EFFECT OF REGENERATION OF HEMATOPOIESIS ON THE COURSE OF THE SYSTEMIC GRAFT VERSUS HOST REACTION

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Reparative regeneration of the spleen increases the resistance of (CBA × C57BL/6) F_1 hybrids to the systemic graft versus host reaction (GVHR) [3]. This phenomenon is accompanied by changes in the functional properties of the lymphoid cells in connection with the heterogeneity of the tissue composition of the spleen. It was decided to study whether such an effect is the result of regeneration of its hematopoietic or its lymphoid component.

For this purpose the course of the systemic GVHR and the hematologic status were investigated in recipients, and the changes in the functional properties of their spleen also were studied during regeneration of hematopoietic tissue induced by acute blood loss.

EXPERIMENTAL METHOD

Experiments were carried out on 155 inbred female (CBA × C57BL/6)F_1 mice weighing 18-20 g and 100 female C57BL/6 mice weighing 20-23 g, obtained from the Inbred Animals Nursery of the Academy of Medical Sciences of the USSR (Stolbovaya). Altogether three series of experiments were carried out on 93 F_1 hybrids. In series I the experimental animals were bled once from the retro-orbital venous sinus in a volume of 0.5 ml 3 days before induction of the GVHR. In series II, the animals were bled twice on this same day, 0.5 ml each time, with an interval of 1 h. In series III the animals were bled three times, in a volume of 0.5 ml each time, daily for 3 days before induction of the GVHR.

The donors for induction of the GVHR were female C57BL/6 mice. A suspension of spleen cells was prepared in the cold by homogenization, filtration twice through a fine-pore Kapron filter, and centrifugation twice at 1000 rpm for 7 and 5 min. The number of viable cells was counted with the aid of trypan blue. Spleen cells thus prepared were injected, in a dose of $(75-100)\cdot 10^6$ into the recipient's retro-orbital venous sinus in a volume of 0.4 ml. Intact animals in which a GVHR was induced, and also animals exposed only to bleeding without induction of the GVHR, served as the control.

TABLE 1. Effect of Acute Blood Loss before Induction of GVHR on Mean Life Span of Recipients

Series of Experiments	Groups of recipients	Number of bleedings	Total loss of blood, ml	Dose of parental cells injected, $\times 10^6$	Number of recipients	Life span of animals	
						$M \pm m$	P
I	Experimental	1	0,5	75	14	26,6±0,99	>0,05
	Control	1	0,5	75	11	28,2±0,98	
II	Experimental	2	1,0	100	12	27,1±0,97	>0,05
	Control	2	1,0	100	11	23,0±1,33	
III	Experimental	3	1,5	75	14	22,6±1,00	<0,001
	Control	3	1,5	75	10	29,5±0,40	

Legend. Period of observation, 30 days.

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TABLE 2. Number of Exogenous CFU in Spleens of F_1 Hybrids 2 and 3 Days after Acute Blood Loss (M ± m)

Time after bleeding, days	Number of animals	Number of exogenous CFU	P
2	10	29,1±2,82	<0,001
3	10	30,8±6,28	<0,001
Intact mice	10	10,3±1,08	—

*Per 10^6 injected spleen cells.

TABLE 3. Degree of Enlargement of PLN Induced by Injection of Spleen Cells of Intact Donors and Three Days after Acute Blood Loss (M ± m)

Group of recipients	Number of animals	Dose of parental cells injected, × 10^6	Degree of enlargement of PLN	P
Experimental	15	10	2,36±0,199	<0,02
Control	15	10	3,26±0,271	—

The peripheral blood of the hybrids of all groups was studied. Blood films were stained by the May–Grunewald method. The state of the stem cells was judged by determining the number of exogenous CFU in the spleens of lethally irradiated mice, receiving $1 \cdot 10^6$ spleen cells of syngeneic donors 2-3 days after bleeding, by the method of Till and McCulloch [6]. The recipients were irradiated on the RUM-17 apparatus in a dose of 900 rads 2 h before injection of the cells. The number of exogenous colonies was counted on the 8th day, and the spleens were fixed in Clarke's mixture (1 part glacial acetic acid and 3 parts ethanol).

