

IMMUNOGENESIS AND NONSPECIFIC FACTORS OF NATURAL RESISTANCE

COMMUNICATION III. INFLUENCE OF ACTIVE IMMUNIZATION ON THE LYSOZYME CONTENT OF BLOOD

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In previous communications we have reported evidence of a negative influence of active immunization on the lysozyme content of body fluids. Thus D. F. Pletsityi, A. M. Monaenkov, Yu. B. Ostrovskii, and P. T. Boinik [1] showed that after vaccination of dogs by a tetravaccine against intestinal infections there was a marked fall in the lysozyme content of the saliva, and that the reduction was maintained for a long time.

The object of the present investigation has been to study the changes of lysozyme activity in the blood of animals at various times after immunization with various vaccines.

Numerous reports concerning this problem have been made but the results are contradictory. Myrvik [3] showed that when rabbits receive the BCG vaccine the lysozyme content of pulmonary extracts is increased, and L. G. Élkonin [2] found that as far as the blood was concerned repeated injection of rabbits with large doses of heated vaccine against intestinal infection at first caused a small increase in the amount of lysozyme in the blood and that it was followed by a fall in titer. There was an even stronger tendency for the lysozyme of the blood to fall when the present author immunized rabbits with an alcoholic dysenteric monovaccine.

EXPERIMENTAL METHOD

Observations were made on chinchilla rabbits weighing 2-3 kg. A study was made of the influence of the following vaccines: native tetanus anatoxin, chemical divaccine, and microbial tetravaccine against intestinal infections, sorbed diphtheritic anatoxin, gangrene-tetanus tetra-anatoxin. One or two subcutaneous injections of the preparations were given. The amount of lysozyme in the blood was determined from the third day after the injection onwards. The initial titer (before immunization) was usually determined several times immediately before the vaccine or anatoxin. To determine serum lysozyme we used the generally accepted direct method for the quantitative indication of this enzyme in fluids, based on the high sensitivity of *Micrococcus lisodeicticus* to the action of lysozymes. The serum was diluted with a 0.5% sodium chloride solution and brought to concentrations from 1:10 to 1:320. To a test tube containing 1 ml of the diluted serum we added 1 ml of a suspension of a live culture of the micrococcus (one billion microbial cells). The results of the reaction (degree of lysis) were determined after the test tubes had been kept for three hours in a thermostat at 37°. A test-tube with a solution of sodium chloride and the same amount of microbial suspension was used as a control. The last dilution which produced lysis was taken as the titer of the lysozyme. The experiments were carried out on 40 rabbits.

EXPERIMENTAL RESULTS

In the first set of experiments we investigated the influence on the blood lysozyme of sorbed gangrene-tetanus tetra-anatoxin (the preparation was obtained from the Department of Wound Infection, N. F. Gamalei Institute of Epidemiology and Microbiology, AMN SSSR). Repeated subcutaneous injections of 1 ml of the anatoxin were given.

TABLE 1. Change in the Lysozyme Content of Rabbit Blood under the Influence of Gangrene-Tetanus Tetra-Anatoxin Vaccine

Number of rabbit	Titer of lysozyme							
	Before immunization	After immunization						
		on the 3rd day	on the 5th day	on the 7th day	on the 12th day	on the 20th day	on the 30th day	on the 40th day
2644	1:160	1:80	1:40	0	1:5	1:10	1:80	1:16
2747	1:80	1:40	1:20	0	1:5	0	1:40	1:80
2695	1:80	1:40	1:20	1:10	1:5	0	1:80	1:80
2678	1:80	1:40	1:20	0	1:5	0	1:80	1:80
224	1:80	1:5	1:5	1:5	1:5	0	1:80	1:80
315	1:80	1:10	1:10	0	0	1:5	1:80	1:80
280	1:80	1:10	1:10	1:5	0	0	1:80	1:80
143	1:40	1:5	1:5	0	0	0	1:40	1:40
Mean	1:85	1:28	1:15	1:2	1:3	1:2	1:70	1:85

As can be seen from Table 1, even by the third day after the first injection of the anatoxin there was a marked reduction in the titer of lysozyme, a change which was most marked by the 7-20th day after immunization. A very low lysozyme content in the blood was found for 20-25 days. Subsequently it gradually rose and reached its original level on the 30-40th day.

