DETECTION OF ANTIBODY-FORMING CELLS IN THE TURTLE SPLEEN BY A MODIFIED METHOD OF LOCAL HEMOLYSIS IN GEL

L. F. Kassin and L. A. Pevnitskii

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The spleen of turtles (Testudo horsfieldii) immunized with sheep's erythrocytes contains antibody-forming cells which can be detected by the reaction of local hemolysis in gel only if serum against turtle serum proteins is used. This evidently shows that antibodies in turtles against sheep's erythrocytes are largely incomplete.

* * *

The study of antibody formation in cold-blooded animals, the foundations of which were laid originally by Mechnikov and his school [3], is being conducted vigorously at the present time. The material accumulated, however, is contradictory in many respects. Some workers deny that antibody formation can be induced in cold-blooded animals in general (and in turtles, in particular) against particular antigens [1]. In most investigations of this type, the tests used to detect antibody formation have mainly been limited to determination of antibodies in the blood by the usual method (precipitation, agglutination, and complement fixation reaction in vitro). More recently, however, several new and highly sensitive immunologic methods have been suggested and used, including Jerne's method, which is now widely used in investigations on mammals and birds [7, 11].

In this investigation an attempt was made to detect antibody-forming cells in the spleen of immunized turtles by the method of local hemolysis in gel.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out during the autumn-winter period on land turtles of the species Testudo horsfieldii, kept in an incubator at 37°C. Sheep's erythrocytes or, in some cases, the stroma of the erythrocytes, was used for immunization. The first injection of antigen was given into the heart, subsequent injections into the jugular vein. Hemagglutinins and hemolysins in sera heated to 45° for 45 min to destroy nonspecific lytic properties [1] were determined by the usual method and also by a modified method. Dried guinea pig complement in dilutions of 1:5-1:10 was used in the hemolysis reaction.

The electrolyte content in the turtle plasma differs only very slightly from that in mammals [9], so that when suspensions of cells of the organs and the agar for Jerne's reaction were prepared, isotonic Hanks's solution for warm-blooded animals was used. The technical details of Jerne's reaction have been described previously [4], and the only difference in this case was that the incubation time of the dishes after the mixture of spleen cells and erythrocytes had been poured into them was increased (2 h at 37° instead of 1 h) and a more concentrated guinea pig complement was used (1:2.5 instead of 1:5). In the experiments using antiglobulin serum (see below), before addition of the complement, 2.5 ml of this serum (1:5) was poured into the dishes, which were incubated at 37° for 1 h, the serum was poured off, the surface of the agar was washed with physiological saline, and the complement was then poured on.

The turtles were immunized once, twice, or many times (3-7 injections) at intervals ranging from 4 to 52 days, with a total duration of the course of up to 113 days. The dose of erythrocytes per injection was usually 2 billion cells, but in some experiments it was 500 million. Antibodies in the blood were determined at intervals of 3-7 days throughout the course of immunization.

Laboratory of Immunologic Tolerance, Department of General and Radiation Immunology, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow; Department of Histology, Perm' Medical Institute. (Presented by Active Member of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 70-74, March, 1969. Original article submitted May 31, 1968.

TABLE 1. Effect of RAGS on Antibody Titers in Sera of Turtles Immunized with Sheep's Erythrocytes

No. of sera	Hemagglutinins titer		No. of sera	Hemolysins titer	
	without RAGS	with RAGS		without RAGS	with RAGS
2	<1:10	1:10—1:20	9	<1:10—1:10	<1:10—1:80
6	1:10—1:40	1:20—1:1 280	6	1:20	1:40—1:640
6	1:80—1:160	1:160—1:2 560	1	1:40	1:2 560
4	1:320—1:640	1:1 280—1:2 560	1	1:160	1:640

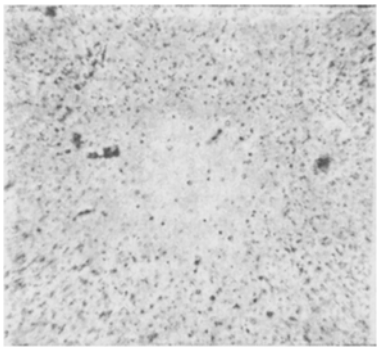

Fig. 1. Zone of hemolysis seen under the microscope. 63 ×.

The first stage of the work was to study the dynamics of the antibody titers in the blood, which were determined by the usual methods.*

The blood antibody titer varied considerably even in turtles immunized by the same scheme. As a rule hemolysins appeared in the blood by the 4th day after a single injection of 2 billion erythrocytes (or stroma obtained from 10 billion cells) in a titer of 1:10-1:40. During the next 10 days their level did not rise significantly, and sometimes it actually fell. Only in a few cases did subsequent injections of antigen cause an increase in titers to 1:40-1:160.

