

EFFECT OF IMMUNIZATION ON MITOTIC ACTIVITY OF THE THYMOCYTES AND THE LEVEL OF HUMORAL ANTIBODIES

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Experiments on mice immunized with sheep's erythrocytes showed that the small lymphocytes from the cortex of the thymus react to immunization by doubling their level of mitotic activity during the next 20 days of the experiment. The rise in mitotic activity is followed by a corresponding increase in humoral antibody production.

Investigation of cell proliferation in the thymus is interesting from the point of view of elucidating the principles governing tissue removal in that organ and its role in immunogenesis. Despite past investigations of cell division in the thymus [3-6], the link between its functional activity and cell renewal processes and its reactions to antigen injection in vivo have not yet been explained.

The view is held that, unlike other lymphoid organs, the thymus plays no part (at least directly) in the immune reactions of the organism [8], because of the existence of a blood-thymus barrier, described by Weiss [9], which is impermeable to antigen. At the same time, thymus cells have been shown to react to an antigen (tetanus toxin, human serum, tuberculin), when injected intraperitoneally or intravenously, by the liberation of lysine-rich histones from their nuclei [7]. This conflicts with the notion of absolute impermeability of the blood-thymus barrier to antigen.

TABLE 1. Dynamics of MA of Thymocytes (in $0/00$) at Various Times after Immunization

Time after immunization (in days)	Control	Immunization	P
2	2,4	2,6	—
4	2,2	4,2	0,002
6	2,2	1,8	—
8	2,7	2,0	0,071
10	2,6	3,8	0,034
15	2,7	1,5	0,012
20	2,7	1,9	0,12

Note. For the series of experiments with immunization, 2nd-4th day $P=0.016$; 4th-6th day $P=0.001$; 8th-10th day $P=0.002$; 10th-15th day $P=0.001$.

It appeared interesting to study whether changes in mitotic activity (MA) of the thymocytes take place in response to immunization, and, if so, to determine the dynamics of these changes at various times after injection of antigen.

A preliminary investigation [2] showed that 3-4 days after injection of foreign protein into C57BL mice there is an increase in MA of the thymocytes and lymphocytes of the inguinal lymph glands.

In the present investigation, the MA of the small lymphocytes in the cortex of the thymus was studied and the humoral antibody level was also determined at various times after injection of antigen.

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EXPERIMENTAL METHOD

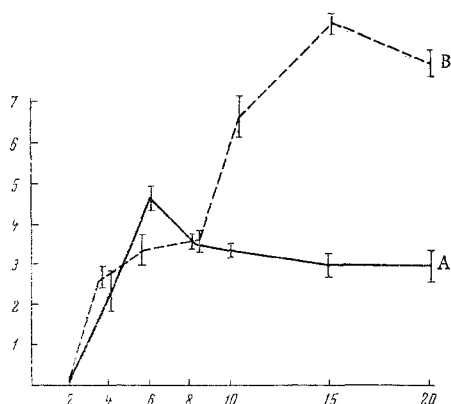


Fig. 1. Dynamics of changes in titers of hemolysins (A) and hemagglutinins (B) at various times after immunization. Abscissa, days after immunization; ordinate, antibody titer in log₂ units.

Experiments were carried out on male albino mice weighing 20-22 g. The animals were immunized by a single intraperitoneal injection of 1 ml of a 5% suspension of sheep's erythrocytes (10^9 cells) and were sacrificed parallel with control animals after 2, 4, 6, 8, 10, 15, and 20 days in groups of 6-7 mice at the same time of day. The thymus and spleen were removed and weighed, and the blood was collected. The organs were fixed in Carnoy's fluid and embedded in paraffin wax. Sections, 4μ in thickness, were stained with Carazzi's hematoxylin and examined under immersion with a binocular attachment (10 \times), and mitoses were counted in 15,000-18,000 cells in the inner zone of the thymus cortex. Statistical analysis was carried out by the Fisher-Student method.

The titers of hemolysins and hemagglutinins in the serum of the immunized animals were determined in the usual manner. The original dilution of serum was 1:10. Titers were expressed in log₂ units.

EXPERIMENTAL RESULTS

A marked response of the thymus cortex to injection of the antigen was observed in the immunized mice, in the form of a bimodal increase in the MA level of the thymocytes with maxima after 4 and 10 days, followed by a decrease after the 15th day (Table 1). Differences between the values of MA in the control groups were not significant (for the 4th-8th days, $P=0.097$).

It will be clear from Fig. 1, showing the dynamics of hemolysin and hemagglutinin formation, that the production of serum hemolysins reached a maximum 6 days after antigenic stimulation. The titers of hemagglutinins reached their maximum 15 days after injection of sheep's erythrocytes.

The weight of the thymus and spleen showed no significant change throughout the period of the experiment. The mean weight of the thymus of the control mice was 57 mg, and of the experimental mice 51 mg, while the weights of the spleens were 137 and 158 mg respectively.

The view is firmly held in the literature that the existence of the blood-thymus barrier, described by Weiss, is an obstacle to penetration of antigen into the tissues of the thymus. However, in more recent investigations it has been shown that foreign protein can in fact enter the thymus [1, 7].

A no less important discovery is that cortical cells of the thymus react to injection of antigen into the body by a change in their MA. Recent work has shown that the thymus responds to intraperitoneal injection of sheep's erythrocytes by an increase in its MA after 4 and 10 days, and in both cases the increase is followed by a decrease in MA, becoming significant after 15 days. The most interesting feature is the existence of correlation between changes in the MA of the thymus cells and in the functional activity of the antibody-producing cells, manifested by variations in the titers of humoral antibodies.

The thymus thus responds to injection of antigen by a change in the proliferative activity of its small lymphocytes, and on the other hand, there is a close connection between variations in MA of the thymocytes and the antibody concentration in the blood serum, manifested by a preliminary increase in the activity of cell division and a subsequent increase in the serum antibody titers.

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