Restoration of Hemopoiesis and Immunopoiesis with Small Intestinal Epitheliocytes in Lethally Irradiated Mice

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We studied hemopoiesis- and immunopoiesis-restoring activity of small intestinal epitheliocytes in lethally irradiated mice. The populations of peripheral blood cells and parameters of humoral and cellular immune response in lethally irradiated mice returned to normal 6 months after transplantation of cells from the small intestinal epithelial layer. Study of the distribution of intestinal cells in the body after transplantation showed long-term persistence of the donor cells in tissues of lethally irradiated animals. These data attest to high hemopoietic potential of small intestinal cells.

Key Words: *small intestinal epithelial cells; hemopoiesis; humoral and cellular immune response*

Numerous studies showed that somatic stem cells can differentiate into cell elements of other tissues [14]. Many adult stem cells possess a hemopoietic potential [5,6,9,10]. Small intestinal epithelial cells formed hemopoietic colonies (CFU-9) in the spleens of lethally irradiated mice [1], which can be regarded as a manifestation of "plasticity", *i.e.* stem cells of one tissue can form differentiated cells of another tissue [8]. However, we failed to find reports about restoration of hemo- and immunopoiesis by intestinal cells in lethally irradiated recipients. Here we studied the hemopoiesis- and immunopoiesis-restoring activity of small intestinal epitheliocytes in lethally irradiated recipient mice.

MATERIALS AND METHODS

Experiments were carried out on 3-6-month-old $(CBA \times C57Bl/6)F_1$ mice. The animals from Research Laboratory of Experimental Biomedical Modeling, Tomsk Research Center, receiving standard balanced ration, were sacrificed by cervical dislocation. Irradiation was carried out on a RUM-25 device; the lethal dose was 8.5-9.0 Gy. Small intestinal epithelial layer cells were isolated [12]. The purity of the isolated population was evaluated morphologically, viability was evaluated by trypan blue exclusion. Bone marrow cells were mechanically isolated [2]. Small intestinal epitheliocytes were intravenously injected to syngeneic lethally irradiated recipient mice in a dose of 1.5×10^6 cell/mouse, bone marrow cells in a dose of 1.5×10^4 cell/mouse. Intact mice served as the control.

The dynamics of peripheral blood values (erythrocyte and platelet counts, total leukocyte count, hematocrit) was studied on a CELL-Dyn 900 automated hematological analyzer 1, 3, and 6 months after transplantation. Cellular immunity was evaluated by the delayed-type hypersensitivity (DTH) reaction [15]. Humoral immune response was evaluated on day 5 by the number of local hemolysis zones (IgM-antibody producing cells) in liquid medium after intraperitoneal injection of sheep ery-

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throcytes [7]. Stem cell distribution *in vivo* is sometimes evaluated by detection of labeled donor cells in various tissues of irradiated recipient [13].

We studied the distribution of donor intestinal cells in tissues of irradiated recipient using Y chromosome marker *sry* gene, which was detected by "nested" PCR. DNA was isolated from splenic, bone marrow, liver, intestinal, skin, renal, lung, and Peyer patch cells of recipient females 2, 4, and 6 months after transplantation of 1.5×10^6 cells of male intestinal cells per mouse. DNA samples were amplified in a reaction mixture containing mouse primers specific for Y chromosome [11]. PCR was carried out under the following conditions: denaturation at 95°C for 3 min and 30 cycles of the following protocol: 40 sec at 94°C, 30 sec at 52°C, and 40 sec at 72°C. This was followed by PCR with nested primers [6]. Amplified mixture of primary PCR (1 μl) served as the matrix. Amplification conditions were the same as for the initial PCR, but only 20 cycles were carried out. Amplification yielded a fragment of 320 b.p. Tissue sample from intact males and females served as positive and negative controls, respectively. The data were reproduced in 3-10 similar experiments with 12-20 animals per group. The results were processed using Statistica software. The significance of differences between the groups was verified using nonparametric Mann—Whitney and Kruscal—Wallis ANOVA tests. The data were presented as the means.

RESULTS

Cells containing *sry* gene (Y chromosome marker) were detected by PCR analysis in the bone marrow, spleen, liver, Peyer's patches, intestine, skin, kidneys, lungs, and heart of recipient mice (Fig. 1). The data attest to engraftment of small intestinal epithelial cells in lethally irradiated recipients.