On the other hand, the titer of hemagglutinins rose slowly throughout the course of injections (from 1:10-1:20 after the first injection to 1:40-1:1280 after the 4th-5th injection). Even here, however, very great variability of titers was observed. Sometimes during the course of injections of erythrocytes the antibody titer fell and then increased again after the next injection.

The attempt to stimulate antibody formation against erythrocytes by using O-antigen of Salmonella typhi proved ineffective.

The next series of experiments was carried out to detect antibody-forming cells in the turtle spleen by Jerne's method. Animals immunized with sheep's erythrocytes 3 or 4 times at intervals of 14-25 days were used. On the day of the experiment (10-12 days after the last injection) the hemolysin titer in the blood of these animals was 1:40-1:160. The experiments, carried out by the usual method (without antiglobulin serum), gave negative results.

It was then decided to use antiglobulin serum, having regard to published data on its effectiveness for the detection of some types of antibodies [5, 6, 10, 13]. Antiglobulin serum was obtained from a rabbit after repeated inoculation with turtle serum. Before use it was absorbed with sheep's erythrocytes. Subsequently, to shorten the description, it will be described as rabbits antiglobulin serum (RAGS). In the ring precipitation test, RAGS reacted with turtle serum in dilutions of 1:320-1:640.

The action of RAGS was investigated by the hemolysis and hemagglutination reactions. RAGS was added to the reaction mixture (turtle immune serum + erythrocytes) in final dilutions of 1:15-1:20, while in the control the RAGS was replaced by physiological saline. In the hemolysis reaction, after addition of RAGS and subsequent sedimentation of the erythrocytes, the supernatant was replaced by physiological saline and complement was added.

The results showed that RAGS increases the antibody titer in the sera of immunized turtles in the hemolysis and hemagglutination reactions (Table 1).

Having obtained these results, the next step was to carry out experiments to determine hemolysin-forming cells in turtles by the reaction of local hemolysis in gel using RAGS. Antibody-forming cells giving characteristic zones of hemolysis were detected in the spleen of the immunized animals. Usually a single cell was found in the center of these zones (Fig. 1). The reaction was specific, for when rat's erythrocytes were used no zones of hemolysis could be detected. The spleen of unimmunized animals contained no antibody-forming cells.

*This part of the work was carried out with the assistance of L. S. Bank.

TABLE 2. Content of Antibody-Forming Cells in Spleen of Turtles Immunized with Sheep's Erythrocytes

Turtle no.	Scheme of immunization*	Day of investigation after final injection	No. of antibody-forming cells	
			per million cells	in spleen
1	2—2—2—2—2—2—2 / 4 4 15 26 18 31	15-й	4,8	180
2	2—2—2—2—2 / 14 34 18 31	15-й	1,5	70
3	2—2—2—2 / 14 30 16	13-й	28,2	1 162
4	0,5—0,5—0,5—2—2—2 / 4 4 31 18 31	15-й	6,9	342
5†	10—10—10—10—10 / 4 4 25 18	16-й	23,2	1 865
6	0,5—0,5—0,5—2—2 / 4 4 15 18	10-й	6,6	335
7	2—2—2—2—2 / 4 4 35 18	14-й	12,7	859
8	0,5—0,5—0,5—2—2 / 4 4 22 18	17-й	27,0	2 279
9	2—2 / 52	15-й	1,7	160
10	2—2 / 52	15-й	4,3	397
11	2—2 / 52	15-й	11,0	1 588
12	2 / 0	14-й	0,8	88
13	2 / 0	14-й	1,1	156
14	2 / 0	14-й	3,1	809
15	2 / 0	14-й	1,0	145
16	2 / 0	15-й	16,6	2 457
17	2 / 0	15-й	1,6	262
18	2 / 0	15-й	1,1	196

*Numerator represents doses of erythrocytes (in billions), denominator intervals between injections (in days).
†Immunization carried out with stroma of erythrocytes.

As Table 2 shows, antibody-forming cells were found in different numbers in all immunized turtles. No relationship could be found between the number of these cells, the immunization scheme, and the titer of hemolysins in the blood on the day of investigation.

In other organs of the immunized turtles—the liver, thymus, or intestine—no antibody-forming cells were found.

The experimental results thus indicate that in turtles, just as in warm-blooded animals, the spleen is the principal organ producing antibodies against sheep's erythrocytes. This conclusion is in agreement with data in the literature concerning the presence of plasma cells in the turtle spleen [8, 12]. It should also be pointed out that, judging from the results now obtained, hemagglutinins and hemolysins of turtles consist

mainly of incomplete antibodies. This may to some extent explain the failure of attempts to demonstrate antibody formation in turtles by the usual test methods. It is possible that in reality the antibody-forming function is not so poorly developed in reptiles as is generally considered [2], although special methods of investigation are needed to detect antibodies in these animals.

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