MICROBIOLOGY AND IMMUNOLOGY

How the Ability of Bacteria to Inactivate Natural Antiinfectious Resistance Factors Affects Their Resistance to the Bactericidal Action of the Blood (Serum)

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 2, pp. 174-176, February, 1996
Original article submitted March 6, 1995

A relationship is found between the resistance of *Escherichia coli, Staphylococcus aureus,* and *Staphylococcus epidermidis* to human serum and whole blood and their ability to inactivate the factors of natural antiinfectious resistance (lysozyme, complement, immunoglobulins, and a bactericidal fraction of leukocytic interferon).

Key Words: *E. coli; staphylococci; ability to inactivate natural resistance factors; resistance to serum and whole blood*

The ability to resist the lethal action of effector mechanisms of immunity confers on pathogens certain advantages for their survival in the host [1]. Gram-negative bacteria, specifically, *E. coli,* which are on the whole sensitive to the bactericidal effect of blood serum (BES), are nonetheless often fairly resistant and therefore capable of initiating infectious processes of some localizations. This singles out BES resistance as an important factor of their virulence [13]. A different situation is observed with gram-positive microorganisms, such as staphylococci, which are commonly little sensitive to BES but are relatively effectively eliminated upon cooperative exposure to opsonins and phagocytes. Resistance to the bactericidal effect of whole blood (BEB) is the marker of their virulence in such cases [6,10].

Study of the nature of these phenomena permitted us to characterize a number of morphological structures and physiological features regulating the survival of bacteria in the presence of host bactericidal systems [11,13]. However, mechanisms directed toward inactivating the natural antiinfectious resistance factors, the principal effectors of BES and BEB, are represented by just a few examples in this series [9]. Hence, this study was aimed at ascertaining the role of a new group of bacterial properties [1] - antilysozyme and anticomplement activities, the ability to inactivate the bactericidal component of an interferon preparation (anti-BCI) of *E. coli, S. aureus,* and *S. epidermidis,* and the known capacity of *S. aureus* for alternative Fc reception of immunoglobulins by protein A - in rendering bacteria resistant to the lethal action of human serum and whole blood.

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MATERIALS AND METHODS

Thirty-nine uropathogenic strains of *E. coli* isolated from patients with pyelonephritis and 22 strains of *S. epidermidis* and 30 strains of *S. aureus* isolated from

