COMPARATIVE STUDY OF THE RESISTANCE OF MICE IMMUNIZED WITH TYPHOID O- AND VI-VACCINES TO TRUE INFECTION

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The role of Vi- and O-antigens of the typhoid bacillus in the formation of immunity has not been conclusively established up to this time.

The presently used tests of determining the immunogenicity of typhoid strains and vaccines in active and passive protection of mice against infection from lethal doses of typhoid bacilli are essentially tests determining the resistance of mice to acute fatal intoxication, i.e. reveal the basically antitoxic side of immunity. This explains the fact that mice are poorly sensitive to the typhoid microbe and only after massive intraperitoneal infection do animals begin to die in the first three days, which speaks for the predominance of intoxication.

Experiments, carried out to study the antibacterial immunity against typhoid fever using nontoxic doses of the bacillus for challenge, also offers indirect evidence of the immune state in animals infected with microbes not pathogenic for them [2, 11, 12].

The insensitivity of mice to the typhoid microbe obliges investigators studying typhoid fever to use species of salmonella naturally pathogenic for mice in their experimentation.

In the Soviet Union such investigation has been undertaken by several authors [3, 5].

Starting from the fact that the Vi-antigen of typhoid and the paratyphoid C microbe are identical, the strain S. paratyphi C, which causes a true infection in mice, was used to reveal the role of Vi and O-antigens in the formation of immunity [7].

Japanese workers [6, 8, 9, 10, 13] carried out a series of experiments exploring the regulation of the development of infection and immunity in experimental typhoid in a model salmonella infection in mice.

In a earlier paper [4] we showed that S. moscow, a microbe having an antigenic composition related to that of the enteric fever bacillus, produces illness in mice of a cyclical course characteristic for the infective process, both after natural infection via the mouth and after intraperitoneal method of infection.

With the development of the infective process the number of microbes in the organs sharply increases, indicating active multiplication of S. moscow in the mouse. Upon morphological study of sections of mouse organs infected

TABLE 1. Immunogenicity of Vi- and O-typhoid Vaccines in Experimental Active Protection of Mice after Double Immunization and Infection with S. moscow.

Vaccine	Strain for challenge	Value of LD_{50} (no. of cells)	No. of LD_{50}'s in infecting dose	Mean value of ED_{50}	m	$\bar{x} \pm tm$
Ty_2 № 4446	S. moscow	17—90	2—8	$49 \cdot 10^6$	±10	49±23
Ty № 5501		17—90	2—8	$27 \cdot 10^6$	±4	27±9

TABLE 2. Immunogenicity of Vi- and O-typhoid Vaccines after Single Immunization and Challenge with S. moscow

ED_{50} of vaccine Ty_2 No. 4446	ED_{50} of vaccine Ty No. 5501	Number of LD_{50}'s in challenging dose	Ratio of immunogenicity $\frac{ED_{50}\ Ty_2\ №\ 4446}{ED_{50}\ Ty\ №\ 5501}$
$52,5 \cdot 10^6$	$12 \cdot 10^6$	2,5	4,5
$162 \cdot 10^6$	$31,4 \cdot 10^6$	4,0	5,0

TABLE 3. Results of Trial Immunogenicity of O-vaccines Under Conditions of Challenge with Toxic Doses of S. typhi

Vaccine	Strain for challenge	Mean value of ED_{50}	m	$\bar{x} \pm tm$
S. moscow	Ty_2 № 4446	$80 \cdot 10^6$	±17	80±51
Same	Ty № 5501	$7 \cdot 10^6$	±2	7±5,1

with S. moscow, inflammatory centers in the liver and spleen were discovered as were a large number of microbes in the blood and internal organs.

In the present paper we study the role of Vi- and O-antigens of the typhoid bacillus in the formation of immunity in mice infected with S. moscow.

METHODS

Vaccines were prepared from strains of S. typhi No. 4446 (V-form), S. typhi No. 5501 (W-form) and S. moscow No. 643 heated to 56° for the typhoid cultures and to 58° for S. moscow for one hour. The turbidity of the microbial suspension was 5-7 milliard.