In the second set of experiments we investigated the influence on the blood lysozyme of a microbial tetra-vaccine against intestinal infections (series No. 208, Gor'ki NII EG). The tetravaccine was given subcutaneously twice at an interval of 16 days. The first injection consisted of 1 ml of tetravaccine (2.5 billion microbial cells) and the second time 2 ml were given.

From the results of Table 2 it can be seen that in this case there was once more a reduction in the blood serum lysozyme content of all the rabbits, although it was much less than when the tetra-anatoxin vaccine was used. Repeated injection of the vaccine caused a rather more marked and prolonged reduction of the serum lysozyme content than did the first injection.

In the third set of experiments with vaccine we used native anatoxin (series No. 232) obtained from the N. F. Gamalei Institute of Epidemiology and Microbiology, AMN SSSR. The anatoxin was given as two subcutaneous injections at an interval of 25 days (1 ml the first time, and 2 ml for the second injection).

As Table 3 shows the results of this set of experiments were similar to those obtained in the previous series.

The fourth set of experiments was carried out with purified diphtheritic adsorbed anatoxin (series No. 8) containing 68 active units per ml. One ml of anatoxin was given to each of eight rabbits after first diluting it twice in physiological saline.

As can be seen from Fig. 1 the blood lysozyme content changed to approximately the same extent as when immunization was produced by tetanus anatoxin.

In the last set of experiments (on eight rabbits) vaccination was made with a chemical dye vaccine in which the amount of typhoid antigen per ml was 0.02 mg, and of paratyphoid 0.5 mg. A single injection of 1 ml of the vaccine was given.

The results illustrated in Fig. 2 show that the chemical divaccine exerted a far smaller influence on blood serum lysozyme content than did vaccine preparations used previously, although in this case the reduction of the lysozyme titer was observed in all the animals.

The results of the experiment indicate that under the influence of immunization the blood serum lysozyme content falls consistently. The greatest action was exerted by tetra-anatoxin whose injection caused practically complete disappearance of the lysozyme from the blood serum. The reduction of the titers of lysozyme was observed to occur 3-5 days after immunization, being most marked on the 12-15th day. Repeated injection of the vaccine as a rule caused a greater fall of lysozyme content to occur at the same time. The observed individual differences were merely quantitative; there was no case when the blood serum lysozyme content failed to fall under the influence of vaccination (even though the fall might be very small and last only a short time).

TABLE 2. Change of Rabbit Blood Lysozyme Content After the Injection of Tetravaccine (Typhoid, Paratyphoid B. Flexner and Sonne Dysentery)

Number of rabbit	Titer of lysozyme												
	Before immunization	After immunization											
		on the 3rd day	on the 5th day	on the 7th day	on the 12th day	on the 16th day	on the 19th day	on the 21st day	on the 23rd day	on the 28th day	on the 36th day	on the 46th day	
449	1:160	1:40	1:20	1:20	1:40	1:160	1:40	1:20	1:20	1:40	1:80	1:160	
226	1:80	1:40	1:40	1:20	1:80	1:80	1:80	1:40	1:20	1:20	1:80	1:80	
460	1:80	1:80	1:40	1:40	1:80	1:80	1:80	1:40	1:40	1:20	1:80	1:80	
468	1:80	1:80	1:40	1:20	1:80	1:80	1:80	1:40	1:40	1:40	1:80	1:80	
3012	1:80	1:80	1:40	1:40	1:40	1:80	1:40	1:20	1:20	1:20	1:40	1:80	
3752	1:80	1:80	1:40	1:40	1:40	1:80	1:20	1:20	1:20	1:20	1:40	1:80	
2743	1:80	1:80	1:40	1:40	1:40	1:80	1:20	1:40	1:40	1:20	1:40	1:80	
2744	1:160	1:160	1:80	1:40	1:80	1:80	1:20	1:40	1:40	1:40	1:40	1:160	
Mean . . .	1:100	1:80	1:42	1:32	1:60	1:90	1:47	1:32	1:30	1:27	1:60	1:100	

TABLE 3. Change in the Rabbit Blood Serum Lysozyme Under the Influence of Tetanus Anatoxin Vaccine