Since intestinal cells can form hemopoietic colonies in the spleen and since recipient survived longer than 6 months, it was interesting to study the dynamics of hemopoiesis recovery in lethally irradiated mice after transplantation of intestinal cells. According to published data, hemopoiesis recovery after bone marrow transplantation includes restoration of the count of cell elements and functional recovery of the bone marrow, specifically, regeneration of the immune system [3]. The next step was study of the dynamics of peripheral blood cell counts and parameters of cellular and humoral immune response in lethally irradiated recipients 1, 3, and 6 months after transplantation of small intestinal epithelial cells. Complete normalization of peripheral blood cell counts (leukocytes, erythrocytes, platelets) in recipients was observed only 6 months after transplantation (Table 1). Total leukocyte count normalized as soon as 3 months after transplantation, erythrocyte and platelet counts 6 months after transplantation. In parallel, we compared hemopoiesis- and immunopoiesis-restoring activities of the intestinal and bone marrow cells. The doses of injected bone marrow cells were selected so that the number of colonies formed in the spleen were equal to the number of colonies forming after intestinal cell transplantation. The counts of leukocytes, platelets, and erythrocytes normalized in experimental animals 6 months after bone marrow cell transplantation. However, the erythroid stem and hematocrit in this group were statistically lower than in experimental mice transplanted small intestinal epithelial layer cells (Table 1). These data

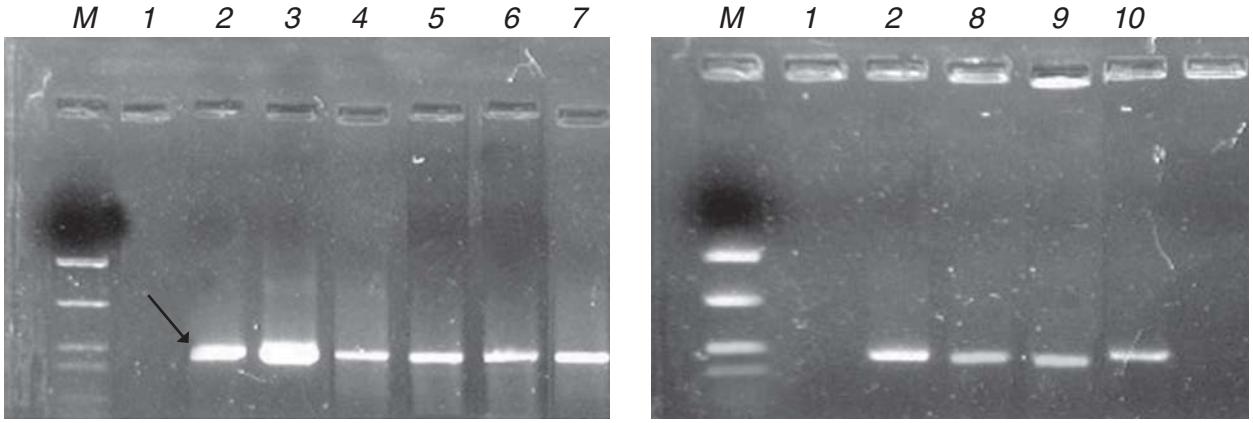

Fig. 1. Detection of *sry* gene (Y chromosome) from cells of lethally irradiated female recipients 2, 4, and 6 months after transplantation of the intestine from male donors. *M*: marker of DNA-pUC19/Kzo91 molecular weight (955, 585, 341, 258 b.p.). Arrow shows a 320-b.p. DNA fragment corresponding to *sry* gene DNA. *1-7*: samples: *1*) negative control (female splenic cells); *2*) positive control (male cells); *3*) bone marrow tissue; *4*) splenic tissue; *5*) liver tissue; *6*) intestinal tissue; *7*) Peyer's patch cells; *8*) renal tissue; *9*) skin cells; *10*) lung tissue.

TABLE 1. Peripheral Blood Values in Lethally Irradiated Recipients

Parameter, group		Month of observation		
		1	3	6
Control		50.9	59.2	51.8
Total content of leukocytes/liter blood, % of control	1	68.2**	83.7	92.6
	2	59.8***	79.1	85.3
Platelet content/liter of blood, % control	1	50.6***	72.7*	89.5
	2	68.2***+	62.5***	94.8
Erythrocyte content/liter of blood, % control	1	84.6***	91.9*	100.9
	2	92.1***+	80.8**	90.2+
Hematocrit, %	1	46.1**	54.3	52.5
	2	48.7	47.9***	47.3**+

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control; +p<0.05 compared to group 1.

