSC in untreated animals did not differ from the baseline. A subcutaneous injection of the G-CSF was followed by a significant increase in the number of bone marrow MSC (by 68%). However, the observed differences were statistically insignificant.

Similar results were obtained in studying the peripheral blood from experimental animals. No differences were found in the number of MSC in intact mice and animals of the saline group. Administration of immG-CSF was accompanied by a 1.6-fold increase in the number of MSC (compared to the baseline). Differences in the number of MSC in blood samples from animals of the control and treatment groups became more significant under these conditions.

The toxic agent caused a progressive decrease in the content of SDF-1 in conditioned media from adherent bone marrow cells, but had no effect on the production of this factor by liver cells (Fig. 3). immG-CSF did not abolish, but significantly potentiated the effect of study agent on bone marrow cells on day 26 of the experiment. However, the product of G-CSF had a strong effect on the production of this factor by hepatocytes. The content of SDF-1 was shown to increase progressively in hepatocyte supernatants (Fig. 3).

We conclude that the production of SDF-1 by cells of damaged organ (*e.g.*, liver cells) does not increase under experimental pathological conditions. It probably contributes to the insufficiency of compensatory mechanisms in this disorder [2]. However, this mechanism is activated after treatment with immG-CSF. It should be emphasized that the concentration of SDF-1 increases in the liver tissue, but decreases in the bone marrow. These changes contribute to the gradient of cytokine concentration, which determines the migration of undifferentiated precursor cells to the liver [8,10]. This mechanism has an important role in the therapeutic action of immG-CSF.

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