SECONDARY IMMUNOLOGIC RESPONSE TO NEONATALLY THYMECTOMIZED MICE AND RESPONSE TO SUCCESSIVE INJECTION OF TWO ANTIGENS

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Immunization of neonatally thymectomized C57BL/6 mice with O-antigen makes these mice capable of giving a primary response to sheep's erythrocytes. Conversely, immunization with sheep's erythrocytes makes it possible to obtain a secondary response to O antigen in thymectomized mice.

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The immunologic response to the first injection of a series of antigens is sharply depressed in neonatally thymectomized mice [7-9]. However, antigens do exist to which neonatally thymectomized mice give a normal primary response [1, 2, 4].

The immunologic response of thymectomized animals to the second and third injections of an antigen has received little study. It has been shown [6] that the response to the second injection of tetanus toxoid in neonatally thymectomized mice of the Swiss line is depressed by 80% compared with controls. Our experiments [2, 4] on C57BL/6 mice have shown that a secondary response to sheeps' erythrocytes equal in magnitude to the secondary response of nonthymectomized mice can be obtained in neonatally thymectomized mice under certain conditions.

In the present investigation the immunologic response of neonatally thymectomized mice to the second and third injections of an antigen was studied.

EXPERIMENTAL METHOD

Mice of line C57BL/6 were used in the experiments. Thymectomy, the verification of completeness of thymectomy, and methods of obtaining blood from the mice and titrating antibodies against sheep's erythrocytes were described previously [2].

Sheep's erythrocytes and Salmonella typhi O antigen were used for immunization. The erythrocytes were injected intraperitoneally in a dose of 0.5 ml of 20% suspension (about $5 \cdot 10^8$ cells), and 2.5 weeks later a second injection of 0.5 ml of 20% suspension was given. One week after each injection blood was taken and the antibodies titrated in the serum. O antigen was injected intravensouly in a dose of 10 μg (twice, at an interval of 2 weeks). One week after the second injection blood was taken and the antibodies titrated in the serum. The third injection of O antigen (10 μg intravenously) was given 2 weeks after the second. One week after the third injection of antigen blood was taken and the antibody titer determined in the agglutination reaction with 0.5% suspension of human (Group O) erythrocytes sensitized with S. typhi O antigen [3].

EXPERIMENTAL RESULTS

Primary immunization of the mice with very small doses of sheep's erythrocytes (approximately $1 \cdot 10^6$ cells) enabled a revaccination response to be obtained to the second injection of this antigen.

We immunized mice with large doses of erythrocytes (approximately $5 \cdot 10^8$ cells). Antibodies were found in high titers in the nonthymectomized mice in response to the first injection of this dose of antigen (see Table 1). Repeated injections of antigen gave no significant increase in the antibody level compared with the primary response, i.e., the nonthymectomized mice gave no marked revaccination response.

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TABLE 1. Formation of Antibodies Against Sheep's Erythrocytes and O Antigen of Salmonella typhi by Thymectomized Mice after Revaccination

Expt. No.	Antigen	Age in weeks	Time after which blood was taken (in weeks)	No. of mice in expt.	Geometric mean titer of antibodies in log (M±m)	t
14	Sheep's erythrocytes	3,5	1 (after 1st injection)	6c	1,602±0,07	6,6
				12TE	0,942±0,07	
			1 (after 2nd injection)	6c	2,204±0,24	0,4
				12TE	2,304±0,11	
31	Sheep's erythrocytes	4—5,5	1 (after 1st injection)	23c	1,602±0,05	13,5
				22TE	0,255±0,10	
			1 (after 2nd injection)	14c	2,377±0,09	1,7
				7TE	2,204±0,06	
38	O antigen	7—10	1(after 2nd injection)	9 c	2,171±0,26	4,1
				9 TE	1,133±0,07	
			1(after 3rd injection)	6 c	2,655±0,18	0,9
				6TE	2,856±0,13	

Legend: C represents nonthymectomized mice (controls), TE thymectomized mice.

Antibody formation in the neonatally thymectomized mice differed considerably from that in the controls: the primary response was almost completely absent, and repeated injection of antigen led to a marked revaccination response (see Table 1). Consequently, a revaccination response may be obtained in thymectomized mice by using doses of antigen ($5 \cdot 10^8$ cells) much larger than the doses required for producing a revaccination response in nonthymectomized mice ($1 \cdot 10^6$ cells).

A marked revaccination response was also possible in the thymectomized mice to O-antigen of Salmonella typhi (see Table 1). In this case, as during immunization with sheep's erythrocytes, the thymectomized mice required a much stronger antigenic stimulus than the nonthymectomized. The control mice gave a revaccination response to the second injection of antigen, and the third injection of antigen did not give rise to a significant increase in the antibody titer. Antibodies were present in low titers in the thymectomized mice in response to the second injection of antigen (see Table 1, experiment No. 38), and not until the third injection of antigen were antibodies produced in considerable titers.

Experiments were carried out to examine the effect of nonspecific immunization on the immunologic response of neonatally thymectomized mice.

