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Effects of Granulocyte-Macrophage Colony-Stimulating Factor Produced by Intestinal Epithelial Cells on Functional Activity of Hemopoietic Stem Cells

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The population of epithelial cells in the small intestine includes precursor cells of hemopoiesis possessing the ability to form splenic colonies on day 8. Intestinal epithelial cells stimulates proliferative and colony-forming activity of hemopoietic stem cells by producing granulocyte-macrophage colony-stimulating factor.

Key Words: *hemopoietic stem cell; intestinal epithelial cells; granulocyte-macrophage colony-stimulating factor*

Phylogenetically, the intestine is the first localized organ for various types of hemopoiesis [2]. Despite evolutional separation of hemopoietic organs, the intestinal epithelium in mammals did not lose the relationship with maturation and differentiation of hemopoietic cells. The intestinal epithelium serves as an organ for the extrathymic development of lymphocytes [4,6]. Previous studies revealed the presence of primary hemopoietic precursors in the epithelium of rhesus monkeys [7]. It is important to study the molecular mechanisms determining maturation and differentiation of hemopoietic precursors in the intestinal epithelium and evaluate the role of intestinal epithelial cells (IEC) in the hemopoietic microenvironment.

Here we estimated colony-stimulating activity of early hemopoietic precursors from mouse intestinal epithelium and evaluated the role of soluble factors produced by IEC in the regulation of functional activity of hemopoietic stem cells.

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MATERIALS AND METHODS

Experiments were performed on 3-6-month-old $(CBA\times C57Bl/6)F_1$ and $(DBA\times C57Bl/6)F_1$ mice obtained from the Laboratory of Experimental Biomedical Modeling (Tomsk Research Center).

The animals were sacrificed by cervical dislocation. The population of IEC was isolated by chemical (1.5 mM ethylenediaminetetraacetic acid, Serva) and mechanical methods [8]. Intraepithelial lymphocytes were removed by centrifugation in a Percoll density gradient (ρ=1.060, Sigma) at 1000 rpm and 4°C for 35 min [4]. The purity of IEC populations was assayed morphologically (98%). Cell viability was determined by trypan blue exclusion (90-95%).

IEC in a concentration of 5×10^6 cells/ml were incubated in culture flasks with RPMI-1640 medium containing 20 mM HEPES, 0.25% bovine serum albumin, and antibiotics at 37°C and 5% CO_2 for 2 h. After incubation conditioned media (CM) were isolated from cells by centrifugation. Fractional separation of CM was performed in an Amicon fractional cell using 30-kDa (Diaflo PM 30, Amicon) and 10-kDa ultrafiltration membranes (PLGC-025-10, Millipore). The content of granulocyte-macrophage colony-stimulating

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factor (GM-CSF) was measured on an Origen Analyzer Igen Inc. electrical chemiluminometer [1]. Biological activity of GM-CSF in fractions was neutralized with anti-GM-CSF (R&D, Mab 415) using biotinylated antibodies in a concentration of 6 μg/ml. After 90-min incubation excess antibodies and GM-CSF were removed with Dynabeads M-280 streptavidin microbeads (Origen). IEC were syngeneically transplanted to lethally irradiated recipient mice. The population of epithelial cells was transplanted in concentrations of 2.5×10^5, 1.5×10^6, and 2.5×10^6 cells/mouse. The population of cryptal IEC isolated in a Percoll gradient was transplanted in a concentration of 2.5×10^6 cells/mouse. The mice were killed 8 days after transplantation. The number of colonies was determined visually.

Colony-stimulating and proliferative activity of hemopoietic stem cells were estimated as described elsewhere [3]. Bone marrow cells from intact mice (10^6 cells/ml) were cultured in a medium containing 20% CM of IEC and its fractions treated or untreated with anti-GM-CSF antibodies for 4 h. Hydroxyurea (1 mg/ml) served as a cytostatic. The cells were transplanted to lethally irradiated syngeneic recipients (5×10^4 cells/mouse). The number of splenic colonies was determined visually on day 8. The ratio of S cells was calculated by the formula

$$\frac{(a\text{-}b)}{a}\times100\%,$$

where a and b are the numbers of hemopoietic colonies from cells incubated without and with hydroxyurea, respectively.

The results were analyzed by Student's t test (Statistica software). The data are presented as means and standard errors.

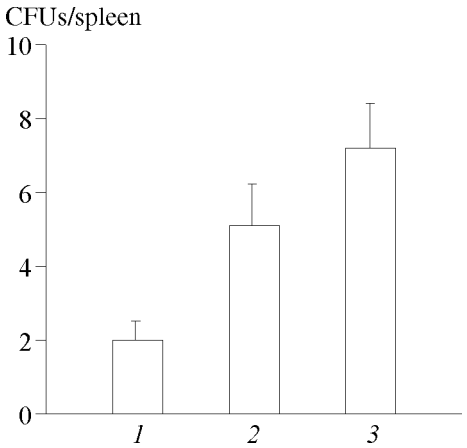

Fig. 1. Number of splenic CFU (CFUs) in lethally irradiated mice on day 8 after syngeneic transplantation of intestinal cells (population of cryptal epithelial cells) in concentrations of 2.5×10^5 (*1*), 1.5×10^6 (*2*), and 2.5×10^6 cells/mouse (*3*).