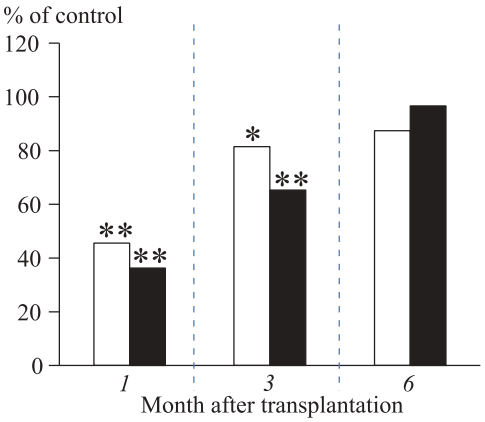

Fig. 2. Intensity of DTH reaction to sheep erythrocytes in lethally irradiated recipients of intestinal epitheliocytes (light bars) or bone marrow cells (dark bars). Here and in Fig. 3: control is taken for 100% (non-irradiated mice). *p<0.05, **p<0.001 compared to the control.

can be explained by high proliferative potential of intestinal cells [4], due to which all hemopoietic stems recover more intensely.

Evaluation of functional activity of immune cells of lethally irradiated mice showed a significant decrease in the intensity of DTH reaction in both experimental groups during the first 3 months, and only 6 months after transplantation the level of cell response did not differ from that in the control group (Fig. 2). No statistically significant differences between the experimental groups were detected. Study of the time course of humoral immunity on the model of primary IgM immune response to T-dependent antigen during the first 3 months after transplantation showed a statistically significant reduction of the absolute counts and percentage of antibody-producing cells (APC) in both experimental groups animals in comparison with the control group (Fig.

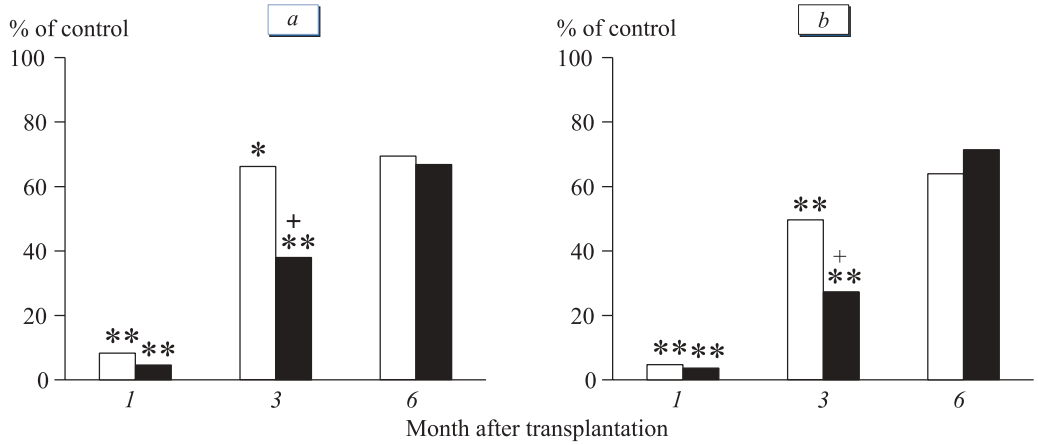

Fig. 3. Percent (per 10^6 cells; *a*) and absolute count (*b*) of IgM-antibody-producing cells in the spleens of lethally irradiated recipients of intestinal cells (light bars) or bone marrow cells (dark bars). +p<0.01 compared to recipients of intestinal cells.

3). However, humoral response was less pronounced in recipients of bone marrow cells than in lethally irradiated mice transplanted small intestinal epithelial cells. Recovery of humoral immunity in both experimental groups was observed only 6 months after transplantation: by this time the level of humoral immune response did not differ from the parameters of mice not exposed to radiation.

Hence, we showed that small intestinal epitheliocytes are not only capable of colony formation in the spleen of lethally irradiated mice [1], but can stimulate hemopoiesis recovery, which is not rapid and reaches the normal level 6 months after transplantation. These data attest to high hemopoietic potential of intestinal cells and prompt the use of small intestinal epithelial layer cells as a model for studies of the phenomenon of "plasticity" of somatic stem cells.

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