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SUPPRESSION OF EPTHELIAL CELL PROLIFERATION IN MICE BY SPLENOCYTES FROM UNILATERALLY SIALADENECTOMIZED SYNGENEIC DONORS

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Splenocytes of the regenerating spleen suppress proliferation of cells of a different histological type in syngeneic recipients [3]. This property of splenocytes coincides in time with their ability to inhibit several immune reactions [4].

To identify the type of lymphoid cells involved in regulating the level of proliferation in nonlymphoid organs and to ascertain any common features of its mechanism for cells of any histological type, the best course to follow is to choose an experimental model in which splenocytes from the undamaged spleen would possess suppressor properties relative to lymphocytes. We considered that an operation on the spleen itself would induce a combination of changes in the properties of its cells, in which their suppressor properties could be the result of many causes, not merely an increase in T-lymphocyte activity.

In the investigation described below the effect on proliferation of hepatocytes of the regenerating liver and of corneal epitheliocytes of the recipients of splenocytes after unilateral sialadenectomy (USE) in mice, which is accompanied by inhibition of the immune response [4], was studied. Both the tissue systems chosen have a high level of proliferation, so that the effect of inhibition of cell division could be distinguished more clearly.

EXPERIMENTAL METHOD

Altogether 395 male CBA mice weighing 18-20 g and obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. USE on the future donors (removal of the submandibular and sublingual glands en bloc) and partial hepatectomy (removal of one-quarter of the liver tissue) on the future recipients were carried out under ether anesthesia. The animals were killed by cervical dislocation. The donors were killed 17, 48, 72, 144, and 168 h after the operation (the donor interval). A suspension of spleen cells was prepared by the method described previously [2]. The suspension was washed twice and centrifuged for 7 and 5 min at 1000 rpm and at 4°C. After determination of the viability of the lymphoid cells they were injected into the retro-orbital sinus in a dose of $7 \cdot 10^6$/ml of medium 199 for each recipient immediately after the operation. The recipients were killed mainly 48 h, but in certain cases 24 and 72 h after transfer (the recipient interval). Partially hepatectomized and intact recipients, which received the same dose of splenocytes either of intact donors (ID) or of donors undergoing a mock operation (MOD), served as the control. Each group consisted of 7-10 mice. Mitotic activity (MA) was determined in paraffin sections through the liver, 5 μ thick, stained with hematoxylin and eosin; the number of dividing cells was counted in 6000 hepatocytes and in total preparations of the cornea of the intact

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TABLE 1. MI (in ‰) of Hepatocytes and Corneal Epitheliocytes 48 h after Partial Hepatectomy and Transfer of ID, MOD, and USD Splenocytes (M ± m)

Test object	Group of animals	Donor interval, h					
		0	17	48	72	144	168
Hepatocytes	Intact	0,0±0	—	—	—	—	—
	Partially hepatectomized	5,3±2,0	—	—	—	—	—
	Partially hepatectomized recipients of splenocytes						
	ID	3,7±1,31	—	—	—	—	—
	MOD	—	3,0±0,27	2,9±0,24	2,7±0,2	3,5±1,0	2,3±0,16
	USD	—	0,64±0,2	16±0,14	2,0±0,38	0,57±0,27	0,25±0,17
Corneal epitheliocytes	Intact	16,0±0,49	—	—	—	—	—
	Partially hepatectomized	8,6±0,65	—	—	—	—	—
	Partially hepatectomized recipients of splenocytes						
	ID	17,0±0,9	—	—	—	—	—
	MOD	—	13,2±1,13	11,4±0,46	14,3±0,49	16,0±0,4	14,7±0,26
	USD	—	3,8±0,66	6,0±0,48	4,9±0,45	14,9±0,6	12,3±1,0

TABLE 2. MA of Hepatocytes and Corneal Epitheliocytes of Partially Hepatectomized Mice under the Influence of USD Splenocytes Depending on Duration of Recipient Interval (donor interval 17 h)

Group of animals	Time after operation, h					
	liver			cornea		
	24	48	72	24	48	72
Intact	0,25±0,08	0,25±0,08	0,25±0,08	17,0±2,12	17,0±2,12	17,0±2,12
Partially hepatectomized	0,7±0,23	3,0±0,16	3,0±0,76	3,6±0,4	16,0±0,96	8,8±1,1
Recipients of splenocytes						
ID	0,6±0,15	2,7±0,26	1,5±0,19	10,0±1,0	16,5±0,74	14,0±0,4
MOD	0,7±0,11	3,0±0,27	1,0±0,19	11,5±1,05	15,0±0,79	16,0±0,55
USD	0,35±0,35	0,0	0,37±0,23	8,9±0,54	8,0±0,57	8,45±1,54

Legend. All experimental and control animals were killed on the same day at 10-11 a.m.

eye. The mitotic index (MI) was expressed in promille. There were altogether three series of experiments, which had different aims.

