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STUDY OF MECHANISM OF INHIBITION OF THE IMMUNE RESPONSE IN PARABIONTS OF DIFFERENT AGES

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When animals of different ages are joined together in parabiosis for a long time with common circulation and joint cell metabolism, the immune response of the younger partner is inhibited considerably whereas the stimulating effect of the younger partner on the older is small [1]. Other investigations [7, 10, 11] of this problem have shown that the older partner or its tissues are carriers of information accelerating ontogenetic development, which "age" the younger partner on close contact with it. As a result the question arises: What is responsible for this inhibitory effect? There is evidence that the old spleen may be a source of suppression factors [5] and that splenectomy in old animals increases the residual life span in mice of certain lines [2].

The effect of some experimental factors on the primary immune response was studied in animals of different ages connected together in parabiosis.

EXPERIMENTAL METHODS

Female CBA mice of different ages obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used. Operations to form parabiotic pairs were carried out on animals aged 2-3 and 22-25 months under intraperitoneal hexobarbital anesthesia by the method of Bunster and Meyer [4]. The parabiotic pairs were separated surgically two months after the first operation. The immune response in the animals of this group was determined 1 and 3 months later. In another experimental group the spleen of the older partners was removed during the operation for forming parabiotic pairs. The older parabionts of the third group were irradiated with x rays in a dose of 1000 R 3-5 days after joining to the younger animals (RUM-17 apparatus, voltage 180 kV, current 15 mA, filters 0.5 mm Cu + 1 mm Al, dose rate 100 R/min). During irradiation the younger animals were screened. The duration of parabiosis was 2 months. The level of the primary immune response was determined on the fourth day after intraperitoneal injection of $5 \cdot 10^8$ sheep's red blood cells, by counting the number of direct [8] and indirect [6] plaque-forming cells (PFC) in the spleen and by measuring the levels of serum hemolysins and hemagglutinins.

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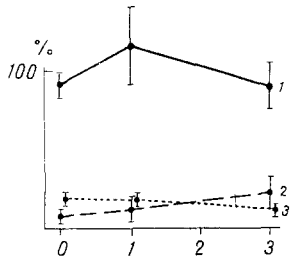

Fig. 1

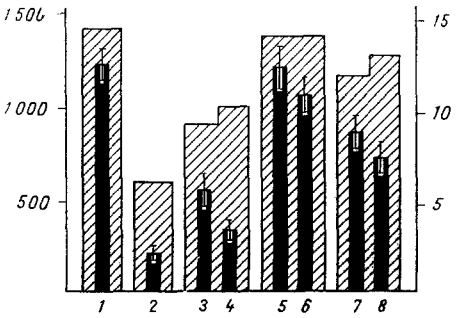

Fig. 2

Fig. 1. Primary immune response in paraboints of different ages at different times after separation. 1) Young animals connected with young; 2) young animals connected with old; 3) old intact animals. Abscissa, time after separation of parabiotic pairs (in months); ordinate, number of direct PFC per 10^6 splenic karyocytes (in percent of immune response of young intact animals).

Fig. 2. Number of direct PFC per 10^6 splenic karyocytes (black columns) and hemolysin titers in serum (obliquely shaded columns) in parabionts 2 months after irradiation. 1) Young intact animals; 2) old intact animals; 3) young animals connected with old (4); 5) young animals connected with young irradiated animals (6); 7) young animals connected with old irradiated animals (8). Ordinate: left, number of direct PFC, right — $\log_2$ of antibody titers.

TABLE 1. Primary Immune Response in Parabionts with Splenectomy on One of the Partners (M ± m)

Index	Group of experimental animals							
	1-		2-		3-		4-	
	Y	O	Y	O	Y	O	Y	O
No. of direct PFC per spleen, thousands	210,6±40,5	21,9±6,0	193,2±22,1	—	66,2±8,5	48,2±6,7	63,6±14,2	—
No. of indirect PFC per spleen, thousands	49,5±8,4	4,2±1,5	67,7±10,7	—	10,8±3,0	7,2±3,1	15,5±4,9	—
Hemolysin titer, $\log_2$	9,8±0,8	2,2±0,7	8,1±0,1	8,4±0,3	5,9±0,6	5,9±0,6	4,4±1,0	3,8±0,7
Hemagglutinin titer, $\log_2$	7,0±1,0	2,0±0,5	6,1±0,3	5,7±0,2	3,7±0,4	4,0±0,7	2,5±0,8	2,3±0,6
No. of animals in group	5	8	7	7	8	8	7	7

Legend. Group 1) single animals; 2) young pairs with splenectomy; 3) parabionts of different ages; 4) parabionts of different ages with splenectomy on older partner; Y) young animals; O) old animals.

EXPERIMENTAL RESULTS

By themseleves the procedures of formation and separation of the parabiotic pairs did not affect the level of the primary immune response, as was confirmed by tests on young animals united in parabiosis with animals of the same age (Fig. 1). Joining the younger partner to an older animal depressed the immune response of the former, as shown by a decrease in the number of direct PFC in the spleen and in the level of antibodies in their serum to those found in the older mice. The immune response had not started to recover even one month after separation of the young animals from the old, and a weak tendency toward recovery only began to be observed after 3 months.

Removal of the spleen did not lead to changes in the immune response if animals of the same age were connected together in parabiosis (Table 1). When animals of different ages were connected together, after removal of the spleen of the older partner the level of the

primary immune response, as reflected in all indices, was the same as in parabiotic partners of different ages in the absence of splenectomy. In this case also the inhibitory influence of the older animal was highly significant ($P < 0.002$). The antibody level in the parabiotic partners did not differ significantly in the pairs either with or without splenectomy.

Exchange of cells has been shown to begin starting with the fourth day of parabiosis [3]. For that reason irradiation at this time creates the conditions for unidirectional cell movement — from the young unirradiated animal to the old irradiated partner. If both animals of the pair were young, the immune response of the unirradiated animal was unchanged whereas the immune response of the irradiated partner was only reduced a little (Fig. 2). On irradiation of the older animals in the pair of different ages, a significant increase in the number of direct PFC was observed in the spleen of both the younger ($P < 0.02$) and of the older ($P < 0.005$) partner compared with the unirradiated parabiont of different ages, although it did not reach the level in the younger parabionts. Similar significant changes also were found in the number of indirect PFC and the antibody titers.

It can accordingly be concluded that depression of the immune function of the younger animals during close contact with an older animal, as the result of which there is an exchange of humoral factors and cells, is stable and continues for at least three months after separation of the parabionts. Removal of the older partner's spleen does not abolish this inhibitory effect, and only total irradiation of the older animal can reduce this substantially. It can be tentatively suggested that irradiation of the old animal causes death of suppressor cells of medullary [9] or thymic [5] origin, which appear during aging, or that it damages certain suppressor mechanisms, as the result of which the influence of the younger animal becomes dominant.

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