

# MICROBIOLOGY AND IMMUNITY

## RELATION BETWEEN THE LEVEL OF SPECIFIC ANTITOXIN IN THE BLOOD AND THE DEGREE OF IMMUNITY TO TETANUS TOXIN IN WHITE MICE IMMUNIZED WITH ADSORBED TETANUS TOXOID

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It is generally agreed that the antibody level of the blood does not reflect completely the state of immunity of the organism as a whole, although the relation between humoral immunity and general immunity has been little studied. Only scattered observations relating to this problem are available [1, 3, 4].

In view of this we studied, in white mice (weight 17-20 g) immunized by a single intra-abdominal injection of purified tetanus toxin adsorbed on aluminum hydroxide (series 10 k), the content of the blood of specific antitoxin and the degree of immunity to tetanus toxin.

### EXPERIMENTAL METHODS

We took blood for the determination of antitoxin from the tail veins, by shaving and cutting off a piece 2-3 cm long (beforehand we put the tail in warm water). We obtained about 5 drops of blood from each mouse; to avoid later loss of blood we attached a ligature. The determination of antitoxin was repeated 3-4 times (the average values are presented) by the usual method [6] on the pooled serum of that group of mice which had been immunized with the same dose of antigen.

We determined the degree of immunity to tetanus toxin by the method previously described by us [2]. As a quantitative measure of the degree of immunity of a group of white mice we used the  $LD_{50}$  (which is the dose of tetanus toxin, in  $MLD_{100}$ , leading to the death of 50% of the mice), which was calculated by the probit method [5] by accumulating deaths and survivals by the method of Reed and Muench.

As a preliminary we examined the influence of blood taking on the degree of immunity to tetanus toxin. In this experiment we immunized 77 mice with 0.05 ml of antigen. After about 21 days the first group (40 mice not bled) had an  $LD_{50}$  of 229  $MLD_{100}$  while in the second group (37 mice with preliminary bleeding) the  $LD_{50}$  was 174  $MLD_{100}$  of toxin. Thus a preliminary bleeding somewhat lowers the degree of immunity of the mice.

### RESULTS OF THE EXPERIMENTS

In Table 1 are presented the changes in average antitoxin levels (AE) in the serum and the degree of immunity to tetanus toxin  $LD_{50}$  in relation to the strength of the antigenic stimulus.

As is seen from Table 1, with increasing doses of antigen the AE and  $LD_{50}$  increase. But with increasing strength of antigenic stimulus the rate of antitoxin production falls behind the rate of increase in degree of immunity to toxin. Thus, on increasing the dose of antigen 2-fold (from 0.005 to 0.01 ml) the  $LD_{50}$  increases 4-fold, but the AE 3-fold; on increasing the dose of antigen 20-fold (from 0.005 to 0.1 ml) the  $LD_{50}$  increases 1780-fold, but the AE only 29.6-fold.

TABLE 1

Level of Antitoxin in the Blood and Degree of Immunity to Tetanus Toxin in White Mice about 21 Days after Immunization with Adsorbed Tetanus Toxoid

Dose of toxoid (ml)	Immunity to toxin			Average level of antitoxin in AE/ml
	Dose of toxin (MLD <sub>100</sub> )	Number of mice*	LD <sub>50</sub> in MLD <sub>100</sub>	
0.005	1	5/10	1	0.0025
	2	4/11		
0.010	1	6/2	4	0.0075
	5	5/3		
0.025	10	1/6	28,8	0.035**
	5	10/0		
	10	9/1		
0.050	20	8/2	158	0.062
	50	5/1		
	100	4/2		
0.100	200	3/2	1780	0.074
	500	10/0		
	1000	9/1		
	2000	3/6		

\* In the numerator, the number of survivors, in the denominator, the number of deaths with symptoms of tetanus within a week after the administration of the toxin.

\*\* Result of another experiment set up a month before the main experiment.

In the following experiment, using another group of mice, we studied the change of AE and LD<sub>50</sub> dynamics of immunity. The results obtained are given in Tables 2 and 3.

TABLE 2

Degree of Immunity to Tetanus Toxin in White Mice at Various Intervals after a Single Immunization with Adsorbed Tetanus Toxoid

Dose of toxoid (ml)	Days after immunization							
	7		14		22		28	
	Dose of toxin (MLD <sub>100</sub> )	No. of mice	Dose of toxin (MLD <sub>100</sub> )	No. of mice	Dose of toxin (MLD <sub>100</sub> )	No. of mice	Dose of toxin (MLD <sub>100</sub> )	No. of mice
0.01	1	2/13	1	8/2	1	8/0		
	2	0/15	5	7/3	5	5/4	5	4/3
0.05			10	4/5	10	4/4	10	3/4
	1	5/4	10	10/0	50	10/0		
	2	1/9	20	9/1	100	9/0	200	6/1
	5	0/10	50	7/2	200	8/1	400	5/3
0.25	10	0/10	100	5/4	400	6/3	800	4/3
	1	9/1	100	10/0	1000	9/0	2000	6/1
	2	4/6	200	10/0	2000	7/2	4000	5/2
	5	0/10	400	9/1	4000	5/3	8000	4/3
	10	0/10	800	6/3	8000	3/5	12 000	3/5

Note: In the columns headed "Number of mice" the numerator and denominator have the same significance as in Table 1.

