PRIMARY IMMUNE RESPONSE IN PARABIOTIC PAIRS OF DIFFERENT AGES

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The functional capacity of the immunity system is known to decline during aging, largely in connection with changes in the immunocompetent cells [9, 11]. Changes in these cells depend in turn on the influence of the environment in which their morphogenesis and differentiation take place [1, 8, 13]. This environmental effect may be mediated by the cellular microenvironment, or by humoral and, in particular, endocrine factors or certain unknown regulatory influences at the cell population level. Changes in these regulatory mechanisms with age probably lead to changes in the immune function during aging [5, 7].

The object of this investigation was to study the mechanisms of development of insufficiency of the immune function during aging. For this purpose an attempt was made to alter the cellular environment by using parabiosis between animals of different ages as the model.

EXPERIMENTAL METHOD

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 and hexobarbital anesthesia in a dose of 110 mg/kg body weight, by the method of Bunster and Meyer [4], during which the peritoneum, muscles of the abdominal wall, scapular muscles, and skin were sutured together.

The parabiotic pairs could survive 2 years or more if both partners were young at the time of the operation, but not more than 7 months if one of the partners at operation was 22 months old and the other 3 months old.

To study the humoral connection between the parabiotic partners one of them was given an intraperitoneal injection of 1% indigocarmine solution. The function of the vascular anastomoses between the partners was assessed on the basis of the rate of disappearance of hens' erythrocytes, injected into one of the partners,

TABLE 1. Colonization of Bone Marrow, Thymus, and Spleen by Parabiotic Partner's Cells (in %)

Test object	Young partner		Old partner	
	its own cells	foreign cells	its own cells	foreign cells
Bone marrow	78±4,6 (3)	22±4,6 (3)	91±1,7 (3)	9±1,7 (3)
Thymus	70±0 (2)	30±0 (2)	69±2,0 (2)	31±2,0 (2)
Spleen	58±2,5 (3)	42±2,5 (3)	67±1,2 (3)	33±1,2 (3)

Legend. Number of parabiotic pairs shown in parentheses

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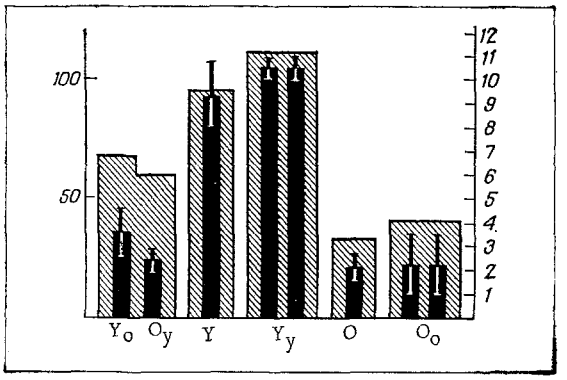

Fig. 1. Number of direct PFC per spleen (black columns) and serum hemolysin titers (shaded columns) in parabiotic pairs. Y) Single young animals; O) single old animals; Y_y) young animals connected with young; O_o) old animals connected with old; Y_o) young animals connected with old; O_y) old animals connected with young. Ordinate: on left, number of PFC (in thousands), on right: $\log_2$ of dilutions of serum.

from the blood stream compared with the rate of their elimination from the blood stream of a single animal. To determine colonization of the lymphoid organs of the animal by circulating cells of the other partner in parabiosis, CBA mice were connected with animals of the subline CBA T6T6. The primary immune response of the parabiotic pair to injection of sheep's erythrocytes was studied 8-10 weeks after the operation. Its magnitude was determined on the 4th day by the number of direct [10] and indirect [6] plaque-forming cells (PFC) in the spleen and by determining the levels of hemolysins [12] and hemaglutinins [2] in the blood serum.