Number of rabbit	Titer of lysozyme													
	Before immuniza- tion	After immunization												
		on the 3rd day	on the 5th day	on the 7th day	on the 12th day	on the 20th day	on the 25th day	on the 28th day	on the 30th day	on the 32nd day	on the 37th day	on the 45th day	on the 55th day	on the 66th day
808	1:160	1:160	1:80	1:80	1:80	1:80	1:80	1:40	1:40	1:40	1:40	1:80	1:160	
1492	1:160	1:160	1:80	1:80	1:80	1:80	1:160	1:80	1:40	1:40	1:80	1:80	1:160	
718	1:80	1:160	1:80	1:80	1:80	1:80	1:160	1:80	1:80	1:80	—	—	—	
1407	1:80	1:80	1:40	1:80	1:80	1:80	1:0	1:80	1:80	1:40	1:40	1:80	1:80	
215	1:160	1:160	1:160	1:40	1:40	1:80	1:20	1:20	1:40	1:40	1:80	1:80	1:80	
300	1:160	1:160	1:160	1:80	1:80	1:160	1:40	1:40	1:40	1:40	1:80	1:160	1:160	
523	1:80	1:80	1:80	1:40	1:40	1:40	1:20	1:20	1:40	1:20	1:40	1:80	1:80	
674	1:80	1:80	1:80	1:40	1:40	1:80	1:20	1:20	1:40	1:20	1:40	1:40	1:80	
Mean	1:127	1:127	1:100	1:65	1:67	1:100	1:77	1:47	1:50	1:40	1:57	1:85	1:118	

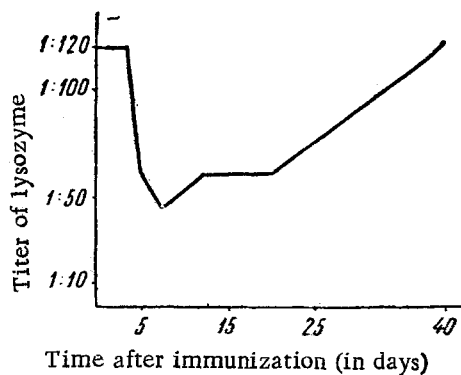


Fig. 1. Change in the amount of lysozyme of rabbit blood serum after the injection of purified adsorbed diphtheritic anatoxin.

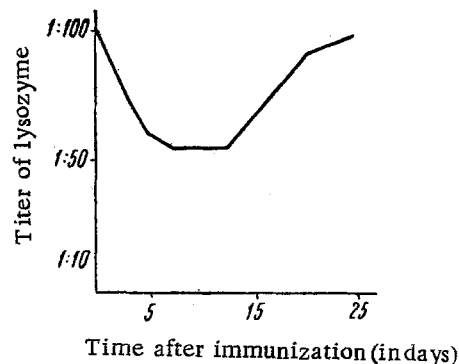


Fig. 2. Change in the blood serum lysozyme content of rabbits after the injection of a chemical divaccine.

An analysis of the results obtained afford a basis for the conclusion that there is a direct relationship between the immunizing power of the vaccines and the extent to which the blood serum lysozyme content of the immunized animals fell. The injection of a highly immune preparation — gangrene-tetanus tetra-anatoxin — caused a considerable reduction in the lysozyme titers in rabbits, while giving the chemical divaccine against intestinal infections, which did not have a high immunogenicity, reduced the lysozyme activity very little.

We must note the definite correlation between the serum content of lysozyme and specific antibodies. Special experiments showed that the maximum reduction in the titers of lysozyme were recorded at times when antibodies accumulated most intensively. In this respect our results correspond completely with those of previous investigations aimed at determining the influence of vaccination on the lysozyme content of saliva of animals and man.

SUMMARY

A study was made of the change in the blood serum lysozyme content of rabbits immunized with the following vaccine preparations: crude toxoid, chemical divaccine, and microbial tetravaccine against intestinal infections, sorbed diphtheria toxoid, gangrene-tetanus tetratoxoid.

It was shown that vaccination caused a distinct reduction of the blood lysozyme content. Restoration of the lysozyme titer to the initial level was observed to occur in most cases not earlier than 20 days after the preparation had been given. There was a direct relationship between the immunizing power of the vaccine (its amount, antigenic activity, and number of administrations) and the extent of the reduction of the lysozyme titer. A definite correlation was noted between the blood content of specific antibodies and lysozyme: the time for the greatest antibody titer rise corresponded to the periods of least lysozyme content.

LITERATURE CITED

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