duction it remained, as in the controls, greatly diminished.

As is evident from the foregoing, the effect of HHT on rats with a one-month inflammation process depends on the treatment conditions. The action of HHT before the induction of inflammation on the whole provides a favorable effect, but the action of HHT after induction, in addition to positive changes, also yields significant negative alterations. First and foremost, there is an exacerbation of the inflammation of the lungs. Based on these data, HHT could be considered as an effective preventive factor. The possibility of its use in treatment of chronic inflammation of the lungs calls for further investigations.

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Characteristics of the Antilysozyme Activity of *Staphylococcus aureus* in Different Types of Experimental Infection

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UDC 616.98.579.861.2] – 092.9 – 036.1

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 178 – 180, February, 1993
Original article submitted October 7, 1992

Key Words: *staphylococcus; antilysozyme activity; population analysis; type of infection process*

According to the laws of classical microbiology, the development of an infection and its type depend upon the characteristics and specific interaction of three forces: pathogenic microorganism, susceptible macroorganism, and environmental conditions. Recently, a great deal of attention has been paid to the factors of microorganism persistence and their role in the formation of different types and forms of infection: lingering, chronic forms, bacteria-carrying forms, hospital infections, etc. [5,7]. The ability of bacteria to inactivate lysozyme, defined as antilysozyme activity (ALA), is considered to be a marker of the persistence of bacteria capable of intracellular parasitism [4]. It has been shown that the ALA of bacteria is a constitutive, secretional factor interacting specifically with lysozyme, which is a crucial element in the natural resistance of the organism [9]. A number of clinical and laboratory investigations have shown a direct correlation between bacterial ALA and the course of infection [6,11]. The role of ALA in the

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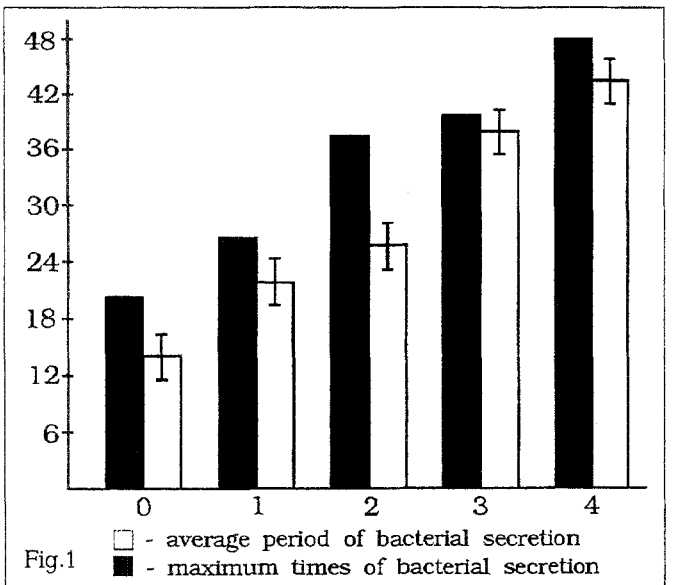

Fig.1

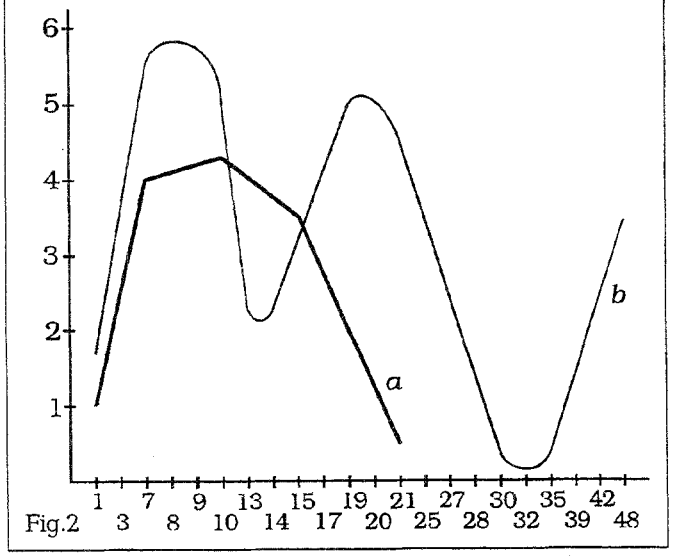

Fig.2

Fig. 1. Period of staphylococcus secretion in mouse kidneys as a function of the initial level of ALA in clones. Abscissa: level of ALA in clones (μg); ordinate: days of observation. Light columns show average period of bacterial secretion; dark columns show maximum times of bacterial secretion.

Fig. 2. Dynamics of microbial insemination in kidneys with respect to initial level of ALA in staphylococcus clones. Abscissa: days of investigation; ordinate: scale of kidney insemination (log CFU/mg). *a*) infection by clone with an ALA of 0 μg; *b*) infection by clone with an ALA of 4 μg.

infection pathogenesis must be investigated using experimental infection models.

The aim of the present study is to assess the antilysozyme activity of *Staphylococcus aureus* in the acute and protracted forms of experimental infection.

