ACTIVATION AND SUPPRESSION OF CELLULAR IMMUNITY DURING REPARATIVE LIVER REGENERATION IN MICE

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Processes of regeneration in vivo are accompanied by morphological and functional changes in the immune system [5]. The method of partial hepatectomy (PHE) on animals is that most widely used for studying the process of regeneration. However, changes in lymphocyte function after PHE have not yet been adequately studied, and in some cases the data are contradictory in character: In some experiments PHE was followed by strengthening of antitumor immunity [2, 4], whereas in others it led to more rapid growth of tumors [11, 12].

The aim of this investigation was to study the effect of PHE on functional activity of various lymphocyte populations *in vitro* and *in vivo*, namely its effect on proliferative, effector, and killer activity.

EXPERIMENTAL METHODS

Female BALB/c, CBA, C57BL/6, and (CBA × C57BL/6)F_1(F_1) mice aged 2-4 months, obtained from the "Stolbovaya" Inbred Animals Nursery, Academy of Medical Sciences of the USSR, were used. PHE involved removal of two-thirds of the liver from mice anesthetized with ether by the usual method [9]. A mock operation (MO) consisting of laparotomy followed by closure of the abdominal would, was performed on the control mice. To determine proliferative activity of the lymphocytes, the mixed lymphocyte culture (MLC) reaction was set up by a modified method in [3]. The reacting cells were spleen cells (SC) from F_1 mice, on which PHE or MO was performed 3, 10, and 22 days before the MLC. A mixture of 50 µl of reacting SC in a dose of $6 \cdot 10^6$ cells/ml and 100 µl of stimulating SC from BALB/c mice, irradiated in a dose of 2000 rads, in a dose of $9 \cdot 10^6$ cells/ml was incubated in a total volume of 200 µl in 96-well flat-bottomed plates (Falcon Plastics, USA) for 5 days at 37°C in an atmosphere of 5% CO_2, in medium RPMI-1640 containing 5% of heated human serum, 2 mM L-glutamine, 10 mM of HEPES buffer, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, and antibiotics (penicillin 100 U/ml, and streptomycin, 100 mg/ml). ^{3}H-thymidine (1 µCi per well in a volume of 50 µl) was added to the medium 16 h before the end of incubation. The samples were transferred by means of a 12-channel device to Synpor-3 filters (Czechoslovakia), treated with TCA and alcohol, and their radioactivity was measured in a β-spectrometer. Pools of SC from two or three mice were tested in three parallel tests for each sample of lymphocytes.

The index of stimulation (IS) of proliferation in MLC was determined by the equation:

$$IS = \frac{a-b}{a} \cdot 100\,\%,$$

where a and b denote incorporation of ^{3}H-thymidine (in cpm) in the experimental and control cultures respectively. The index of inhibition (II) of proliferation was calculated by the equation:

$$II = \frac{b-a}{b} \cdot 100\,\%.$$

To determine suppressor activity of SC from F[mice 10 days after PHE they were added in a dose of $4 \cdot 10^c$ cells"ml and in a volume of 50 µl as the third component in MLC, where

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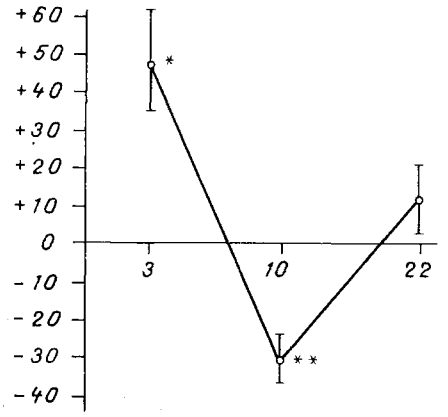

Fig. 1

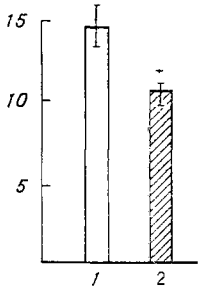

Fig. 2

Fig. 1. Effect of PHE on proliferative activity of SC from F_1 mice in response to BALB/c alloantigens in MLC. Abscissa, days after PHE and MO; ordinate, SI and II (in % of control — level of proliferation of SC from hybrids after mock operation, on each day of testing). *P < 0.02, **P < 0.01. Results of one typical experiment (M ± m).