The antigenic structure of the strains upon determination with monoreceptor serum on glass was the following: S. typhi No. 4446—Vi, d; S. typhi No. 5501—9,12: d; S. moscow No. 643—9, 12: g, q.

Infection was made with strains of S. moscow No. 643, S. typhi No. 4446, S. typhi No. 5501. The LD_{50} in our investigations ranged from 17-90 microbial cells for S. moscow, 27-90 million cells for S. typhi No. 4446, and 630,000 to 1.7 million cells for S. typhi No. 5501 (in 0.4% agar).

Experiments used non-pedigreed mice of both sexes, weighing 14-17 g.

Comparative study of the immunogenicity of the Vi- and O-typhoid vaccines in relation of challenge with S. moscow and crossed studies (immunization with S. moscow vaccine and challenge with strains Ty_2 No. 4446 and Ty No. 5501 to elucidate the importance of the O-antigen in antitoxic immunity) was performed in active immunization experiments on mice.

Mice were immunized subcutaneously twice at weekly intervals.

Vaccine was injected in doses of 500, 100, 20, four and 0.8 million microbial cells per 0.5 ml in a single injection (the number of microbes was measured by an optical standard) into a group of mice with 10-15 animals receiving each dose. The number of mice for each dosage was the same. Challenge occurred 14 days after the second intraperitoneal injection of a constant dose of the infective culture.

Trial of Vi- and O-vaccines was made always parallel in each experiment; consequently, a single population of cultures was used for infection.

Each experiment was accompanied by a control infected culture. The period of observation after infection was 14 days for S. moscow infection and three days for typhoid culture infections.

The results of the experiments were used in calculating the values LD_{50} and ED_{50} according to the method of Reed and Mench.

RESULTS

Eleven studies of active protection of mice with typhoid vaccine from strain Ty_2 No. 4446 (V-form) and eleven studies with vaccine from strain No. 5501 (W-form) using S. moscow challenge were performed (18 of these with double and four with single immunization).

Table 1 presents a summary of data from 16 investigations. The results of two trials were excluded in accordance with the rule of excluding far scattered values according to the formula $(xi - \bar{x}) > \sigma f$ [1].

It is seen from Table 1 that after immunization of mice with typhoid vaccines the expression of immunity is related to the S. moscow infection.

The absolute values of ED_{50} show the advantage of vaccine Ty No. 5501 as compared to vaccine Ty_2 No. 4446 (ED_{50} of vaccine Ty No. 5501 was less than the ED_{50} of vaccine Ty_2 No. 4446). However, the statistical evaluation of the results obtained does not confirm the validity of the difference between mean values of the ED_{50}.

The second group of experiments was performed according to the method suggested by the L. A. Tarasevich Control Institute for evaluation of the immunogenicity of typhoid strains and vaccines: mice were immunized once with different doses of vaccine and ten days post immunization were challenged with three to five LD_{50} of test-culture.

The results of the single immunization experiments using typhoid vaccines and S. moscow challenge are presented in Table 2.

To study the role of the O-antigen in antitoxin immunity, the following experiments were performed: Immunization was made with the O-vaccine of S. moscow, in two injections at intervals of one week, and challenge—after 14 days with the Vi-strain of Ty_2 No. 4446 and the O-strain of Ty No. 5501.

In Table 3 are presented the data from three experiments using immunization with S. moscow vaccine and infection with Ty_2 No. 4446 and five experiments using S. moscow vaccine and Ty No. 5501 infection. Challenge was made with 0.8-2 LD_{50} of strain Ty_2 No. 4446 and 0.8-5 LD_{50} of strain Ty No. 5501.

Statistical analysis of the results established the validity of the difference of the mean values of ED_{50} for the vaccines of S. moscow after infection with strains TY_2 No. 4446 and Ty No. 5501.

The results of these experiments showed that immunization of mice with VI- and O-typhoid vaccines produce immunity against S. moscow infection. The presence of crossed immunity between the typhoid bacillus and S. moscow indicates the possible use of S. moscow as a test-culture in the study of the anti-infection typhoid immunity in the experimental situation.

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