MATERIALS AND METHODS

Staphylococcus aureus strain № 162, isolated from the anterior portion of the nose of a resident bacteria carrier, was used in the study. This strain possessed the typical biological properties, exhibited an ALA of 4 μg, and belonged to phagotype 53/83a of phagogroup III. Isogenic clones with different ALA levels (0-4 μg) were obtained from this strain by the "replica" method [13]. Animals were inoculated by intraorbital injection of 0.2 ml of a suspension of a 24-hour staphylococcus agar culture with a concentration of 1.0×10^9 CFU/ml into the eye cellular tissue [1]. CBA×C57Bl/6 (F_1) male mice weighing 18-20 g were used for infection. The kidneys were isolated at different times (over 1-48 days) and the tissue homogenates were seeded in 6.5 % salt broth (accumulating medium) and in yolk-salt agar by the sectorial seeding method [10] with an aid of a standard bacteriological loop. The microbial insemination (CFU/mg kidney tissue) was determined in the yolk-salt agar culture. Identification of staphylococci was performed according to Bergey's guide [12], ALA was determined by the method described by Bukharin *et al.* [3]. Identity of isolated cultures was established according to phagotype similarity. Population analysis was carried out in 36 subpopulations isolated from six mice at each point of time. The results were subjected to statistical analysis using the Student-Fisher test and regression analysis [8].

RESULTS

A correlation is found between the staphylococcus secretion from the kidneys and the level of ALA in the clones (Fig. 1). A clone having no ALA exhibited secretion of staphylococcus up to the 20th day from the moment of inoculation, the average period of bacterial secretion lasting 14.1±0.2 days. The clones with an ALA within the range of 1-4 μg showed a significant prolongation of staphylococcus secretion from the kidney tissue: when the infection was produced by clones with an ALA of 1, 2, 3, and 4 μg, the average period of bacterial secretion was 20.6±0.2, 26.1±0.7, 39.8±0.1, and 43.1±0.2 days, respectively ($p<0.05$). The results were subjected to mathematical analysis by the method of least squares [8], yielding a correlation between the period of bacterial secretion and the initial level of ALA in staphylococcus clones:

$$y = 13.3\pm7.72x,$$

where y is the period of bacterial secretion and x is the initial level of ALA in the clones. According to this formula, a prolongation of the experimental infection by an average of 7.7 days was observed when the initial level of ALA increased by 1 μg. The coefficient of correlation is 0.984.

It is shown in Fig. 2 that the dynamics of the microbial insemination in the kidney tissue depends upon the initial level of ALA in staphylococcus