Fig. 2. Detection of suppressor activity of SC from F_2 mice 10 days after PHE in MLC. Ordinate, incorporation of ^{3}H-thymidine in MLC (in cpm × 10^3). 1) SC from F_1 mice added in MLC 10 days after MO (control); 2) SC from F_1 mice added to MLC 10 days after PHE. *P = 0.02. Results of three experiments (M ± m).

the reacting cells were lymphocytes of intact F_1 hybrids, and the stimulators were irradiated SC of BALB/c mice. SC from mice undergoing the mock operation were added to the control wells in the same dose. Suppression was estimated by the reduction of incorporation of ^{3}H-thimidine in the experimental wells compared with the controls.

A regional graft versus host reaction (rGVHR) was induced in the popliteal lymph nodes (PLN) by subcutaneous injection of $5 \cdot 10^6$ parental SC from C57BL/6 or CBA mice in a volume of 50 µl of medium 199 into the right foot by the method described previously [8]. An injection of $5 \cdot 10^6$ syngeneic SC from F_1 mice was given into the left paw. PHE and MO were performed on the CBA donors or recipients 10 days before induction of the rGVHR. Seven days later the PLN were removed from the recipients, dehydrated in acetone, and weighed with an accuracy of 0.01 mg. The strength of the rGVHR was judged by the degree of hypertrophy of PLN, determined by the formula:

$$\frac{\text{weight of right PLN}}{\text{weight of left PLN}}.$$

Activity of immune and normal killer cells was determined in the membrane-toxic test with ^{3}H-uridine by a modified method in [6]. Immune killer cells were obtained by intraperitoneal injection of $25 \cdot 10^6$ thymoma E1-4 cells, which were maintained by weekly passage through C57BL/6 mice, into BALB/c mice. SC from immune BALB/c mice with or without PHE (immune killer cells) and SC from normal BALB/c mice with PHE 11 days previously or without the operation (normal killer cells) were used in the experiments 11 days later. E1-4 target cells were labeled with ^{3}H-uridine (3 µCi/ml, specific activity 24 Ci/mmole) in a dose of $3 \cdot 10^6$ ml for 1 h at 37°C, and washed three times. The killer cells were treated with actinomycin D (from West Germany, concentration 1 µg/ml) for 1 h at 37°C and washed three times. The test was wet up in round-bottomed plastic plates in a volume of 0.2 ml of medium RPMI-1640 with the addition of 10% fetal calf serum and 10 mM HEPES buffer. Into each well was poured 10^4 labeled target cells, different numbers of killer cells or medium, and pancreatic RNase (from Calbiochem, USA) in a final concentration of 24 U/ml. Incubation lasted 4 h at 37°C. The cells were harvested and the samples counted as described in the MLC method. The cytotoxicity index (CTI) was calculated by the equation:

$$\text{CTI} = \left(1 - \frac{\text{number of counts in test well}}{\text{number of counts in well without killer cells}}\right) \times 100\%.$$

TABLE 1. Testing Strength of rGVHR in F_1 Mice 10 Days after PHE on Donors or Recipients

Expt. No.	Number of mice	Strain of donor	Type of operation		PLN (expt), mg/ PLN (control), mg	P
			on donor	on recipient		
1	23	C57Bl/6	—	MO	1,86±0,10	
2	23	C57Bl/6	—	PHE	1,49±0,13	<0,05
3	15	CBA	MO	—	2,23±0,20	
4	15	CBA	PHE	—	1,82±0,16	>0,20