Mice aged 6-7 weeks were immunized with sheep's erythrocytes (0.5 ml of a 20% suspension). One week after immunization the thymectomized mice had much lower antibody titers than the controls (see Fig. 1, experiment No. 2). Three weeks after immunization with sheep's erythrocytes, immunization was carried out with O antigen (twice in doses of 10 μg with an interval of two weeks).

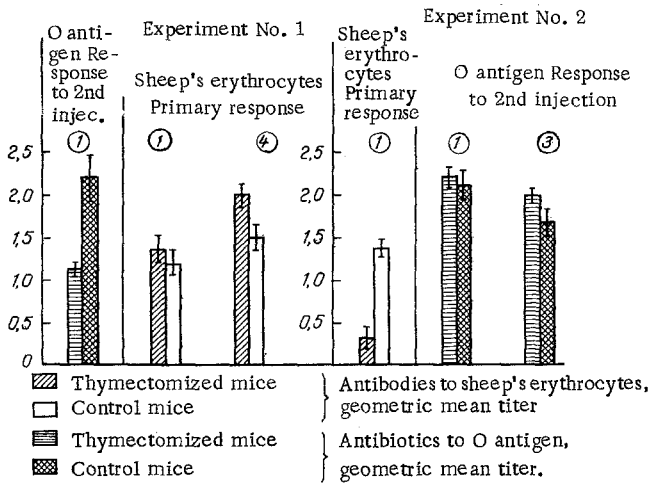

Fig. 1. Antibody formation by thymectomized and control mice in response to successive injection of two antigens. Ordinate – antibody titers (in log). The numbers in circles denote time after injection of antigen when blood was taken (in weeks).

Titers of antibodies against O antigen were determined 1 and 3 weeks after the second injection of antigen. In this experiment antibodies were found in the thymectomized mice in response to the second injection of antigen in the same high titers as in the nonthymectomized mice. Furthermore, the level of the antibodies fell rather more slowly in the thymectomized mice than in the controls.

In experiment No. 1, illustrated in Fig. 1, mice aged 7-10 weeks were immunized intravensouly with O antigen (twice in doses of 10 μg with an interval of two weeks). One week after the second injection of antigen blood was taken and the antibody titers in the serum determined. In thymectomized mice the antibody titers were significantly lower ($P < 0.01$) than in the controls. Two weeks after the second injection of antigen, the mice received a third injection of O antigen (10 μg, intravenously). The antibody titer in the thymectomized mice was slightly higher than in the controls (see Table 1, experiment No. 38). Later, three weeks after the third injection of O antigen, the mice were immunized for the first time with sheep's erythrocytes (0.5 ml of a 20% suspension, intraperitoneally), and this led to antibody formation in the same titers as in the controls (see Fig. 1, experiment No. 1). Usually the response of thymectomized mice (one week after immunization) to the first injection of sheep's erythrocytes is suppressed [2]. Four weeks later immunization with sheep's erythrocytes the titers of antibodies against sheep's erythrocytes continued to rise. During this same period the antibody titer of the control mice remained unchanged.

I mentioned above that the decrease in the level of antibodies against O antigen in thymectomized mice takes place more slowly than in nonthymectomized mice. These facts show that not only the mechanism of induction of the immunologic response, but also the mechanism of its cessation at a certain level is disturbed in thymectomized animals.

Using sheep's erythrocytes and O antigen of S. typhi as examples, we were able to show that the formation of an immunologic memory and a secondary immunologic response are possible in neonatally thymectomized mice. However, to obtain a secondary immunologic response in neonatally thymectomized mice a stronger antigenic stimulus was required than in nonthymectomized animals. The immunologic readjustment after antigenic stimulation took place more slowly in the thymectomized mice, but after its completion antibodies could be found in the thymectomized mice in the same titers as in the nonthymectomized mice.

After immunization with O antigen a normal primary response may take place to sheep's erythrocytes and, conversely, after immunization with sheep's erythrocytes a normal secondary response to O antigen is possible.

The small lymphocytes are known to play an important role in the induction of the primary [5] and secondary response. Neonatal thymectomy leads to a considerable reduction in the number of small lymphocytes in the blood and lymphoid organs [7-9].

Besides changes in the relative numbers of plasma cells and lymphocytes, some of the small lymphocytes in thymectomized mice are imperfectly formed, for in the opinion of most authorities, these cells undergo "training" in the thymus [10]. The need for powerful antigenic stimulation in order to cause induction of the immunologic response in thymectomized mice and the delayed immunologic readjustment after primary injection of antigen are evidently associated with this phenomenon.

It must be noted that in immunized neonatally thymectomized mice, we observed a gradual restoration of the normal morphological pattern in the lymph nodes, and especially in the regional lymph nodes for the place of injection of the antigen – a normal number of lymphocytes, and the presence of lymphoid follicles with centers of proliferation.

This evidently explains the effect of nonspecific immunization on restoration of the normal primary and secondary immunologic response to sheep's erythrocytes and to O antigen.

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