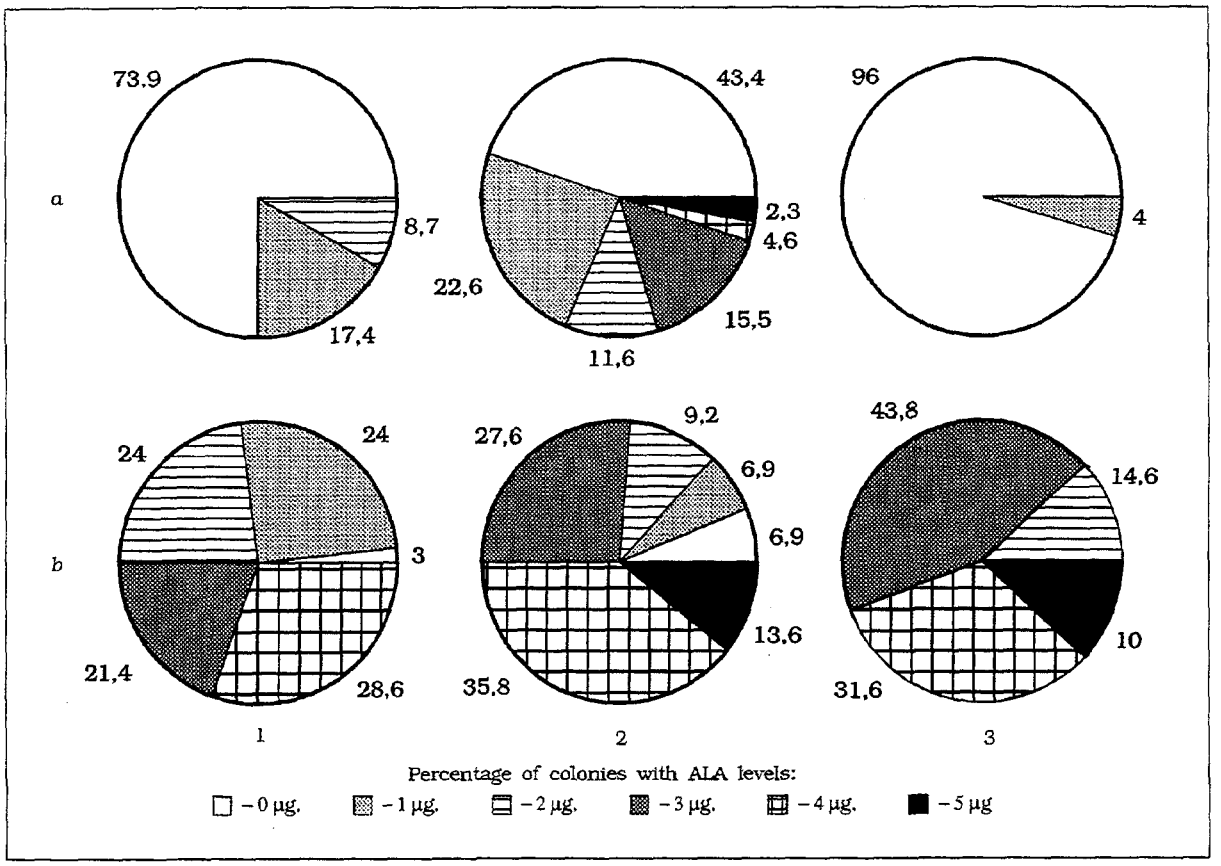

Fig. 3. Dynamics of staphylococcus population structure with respect to level of ALA in diverse types of infection. *a*) acute course; *b*) protracted course. 1) initial population structure; 2) population structure during the course of infection (2 weeks); 3) population structure at the end of the period of investigation.

clones. The dynamics of the microbial insemination in the tissue has a cyclic character [2]. The staphylococcus clone having no ALA and the clones with a low ALA (1-2 μg) induced a cyclic development of the bacteria population in the kidney tissue, this expressed by a regression function of a parabolic type. The results attest to a subsiding type of course of experimental infection in this case. At the same time, a complex function consisting of two curves represented the dynamics of the microbial insemination in the kidneys of mice infected by the staphylococcus clone with an ALA of 4 μg. From the 1st to the 14th day the curve was a parabolic function with a single maximum, whereas on days 14-48 the curve assumed a distinct cyclic character; the rhythm period was 28 days; the average level of microbial insemination was 2.62 log CFU/mg. The amplitude of fluctuations of the microbial insemination was 2.54 log CFU/mg. The clone with an ALA of 4 μg induced a marked infradian rhythmic character of the dynamics of the microbial insemination in the kidneys beginning from 14th day, this showing the development of a subsiding type of infection course.

In order to assess accurately the level of staphylococcus ALA in the diverse types of infection process, the population structure of the clones was investigated with regard to the initial level of ALA in the dynamics of the experimental infection. Variations in the population structure dynamics of the clones with different initial ALA were established for different types of infection course. The initial population of the clone having no ALA consisted 73.9% of subpopulations with a zero level of ALA, 17.4% of colonies with an ALA of 1 μg, and 8.7% of colonies with an ALA of 2 μg (Fig. 3). In the course of infection (during two weeks) the population structure changed significantly in terms of the level of ALA. The heterogenity of the population increased, and subpopulations with a higher ALA were found: 15.5, 4.6, and 2.3% with an ALA of 3, 4, and 5 μg, respectively. However, in the final stage of infection the population heterogenity fell; the majority of subpopulations represented colonies having no ALA (96% of colonies), and 4% of the colonies had a low ALA (1 μg).

Another type of dynamics was observed in the population structure of the clone with an initial ALA of 4 μg. The population structure before infection was represented 50% by colonies with a low or zero ALA and 50% by colonies with a high ALA (3-4 μg). In the course of infection the population

heterogenity increased due to the appearance of a significant fraction (13.6%) of colonies with a high ALA (5 μg). Upon further investigation, the population heterogenity was still quite high (4 types of colonies with different ALA levels), and colonies with zero ALA and with an ALA of 1 μg were eliminated. At the same time, the specific weight of subpopulations with a high ALA (3-5 μg) rose from 50% in the initial population to 85.4% in the 5th-6th week of the experimental infection.

Analysis of the dynamics of the population structure of the clones with respect to the microbial insemination of the kidney tissue showed a dependence of the colony distribution in the population on the scale of insemination. When the level of insemination rose, an increase of the fraction of colonies with a low ALA and a decrease of the number of colonies with a high ALA were observed simultaneously. On the other hand, when the level of microbial insemination dropped, there was an increase of subpopulations with a high ALA and a decrease of subpopulations with a low or zero ALA.

Thus, a correlation was revealed between the level of staphylococcus ALA and the type of course of the experimental infection. Staphylococcus clones with different levels of ALA induced different types of infection course in mice. A subsiding type of infection developed in the case of an infection induced by clones with a low ALA, while in the final stage of the infection a shift occurred in the direction of a decrease in the heterogenity of the population structure with respect to the level of ALA: only colonies with a zero or low ALA remained. A protracted form of infection was caused by a clone with a high ALA, while the dynamics of the microbial insemination demonstrated an infradian rhythmic character, and the population structure of staphylococcus preserved a high heterogenity with respect to the level of ALA. The selection of colonies with a high ALA was observed.

The results thus suggest that the ALA of staphylococcus plays an important role in the pathogenesis of the persistence phenomenon, the period of pathogen survival in the organism being determined by the level of ALA. The findings of the present study may provide an impetus for the development of new methods of prognosis of staphylococcus infections.

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