In series I the level of activity of the donor's splenocytes was studied, depending on the length of the donor interval. The times of death of the donors were chosen allowing for data on the immune reactivity of their lymphocytes to sheep's red blood cells at different times after removal of the submandibular salivary gland [1].

In series II the proliferative activity of hepatocytes and corneal epitheliocytes of partially hepatectomized mice under the influence of splenocytes possessing maximal suppressor activity (17 h) was studied, depending on recipient interval. The recipients were killed 24, 48, and 72 h after transfer of the lymphocytes.

In series III an attempt was made to solve the problem of the effect of an operation on the recipient on its sensitivity to the action of splenocytes. The principal test object was the cornea, as a tissue with normally high level of MA. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results of the determination are given in Table 1, and they show that removal of one-quarter of the liver tissue increased MA of the hepatocytes to 5.3‰ 48 h after the operation. Injection of splenocytes from ID and MOD, isolated at different times after the mock operation, into the hepatectomized recipients immediately after the operation, did not lead to any significant decrease in MI of the hepatocytes of the regenerating liver. Meanwhile, splenocytes from unilaterally sialadenectomized donors (USD), isolated from the spleen 17, 144, and 168 h after the operation, sharply reduced MI in the regenerating liver 48 h after transfer. Reduction of the proliferative activity of the hepatocytes under the influence of splenocytes isolated 48 and 72 h after the operation had no significant effect on the level of proliferation in the regenerating liver of mice not subjected to any additional procedures. Meanwhile, the inhibitory activity of USD splenocytes was significantly stronger (except after 72 h) than the MOD splenocytes. It is interesting to note that it was at these times (48

and 72 h) that a significant but moderate increase in the ability of the splenocytes to respond to the antigenic stimulus during immunization of mice with sheep's red blood cells at the time intervals indicated [1] was observed.

Partial hepatectomy itself caused a decrease in MI in the cornea 48 h after the operation. Injection of ID and MOD splenocytes into the hepatectomized animals significantly reduced this effect of inhibition of proliferative activity; USD splenocytes possessed the same properties, when isolated 144 and 168 h after the operation. Meanwhile, 17, 48, and 72 h after USE, splenocytes injected into partially hepatectomized recipients gave rise to even deeper inhibition of MA of the corneal epithelium than the operation itself. The results of series II are given in Table 2. No changes in MA in the hepatocytes of the recipients took place 24 h after the operation and after a combination of operation with transfer, irrespective of whether the donors had undergone an operation or not. A real increase in hepatocyte proliferation was present 48 h later in the regenerating liver, and was unchanged if ID and MOD splenocytes were injected into the partially hepatectomized animals, also killed 17 h after the operation. Meanwhile USD splenocytes induced a sharp fall (to O) of MI of the hepatocytes, which still persisted 72 h after transfer. This indicates the duration of the suppressor effect of USD splenocytes.

The effect observed in series I was repeated in the cornea. The operation on the liver led to a sharp decrease in MI in the cornea. Injection of ID, MOD, or USD splenocytes into partially hepatectomized recipients weakened the effect of inhibition of MA. At a later stage after the operation (48 h) MI in the cornea returned to the normal value. Injection of ID and MOD splenocytes was not significantly reflected in proliferation of the corneal epithelium. Meanwhile, under the influence of USD splenocytes, it continued to remain at a low level (compared with the control) until the end of the time of observation.

The experiments of series III showed that injection of USD splenocytes into intact recipients did not reduce MI 48 h after transfer (17.0 ± 2.2% in the control, 17.0 ± 0.9% in the experiment), nor did injection of ID and MOD splenocytes (16.5 ± 0.74 and 15.0 ± 0.79%). The data given above are evidence that during an operation on an epithelial organ splenocytes acquire the ability to suppress proliferation of the cells of nonlymphoid organs. This property coincides in time with the corresponding property of giving an immune response.

Inhibition of proliferation does not possess organ specificity, for after an operation on a salivary gland, MI is reduced in other tissue systems (in the liver and corneal epithelium). The inhibitory effect depends on the donor and recipient intervals and on the recipients' sensitivity.

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