To study the immunocompetence of the T cells in the spleens of animals with regeneration of hematopoiesis the method of testing in the local GVHR in the popliteal lymph nodes (PLN) was used. For this purpose, C57BL/6 mice were bled acutely in a volume of 1 ml (0.5 ml twice, with an interval of 1 h). The donors were killed 3 h later and a suspension of their spleen cells prepared by the method described above. Intact (CBA × C57BL/6)F_1 hybrids served as recipients. A local GVHR was induced by the method in [7]. For this purpose, 10^7 living parental lymphoid cells in a volume of 0.02 ml were injected (0.01 ml was injected twice at an interval of 10 min) into the footpads of the hind limbs of the hybrid mice. The experimental results were read on the 7th day: the PLN were removed, dehydrated in acetone, and weighed with an accuracy of 0.1 mg. Depending on the degree of hypertrophy of the PLN, the intensity of the GVHR and the immunocompetence of the injected cells were estimated. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

As Table 1 shows, bleeding once and twice in a volume of 0.5-1 ml 3 days before induction of the GVHR had no significant effect on the course of the reaction or on the mean life span of the recipient. If, however, the animals were bled three times in a volume of 0.5 ml daily for 3 days before induction of the GVHR, intensification of the GVHR and a significant decrease in the mean life span of the animals were observed ($P < 0.001$). It will be noted that in the experimental group the recipients began to fall ill and to die much sooner (from the 17th to the 25th day of the GVHR) than in the control group (from the 26th day). Nevertheless, this was not significantly reflected in the cell composition of the peripheral blood. Ten days after induction of the GVHR the number of erythrocytes and leukocytes in the experimental and control animals was roughly the same. Only a small increase in the number of monocytes was observed in the mice of the experimental group (16.0 ± 2.19 compared with 9.8 ± 1.28 in the control; $P < 0.05$).

Much more marked changes were found in the cell composition and functional properties of the spleen cells. On the second and third day after the beginning of regeneration of the blood in the experimental mice the number of stem cells was significantly higher than in the control animals (Table 2). This could indicate both an increase in their absolute number in the body and also an increase in their migration capacity [1, 2, 4, 5].

The study of the immunologic competence of T cells from the spleens of animals with regeneration of the hematopoietic tissue gave the following results (Table 3). The degree of enlargement of PLN in the experimental animals on the third day after acute blood loss was lower than in the intact hybrids. This indicates that the functional activity of the T cells falls during regeneration of the blood. Such changes in the functional state of the splenic T cells of recipients with reparative regeneration of hematopoiesis evidently aggravate the severity of the course of the GVHR.

The results thus indicate that massive blood loss, carried out before induction of the GVHR, aggravates the course of that reaction. This suggests that regeneration of hematopoietic tissue, unlike regeneration of the spleen, does not increase the resistance of mice to the GVHR. Consequently, it can be tentatively suggested that the increase in the resistance to the GVHR during regeneration of the spleen is due primarily to changes in the lymphoid, and not the hematopoietic, elements of that organ.

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EFFECT OF URETHANE ON PROLIFERATIVE ACTIVITY OF THE EPITHELIUM OF EMBRYONIC MOUSE LUNG ORGAN CULTURES

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Previous investigations have shown that under normal conditions, despite the similarity in their morphogenesis, the survival rate of organ cultures of normal embryonic tissues and, in particular, of the lungs, varies considerably depending on the species, strain, and age of the donor animals [3, 11]. Under the influence of transplacental exposure to carcinogens the survival rate of explanted tissues is considerably increased (the growth-stimulating effect), and specific morphological changes develop in the tissue, culminating in tumor formation [2-5, 7-11]. Interlinear differences are observed in the survival rate and sensitivity to carcinogens of embryonic tissues from mice with high and low predisposition to cancer [3-5, 8, 9].

To study the nature of the growth-stimulating effect of carcinogens comparative investigations were undertaken of the proliferative activity of organ cultures of normal embryonic tissues and of tissues exposed to the action of carcinogens. This paper gives the results of a study of the proliferative activity of the epithelium of embryonic lung organ cultures from two lines of mice: one genetically resistant (C57BL) and one predisposed (A) to the development of lung tumors, under normal conditions and after transplacental exposure to urethane, a carcinogen with affinity for the lungs.

EXPERIMENTAL METHOD

Experiments were carried out on organ cultures of the lungs of 21-day embryos of intact and experimental mice of strains A and C57BL. Urethane was injected subcutaneously, in 10% physiological saline, into the pregnant females once or three times in a dose of 1 mg/g body weight per injection. The effect of a single dose of urethane (1 mg/g body weight) on the 18th and 20th days of pregnancy, i.e., 3 days or 1 day before explantation of the embryonic lungs into culture, was studied in mice of the A strain. The effect of three doses of urethane (3 mg/g body weight) on the 18th, 19th, and 20th days of pregnancy was studied on explants of embryonic lungs of mice of both strains. On the 21st day of pregnancy the intact and experimental females were killed and the lungs of the embryos were explanted into organ culture by the method described previously [2], and studied on the 1st, 7th, 15th, and 21st days in culture. Proliferative activity of the epithelium was judged by the fraction of DNA-synthesizing cells in the explants by an autoradiographic method [6]. ^{3}H-Thymidine was added to the nutrient medium of the cultures 24 h before fixation of the explants, in a concentra-

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