# IMMUNOLOGIC RESPONSE OF MOUSE SPLEEN CELLS DURING INDUCTION OF TOLERANCE IN THE EARLY POSTNATAL PERIOD

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Partial or complete immunologic tolerance in mice to sheep's erythrocytes can be produced by injecting the antigen into the animals before the 7th day after birth. This is in agreement with previous findings showing that the antibody-synthesizing ability of mouse spleen cells appears on the 7th day of life, and at this same time new antigenic components can be detected in the spleen.

Previous investigations [1, 2, 4] have shown that in the "adaptive period" in mice the development of the immunologic function of the spleen bears a definite relationship to postnatal formation of its antigenic structure. The most favorable times for induction of immunologic tolerance are the first few hours or days after birth of the mice [3]. However, the relationship between the development of a state of tolerance and the antibody-synthesizing function of the spleen cells has not been adequately investigated [5-8].

The object of this investigation was to study the formation of tolerance in mice at various times of their postnatal development, concentrating on the ability of the animals to produce antibody-synthesizing cells in the spleen and to synthesize humoral antibodies.

## EXPERIMENTAL METHOD

Mice of line A were immunized with sheep's erythrocytes on the 3rd, 6th, 10th, 14th, 30th, and 90th days of life, i.e., at times when the antibody-synthesizing function and antigenic structure of the spleen were studied previously. Immunization was by a single intraperitoneal injection of a 50% suspension of cells in physiological saline in a dose of between 0.02 and 0.2 ml depending on the animal's weight and age.

To detect a state of tolerance, 30 days after the first immunization the mice were reimmunized with the same antigen (0.2 ml of a 50% suspension of cells).

On the 4th day after reimmunization, the mice of each age group were sacrificed and the spleen removed. A suspension of spleen cells was prepared in Hanks's solution, pH 7.0-7.2, and the number of cells producing antibodies was determined by the Jerns – Nordin method of local hemolysis in agar. Meanwhile the level of hemolysins in the sera of these mice was studied. The experimental results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

Animals in which the number of antibody-synthesizing cells in the secondary response did not exceed their number in intact mice, i.e., it did not exceed 10 cells per $6.5 \cdot 10^6$ spleen cells, were regarded as completely tolerant. Animals in which the number of antibody-synthesizing cells did not exceed 500 were regarded as partially tolerant. This criterion was sound, because in the control the number of antibody-synthesizing cells after the second injection of antigen reached 1500-2000. If the number of antibody-producing cells exceeded 500, it was considered that tolerance had not developed.

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TABLE 1. Immunologic Response of Mouse Spleen in Early Postnatal Period to Repeated Injection of Sheep's Erythrocytes

| Time of first immunization (days of life of mice) | No. of animals used | No. of mice with different levels of antibody-synthesizing cells (per $6.5 \cdot 10^6$ spleen cells) | | | |
|---|---|---|---|---|---|
| | | < 10 | 11-50 | 51-500 | > 500 |
| | | complete tolerance | partial tolerance | | |
| 3 | 30 | 10 | 1 | 3 | 16 |
| 7 | 30 | 8 | 2 | 6 | 14 |
| 10 | 30 | – | – | 5 | 25 |
| 14 | 30 | – | – | 4 | 26 |
| 30 | 30 | – | – | – | 30 |
| 90 (control | 30 | – | – | – | 30 |

As Table 1 shows, in mice receiving the first injection of antigen at the age of 3 days, absence of immune response after the second injection of sheep's erythrocytes was observed in 33% of cases. Partial suppression was observed in 13% of mice.

At the age of 7 days, complete tolerance was induced in 27%, and a partial decrease in the number of plague-forming cells was observed in the same percentage of cases.

Starting from the 10th day after birth, it was no longer possible to produce complete suppression of the antibody-synthesizing function of the spleen, although about 17% of mice still showed partial tolerance.

On the 30th day the immune response of the spleen to a second injection of antigen was almost equivalent to the control (the difference between the number of antibody-producing cells in the group of adult immune animals and in the group of mice receiving the first injection of antigen on the 30th day of life was not statistically significant, $P > 0.05$).

Parallel determination of the titer of hemolysins in the mouse sera showed that none could be detected in the completely tolerant animals, while in the partially tolerant animals the titer did not exceed 1 : 8. Meanwhile, in the control mice the titer of hemolysins was between 1 : 128 and 1 : 256.

It thus follows from these results that it is easiest to induce tolerance if a single injection of sheep's erythrocytes is given to the animals on the 3rd-7th day of life.

These results are in agreement with previous findings indicating that the antibody-synthesizing function of the spleen becomes apparent for the first time on the 7th day of life in mice, and at the same period the specific antigenic components, which are characteristic of this period of development of the animals only, begin to make their appearance.

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