

IMPORTANCE OF SURVIVAL OF FOREIGN ANTIGEN IN THE ORGANISM FOR THE PHENOMENON OF IMMUNOLOGICAL TOLERANCE

(UDC 612.017.1:612.017.12)

B. N. Sofronov

Division of Microbiology (Head, Corresponding Member AMN SSSR V. I. Ioffe),

Institute of Experimental Medicine (Director, Active Member AMN SSSR

D. A. Biryukov) of the AMN SSSR, Leningrad

(Presented by Active Member AMN SSSR D. A. Biryukov)

Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 58, No. 12,

pp. 56-60, December, 1964

Original article submitted May 27, 1963

According to Burnet's clonal selection theory, the presence of an antigen is not an essential condition for the preservation of tolerance to that antigen. Yet there is increasing evidence that the state of immunological tolerance is associated with the presence of the antigen in the organism. Calculations have even been made showing that for tolerance to be maintained, 10^{10} - 10^{12} molecules of protein antigen are necessary [2-4].

We have previously suggested [1] that in tolerant animals, the amount of antigen injected and the changes in its dynamics not only reflect antibody production in the organism, but also may be classed as factors of the first order, determining the state of tolerance. In this paper we present findings indicating the importance of survival of antigen in the organism in the phenomenon of tolerance.

METHOD

The phenomenon of immunological tolerance was reproduced by injecting young rabbits immediately after birth intraperitoneally with 0.5-1.0 ml of horse serum or with a corresponding dose of horse serum albumin. The injections were repeated 24 h later. At various intervals after birth the rabbits were given a further injection of antigen, which demonstrated the ability of the animal organism to give an immunological response. Foreign antigen and antibodies to it in the blood were determined by the slow complement fixation reaction at 4°. Our immune sera were capable of determining antigen in a dilution of 1:4,000,000.

RESULTS

Comparative duration of survival of foreign antigen in the blood after injection into the adult and newborn animal. Horse serum, injected into a normal adult rabbit, could be detected in the blood for 8-9 days (dose of serum injected 0.2-0.3 ml/kg body weight). For the first 5-7 days the fall in the blood antigen level took place slowly, but this was followed by a sharp drop, coinciding with the period of appearance of antibodies in the blood. By the 10th day, as a rule, the injected antigen could no longer be detected. In tolerant rabbits, as we shall describe below, the injected antigen could be found in the blood stream for a much longer time—for up to 20 days.

In contrast to the picture observed in the adult animals, antigen injected into the newborn rabbit could be detected after a much longer period. As Table 1 shows, the sera of rabbits over 2 months old in some cases contained small amounts of antigen, and not until after this age could it be reliably said that no antigen could be detected in the sera. The young rabbits received injections of 1-2 ml of serum at birth; the adult rabbits in the experiments referred to above were injected with approximately the same dose. When calculated per unit body weight at the moment of injection, the dose given to the newborn animal was 30-40 times greater than that given to the adult. However, when antigen was detected for the last time, the rabbit had attained a weight of about 2 kg, and the doses of antigen injected could also be related to this weight. Nevertheless, it was interesting to study the curve of titer of antigen in the adult rabbit after receiving an injection of serum in a dose of 1.5-2.0 ml/50 g body weight, equal to that received by the newborn animal.

TABLE 1. Determination of Antigen Injected into a Newborn Rabbit at Various Periods after Birth

| Result of determination | Time of investigation (in days) | | | | | |
|-------------------------------------------------|---------------------------------|----|----|----|----|----|
| | 22-30 | 36 | 42 | 55 | 70 | 85 |
| Total No. of tests | 4 | 4 | 12 | 4 | 6 | 4 |
| Antigen not found | — | — | — | — | 4 | 4 |
| Antigen found in sera diluted 1:5 (++) and +++) | — | — | 3 | 1 | 2 | — |
| Antigen found in sera diluted 1:5 (++++) | — | — | 4 | 3 | — | — |
| Antigen found in sera diluted 1:10 or more | 4 | 4 | 5 | — | — | — |