EXPERIMENTAL RESULTS

Investigation of the humoral connection between the partners by injection of indigocarmine showed that 18 h after the operation soluble substances could pass from one animal to the other. After 2 months, nucleated erythrocytes injected intravenously into one partner were found in the blood stream of the other partner. It was calculated that 1% of the blood per minute was exchanged through the vascular anastomoses of the partners. As a result of these anastomoses, circulating cells were exchanged. This was confirmed by investigation of parabiotic CBA–CBA T6T6 pairs. Data on colonization of the bone marrow, thymus, and spleen by the partner's cells 6 months after the operation are given in Table 1. No difference was found in colonization of the thymus by "foreign" cells of the old and young partners. Bone marrow and spleen of the young partner were colonized to a greater degree by these cells than in the old animal. Different organs of the immunity system were colonized to different degrees by "foreign" cells.

Investigation of the primary immune response showed (Fig. 1) that it was several times weaker in the old than in the young animals. Operations to form parabiotic pairs affected neither the antibody level nor the number of PFC in the spleen. Antibody titers were identical in both parabiotic partners. In the young partner connected with an old animal, the number of both direct and indirect PFC was considerably less than in young control animals and was nearer to the level found in old animals. Meanwhile, the number of PFC in the spleen of the old partner was unchanged or, in some groups, it showed only a tendency to increase. Direct correlation was found between the magnitude of the immune response of the old animal and that of its young partner ($\rho^S = 0.82$; $P < 0.001$). The antibody level correlated with the number of PFC, and in parabiotic pairs of different ages its value was intermediate between the antibody levels in the young and old control animals.

The immune response was studied in animals of three groups differing in the time they were obtained and in the season of the investigations. Initial levels of the immune response in young and old animals in these groups differed a little. However, in all three groups the change described above in the immune response of partners of different ages was observed (Fig. 2). The immune response of the young partner depended on the immune response of the old partner to a greater degree.

The results show that in a system of parabiosis between animals of different ages the old animal has the determining influence on the immune response of the young partner. Meanwhile, the young partner has no

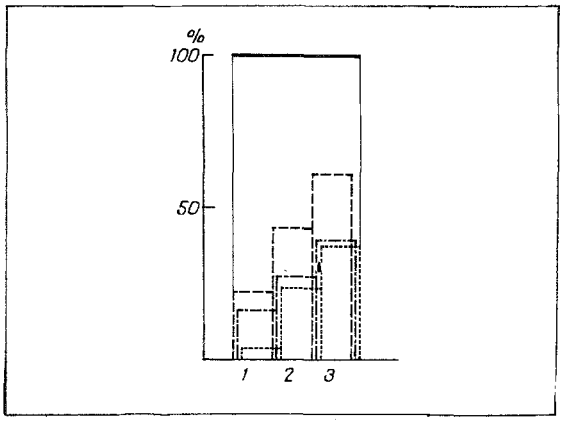

Fig. 2. Immune response of young parabiotic partners depending on initial level of immune response of old animals. Magnitude of immune response of young control animals taken as 100%. Abscissa, group of animals. Continuous line represents single young animals, broken line young animals connected with old, line of dots and dashes old animals connected with young, dotted line single old animals.

clearly marked stimulating effect on the old. If the phenomena observed during aging were simply the result of a decrease in the number or activity of particular types of immunocompetent cells, this defect in the old partner could be compensated by the young partner, for in order to restore the immune potential of a lethally irradiated animal it is sufficient to inject 10^7 bone marrow cells [1], i.e., about one-fortieth of the total number of bone marrow cells in a mouse [3, 14]. For the same reason, the reduction in the immune response of the young partner cannot be regarded as the result of simple redistribution of its immunocompetent cells between the two partners. More probably, active inhibition of the immunity system of the young partner by the old takes place. An investigation by Tauchi and Hasegawa [15] showed that changes characteristic of the liver cells of an old animal take place in the liver of a young parabiotic partner. In that case there were only humoral influences. It can consequently be suggested that substances with a suppressor effect on the immune response of the young partner circulate in the blood of the old partner.

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