RESULTS

Transplantation of the total population of epithelial cells from the small intestine to lethally irradiated recipients showed that the intestinal epithelium includes cells capable of forming splenic hemopoietic colonies on day 8 (colony-forming units of the spleen, CFUs, Fig. 1). Our results are consistent with published data on the presence of early hemopoietic precursors in the epithelium of rhesus monkeys [7]. CFUs-8 were not found in the control and after administration of cryptal IEC in a concentration of 2.5×10^6 cells/mouse.

The influence of soluble factors from IEC on functional activity of hemopoietic stem cells was studied by the method of exogenous colony formation in the spleen and cell self-destruction [3]. The bone marrow from intact mice served as the source of hemopoietic stem cells.

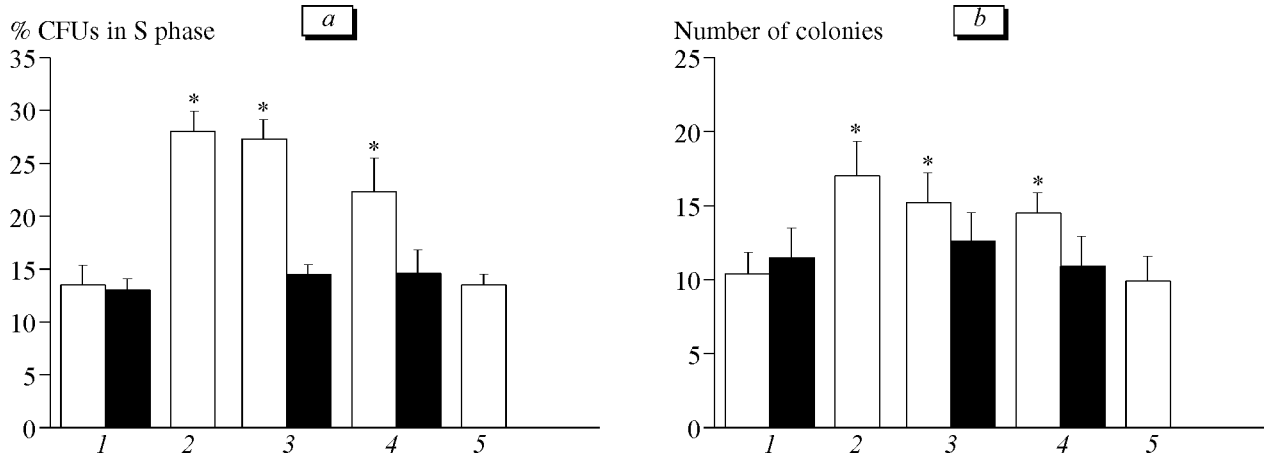

Fig. 2. Effect of the conditioned medium from intestinal epithelial cells and its fractions on proliferative (*a*) and colony-forming activity (*b*) of CFUs-8 before (light bars) and after (dark bars) treatment with biotinylated antibodies to granulocyte-macrophage CSF in a concentration of 6 μg/ml: culture medium (control, *1*), conditioned media (*2*), and fractions 1 (*3*), 2 (*4*), and 3 (*5*). *$p<0.05$ compared to the control.

Culturing of bone marrow cells in the medium containing CM of IEC was followed by a significant increase in proliferative and colony-forming activity of CFUs-8 (Fig. 2). CM was separated into fractions with different molecular weights. Fractions 1 and 2 containing soluble factors with molecular weights of more than 30 kDa and 10-30 kDa, respectively, produced a regulatory effect (Fig. 2).

The quantitative analysis of cytokines in CM and fractions by the method of electrical chemiluminescence [1] revealed GM-CSF in CM and fractions 1 and 2 (37.2, 28.3, and 5.4 ng/ml, respectively). The presence of GM-CSF in 2 fractions resulted from different degree of protein glycosylation. GM-CSF was not found in fraction 3. Previous experiments showed that only human intestinal epitheliocytes produce this cytokine [5].

We evaluated the role of GM-CSF in activation of proliferation and colony formation. GM-CSF-containing fractions 1 and 2 were incubated with biotinylated antibodies to GM-CSF. Antibodies and their complexes with GM-CSF were removed with streptavidin microbeads. This treatment abolished the stimulatory effect of fractions (Fig. 2). In this series the culture medium subjected to similar treatment served as the control.

Our results show that the population of epithelial cells of the small intestine includes precursor cells possessing the ability to form splenic colonies of hemopoietic cells on day 8. Epithelial cells can modulate functional activity of hemopoietic stem cells by producing GM-CSF.

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