TABLE 2. Result of Immunization of Tolerant Rabbits of Different Ages with Horse Serum

| Result of experiment | Age of rabbit tested, in months | | | |
|------------------------------------------------|---------------------------------|-----|-----|---|
| | 2 | 4-5 | 6-7 | 8 |
| Total No. of rabbits | 14 | 8 | 8 | 4 |
| Antibodies not found | 13 | 6 | 4 | 1 |
| Antibodies found in serum diluted 1:10 | 1 | 2 | 2 | 0 |
| Antibodies found in serum diluted 1:20 or more | 0 | 0 | 2 | 3 |

of life of the animals, with the results of the first tests carried out on the 8-month old rabbits (Table 2) shows that repeated injections of antigen prolonged the existence of the state of immunological tolerance of the animals. Clearly repeated injections of antigen into the tolerant rabbits did not lead to an increase in the antibody titer after each immunization, as was observed in the intolerant animals.

Further evidence of the importance of survival of foreign antigen in the organs for the state of immunological tolerance. The curve of the blood antigen titer of the tolerant animals, when compared with that of the intolerant animal, may be an important sign for determining the state of tolerance. An antigen injected into a tolerant animal was found in every case much longer in the blood than in the control animals. The results of one of the experiments are given in Table 4.

In the tolerant rabbits antigen could be detected until the 10th-18th day, and in the controls only until the 7th day. It was noted that the curve of antigen titer in the control rabbits fell more steeply than in the tolerant animals, demonstrating the absence of an immune phase of elimination of antigen in the latter.

We attempted to obtain direct proof of the presence of antigen in the organism of the tolerant animals at a time when none could be detected in their blood. For this purpose the animals received an intravenous injection of immune serum, and at various intervals thereafter the antibody level in their blood was determined. The assumption was made that, if antigen or its determinant groups were present in the organism, "absorption" of the corresponding antibodies would take place. A preliminary experiment was carried out on mice. The animals were

Duration of survival of a foreign antigen injected in a large dose in the blood of adult animals. Rabbits received an injection of 60-80 ml of horse serum each, or an equivalent dose of horse albumin, and in this case the injected antigen could not be detected after intervals when antigen injected into the newborn rabbit was found: the last time when antigen could be detected when injected into an adult rabbit in this dose was the 24th day after injection. Until the 16th day the titer of antigen in the blood was high, but it then fell sharply, coinciding with the appearance of antibodies in the blood. This experiment also showed that the injection of large doses of antigen into adult animals does not lead to a state of immunological paralysis or unresponsiveness. Antigen injected into 3-4 week old rabbits in a dose equivalent to that injected into newborn rabbits could be found, as it could in adults, for a period of 3 weeks. The fact that the antigen was eliminated from the blood of the tolerant animals after longer intervals led us to examine the duration of the phenomenon of tolerance itself.

Duration of survival of the state of immunological tolerance and its prolongation. Rabbits were prepared by injection of antigen after birth and then tested at various intervals thereafter—2, 4-5, 6-7, and 8 months (Table 2).

Nearly all the rabbits immunized after 2 and 4-5 months were incapable of forming antibodies. When immunized after 6-7 months, only half the rabbits were in this state, and of the 4 rabbits immunized after 8 months, 3 produced considerable amounts of antibodies. The gradual disappearance of the antigen from the blood and the gradual ending of the state of tolerance may have been interconnected. This is confirmed by the slight prolongation of the state of tolerance observed in cases in which the antigen was injected repeatedly every 2-4 months after birth.

Comparison of the results of the 3rd and 4th immunizations (Table 3), carried out on the 8th-9th or 10th-11th months

TABLE 3. Result of Repeated Immunizations of Tolerant Rabbits

| Result of experiment | Immunization | | | |
|------------------------------------------------|--------------|-----|-----|-----|
| | 1st | 2nd | 3rd | 4th |
| Total No. of rabbits | 22 | 21 | 10 | 10 |
| Antibodies not found | 19 | 13 | 5 | 4 |
| Antibodies found in serum diluted 1:10 | 3 | 4 | 5 | 3 |
| Antibodies found in serum diluted 1:20 or more | 0 | 4 | 0 | 3 |