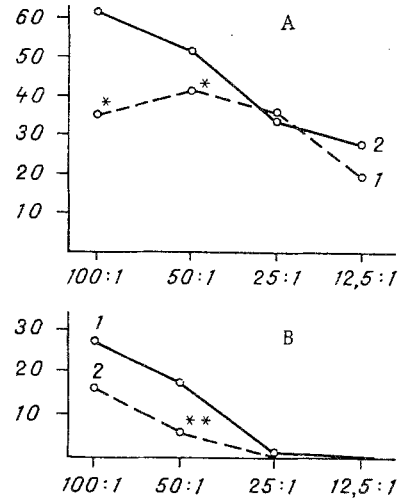

Fig. 3. Testing activity of immune and normal killer cells on E1-4 targets in BALB/c mice 11 days after PHE (immune killer cells were SC from BALB/c mice taken 11 days after immunization by $25 \cdot 10^6$ E1-4 cells). Abscissa, ratio of killer to target cells; ordinate, CTI of immune (A) and normal (B) killer cells (in %). 1) SC from BALB/c mice not undergoing operations (control), 2) SC from BALB/c mice after PHE. *P < 0.01, **P = 0.05. Mean results of 6-9 experiments.

The results were subjected to statistical analysis by Student's t test.

RESULTS

To discover whether the decrease in proliferative activity of the mouse SC 10 days after PHE is connected with the action of suppressor cells, SC from the hepatectomized F_1 mice were added to syngeneic intact cells reacting in MLC. It will be clear from Fig. 2 that incorporation of ^{3}H-thymidine into reacting lymph node cells from F_1 mice was reduced on addition of SC from partially hepatectomized mice compared with that observed on addition of SC from animals which had undergone MO (P = 0.02).

Thus suppressor cells inhibiting proliferation in MLC were found in SC of the mice 10 days after PHE. This is in agreement with recent data on generation of suppressor cells in mouse lymph nodes at this time after PHE [13]. Stimulation of proliferation 3 days after PHE confirms the observed increase in lymphocyte activity in the early stages of regeneration of the liver, obtained on other models [10].

A decrease in activity of mouse lymphocytes 10 days after PHE also was observed in experiments *in vivo*. In partially hepatectomized hybrids rGVHR was significantly reduced compared with animals undergoing the mock operation. After injection of SC from CBA mice into intact hybrids 10 days after PHE a decrease in the strength of the rGVHR was also observed, although it was not significant (Table 1).

The results of the last series of experiments showed (Fig. 3) that activity of immune and normal killer cells also decreases during regeneration of the liver (under these conditions of generation immune killer cells constitute a population of cytotoxic T lymphocytes [7]. The fall in CTI in immune killer cells from partially hepatectomized mice was ob-

served when the ratio of killer to target cells was 100:1 and 50:1, but as regards normal killer cells, a significant decrease in CTI was observed only when the ratio was 50:1 (cytotoxicity was determined 11 days after PHE in BALB/c mice). The results are in agreement with data in [1], according to which, on the 9th day after PHE on CBA mice activity of normal killer cells also was reduced, but they are contradicted by other data [4]. In the latter case, however, the stimulating effect of PHE on normal killer cell function occurred after removal of half of a lobe of the liver from the mice, and not two-thirds of the liver according to the usual method, and this might have affected the results. A decrease in cytotoxicity of T and normal killer cells after PHE may be one cause of the more rapid growth of tumors in animals with a regenerating liver [11, 12].

Depression of killer activity and of the graft versus host reaction after PHE is evidently due to the action of suppressor cells, as is shown by the example of lowering of the helper function in MLC. The authors are grateful to Professor B. B. Fuks for providing the reagents for the membrane-toxicity test.

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INTERLEUKIN 2 PRODUCTION IN PATIENTS WITH THYROID DISEASES

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The T-cell growth factor interleukin 2 (IL 2) plays a key role in maintaining the function and permitting expansion of various classes of cytotoxic lymphocytes [8-10]. During antigenic stimulation, intercellular interactions of complex structure and realized through the lymphokine cascade are activated; one essential component of this cascade is production of IL 2 by antigen-specific T helper cells [6, 12].

One of the factors controlling the level of IL 2 production may be the endocrine system. It has been shown that ACTH and glucocorticoids directly control IL 2 production [7, 11, 12]. Thyroid hormones possible play a similar role as well, and it was therefore decided to study the effect of differences in thyroid function on the IL 2 level, in states of hyper- and hypothyroidism. Mice of strains predisposed to the development of autoimmune diseases are known to have a low level of IL 2 production [4, 5]. In patients with rheumatoid arth-

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