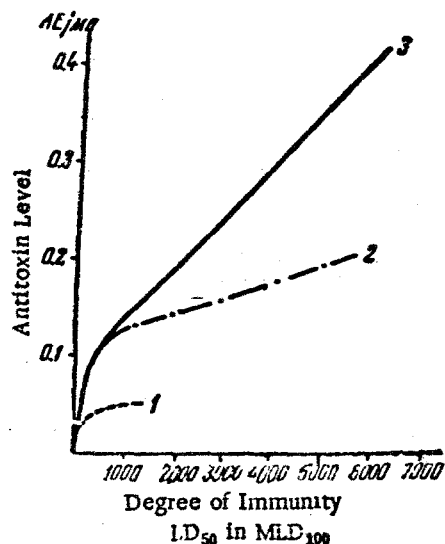
TABLE 3

Dynamics of Antitoxin Levels in the Blood (AE/ml) and Degree of Immunity ( $LD_{50}$ )  
Tetanus Toxin in White Mice Given a Single Immunization with Adsorbed Tetanus Toxoid

Doses of toxoid (ml)	Days after immunization							
	7		14		22		28	
	AE/ml	$LD_{50}$ in MLD <sub>100</sub>	AE/ml	$LD_{50}$ in MLD <sub>100</sub>	AE/ml	$LD_{50}$ in MLD <sub>100</sub>	AE/ml	$LD_{50}$ in MLD <sub>100</sub>
0.01	< 0.0025	0.6	0.011	6.9	0.014	7.6	0.006	7.2
0.05	< 0.0025	1.2	0.044	95	0.117	470	0.145	600
0.25	< 0.0025	1.7	0.053	960	0.190	5000	0.395	6600

The results in Table 3 show that the more marked increase of the  $LD_{50}$  compared to the AE on increasing the dose of antigen, noted in the previous experiment is observed at all the stages of immunization investigated.

However the relation between the  $LD_{50}$  and the AE in the dynamics of immunity is more complicated. Even seven days after a single administration of 0.05-0.25 ml of adsorbed antigen, white mice already have acquired a measurable resistance to tetanus toxin ( $LD_{50} = 1.2-1.7$  MLD<sub>100</sub>), but at that time the antitoxin content of the blood is at about the limit of sensitivity of the method of determination ( $\leq 0.0025$  AE). Between 14 and 22 days the relative increase in  $LD_{50}$  following immunization with 0.05 and 0.25 ml toxoid outstrips the increase of blood AE  $1\frac{1}{2}$  to 2-fold, while between 22 and 28 days the relative increase in the  $LD_{50}$  was the same (for 0.05 ml) or less (for 0.25 ml) than the relative increase in AE.



Dependence of degree of immunity of white mice to tetanus toxin on the level of specific antitoxin.

- 1) Interval after single immunization 14 days;
- 2) 22 days; 3) 28 days.

Thus after immunization with one and the same dose of antigen the relation between the relative tempo of increase in  $LD_{50}$  and AE changes during the development of immunity. From this it follows that the relation between the  $LD_{50}$  and the AE ought to change during the process of immunization. In the figure is shown the relation between the  $LD_{50}$  and the AE at various stages in the development of immunity.

As can be seen from the figure, a given  $LD_{50}$  corresponds to an AE which is larger the longer the interval after the single immunization.

Thus our experiments to study in the same white mice the average levels of antitoxin in the serum and the degree of immunity to tetanus toxin show that the increase in specific resistance of the whole organism accompanies an increase of antitoxin in the blood. But the laws governing the growth of antitoxic and general immunity, both in the dynamics of growth and in their dependence upon the strength of the antigenic stimulus, are different. The relation between the degree of immunity to toxin and the level of specific antitoxin in the blood changes with the dose of antigen and the time after the immunization. All this leads to

the idea that the concentration of antitoxin in the blood is determined not only by the immune state of the organism, but also by other mechanisms regulating the appearance of antitoxin in the circulation.

## SUMMARY

Levels of the specific antitoxin (AE) in blood and the immunity intensity against tetanus toxin ( $LD_{50}$ ) were simultaneously studied on white mice immunized with the adsorbed tetanus toxic. The blood for the antitoxin determination was taken from the blood vessels of the tail (cut off): AE and  $LD_{50}$  increased with an increase of the antigen dose, the growth rate of the latter being much greater. The ratio between the growth rates of AE and  $LD_{50}$  changed in the course of the development of immunity.

$LD_{50}$  being constant, AE level in the blood corresponded to the period elapsed after the antigen injection.

## LITERATURE CITED

- [1] A. Ya. Alymov and D. F. Pletsity, in the book: The Problem of Reactivity in Pathology,\* (Moscow) 1954, pp. 9-18.
- [2] A. A. Vorobyev, Byull. Eksptl. Biol. i Med. 33,43-47, No. 9, 1952.
- [3] D. G. Manolov, Proceedings of the 6th Soviet Anaerobic Conference,\* (Moscow) 1941, pp. 19-21.
- [4] G. V. Shumakova, Problems of Infectious Pathology and Immunology,\* (Moscow) 1954, pp. 127-136.
- [5] C. Bliss, Ann. Appl. Biol. 22, 134, No. 1, 1935.
- [6] G. Istrati, Zentralbl. f. Bakteriol. 143, 106-119, H. 1, 1938.

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\* In Russian.