TABLE 4. Duration of Survival of Horse Albumin in Blood after Injection into Tolerant and Intolerant Rabbits

| Rabbit No. | Time of investigation in days | | | |
|------------|-------------------------------|------|------|------|
| | 7 | 10 | 12 | 18 |
| 606 T | 1:400 | 1:50 | 1:25 | 1:25 |
| 548 T | 1:200 | 1:50 | 1:25 | 1:25 |
| 607 T | 1:100 | 1:50 | 1:25 | — |
| 581 T | 1:200 | 1:50 | — | — |
| 608 T | 1:200 | 1:50 | — | — |
| 353 C | 1:200 | — | — | — |
| 551 C | 1:100 | — | — | — |
| 552 C | 1:100 | — | — | — |
| 373 C | 1:50 | — | — | — |
| 390 C | 1:25 | — | — | — |

Note. The results in the table are the limiting dilutions of serum in which antigen was found; the minus sign denotes that antigen was not found; T) tolerant rabbit; C) control rabbit.

increases, the immunological tolerance begins to disappear, and a further injection of antigen prolongs the maintenance of this state. These results are in complete agreement with those reported in the literature. The fact that antigen was not found in the blood after 2 months does not prove that it was absent either from the organs or from the blood stream, for the method of detection used could not reveal the presence of less than 5 μ g antigen (horse serum) per ml of test substrate.

These results cannot rule out the possibility that tolerance may be associated with the retention of antigen in the organism. At the same time, experiments in which antibodies were injected into tolerant animals showed that the assumed antigen is not eliminated by the action of antibodies and is not blocked by them, nor does it lower the titer of antibodies injected into the animal. In this case the antigen in the organism must be in a bound, isolated form, different from that usually occurring. Whether the prolonged persistence of the antigen in the organism is the cause of the absence of an immunological reaction to it, or whether the absence (or weakness) of the immunological reactions is due to the prolonged circulation of the antigen, are questions to which no answer can yet be given. The conclusion that tolerance is invariably due to the presence of antigen is excessively binding. If there is no other way for the transmission of information about the antigen in a state of tolerance, it is logical to suppose that in acquired immunity the antigen persists throughout the immune state. Otherwise, it would have to be concluded that different ways of transmission of "antigenic information" are possible: one way in immunological tolerance and other ways in acquired immunity. Further facts must be collected.

injected subcutaneously with antigen (horse serum), and immediately afterward received an intravenous injection of immune serum. It was found that the antibody level was lower after the injection of antiserum in the blood of the mice which had received not less than 0.02 ml of horse serum than in the animals receiving smaller doses of horse serum or none whatever. Next, antiserum to horse protein was injected into rabbits prepared with horse serum immediately after birth, and now having reached the age of 3-4 months, and into corresponding control animals. After various intervals of time the antibody titer in the sera of the experimental and control animals was determined. The experiment was repeated twice. In all cases and at all times of investigation no significant difference was found between the antibody levels in the experimental and control animals. One week after injection of the immune serum all the animals were given an injection of antigen, and on this occasion those which had been prepared after birth with antigen proved to be tolerant. This experiment also showed that injection of an immune serum in no way affected the degree of tolerance of the experimental rabbits. Meanwhile, it might be expected that if tolerance were due to the presence in the organism of antigen accessible to the immune serum, injection of the latter may disturb this state. In the next experiment the interval between injection of the immune serum and antigen was shortened to 1 h. In this case the injection of serum likewise had no influence on the tolerance.

The results described above demonstrate that the dynamics of the titer of a foreign protein antigen in animals tolerant to it differs essentially from the changes observed in control animals; antigen injected into an adult, tolerant animal is found in the blood stream for a much longer period than in intolerant animals. As the time from the moment of injection of antigen into the newborn rabbit in-

LITERATURE CITED

1. B. N. Sofronov, In book: Current Problems in Immunology [in Russian], Leningrad (1959), p. 161.
2. N. A. Mitchison, In book: Biological Problems of Grafting, Springfield (1959), p. 239.
3. R. T. Smith, In book: Advances in Immunology, 1, New York (1961), p. 67.
4. R. T. Smith and R. A. Budes, J. exp. Med., 108 (1958), p. 227.