0007-4888/96/0002-0160$15.00

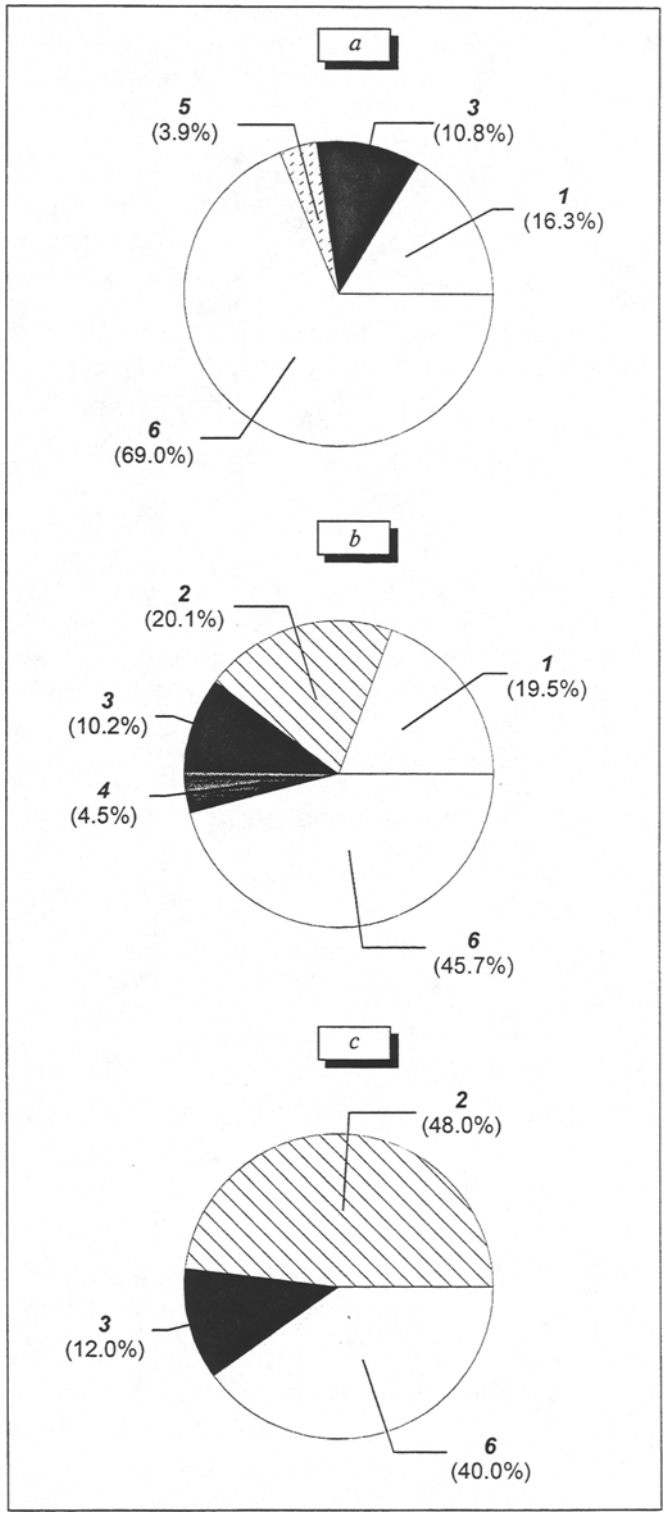

Fig. 1. Effects of antilysozyme and anticomplement activities of *E. coli* (*a*), *S. aureus* (*b*), and *S. epidermidis* (*c*), and of anti-BCI and protein A production on the relative indexes of resistance to BES (BEB). *1*) anticomplement activity; *2*) anti-BCI; *3*) antilysozyme activity; *4*) protein A production; *5*) potentiating effect of anticomplement and antilysozyme activities; *6*) other factors.

patients and bacteria carriers were used in the study. The above samplings were represented by both variants alternative in terms of the anticomplement [2] and antilysozyme [5] characteristics and anti-BCI [8] (and also in terms of protein A production for *S. aureus* [12]), as well as by cultures expressing the above properties to different degrees. The resistance of *E. coli* to *E. coli* BES was assessed as described previously [4], standardized in accordance with the appropriate parameter of *E. coli* strain 212 (L. A. Tarasevich State Research Institute of Standardization and Control of Biomedical Preparations), and expressed as the relative index of resistance to BES (RIR-BES). Staphylococcal resistance to BEB was assessed as described elsewhere [4], standardized by the BEB resistance of *S. aureus* strain 209P, and expressed as the relative index of resistance to BEB (RIR-BEB).

The results were statistically processed using multifactor analysis of variance representing a combination of two-factor analysis of nonorthogonal complexes with assessment of the intensity of the effect of the variability of bacterial characteristics on the variability of RIR-BES/BEB as described previously [7].

RESULTS

Incubation of *E. coli* 212 with native blood serum led to the death of 87.8±3.2% bacteria in the inoculate as soon as by the tenth minute, whereas for uropathogenic *E. coli* this parameter was 34.7±10.2%, on average, with a range of variations from 0 to 87.1%. Hence, the clinical strains of *E. coli* used were 5.28±0.84 times more resistant to BES. The variability of RIR-BES of these bacteria was largely determined by the variability of their antilysozyme and anticomplement characteristics ($p<0.05$) and did not depend on the variability of anti-BCI ($p>0.05$). The absence of an effect of this latter characteristic on BES resistance may be due to the predominantly intracellular localization of BCI excluded from the analyzed system during the preparation of serum samples in this case. The share of RIR-BES variance attributable to variations in the antilysozyme characteristic was as high as 10.8%, and that of anticomplement activity 16.3%. The potentiating effect of the complement and lysozyme during lysis of the gram-negative bacteria evidently determined the low superadditivity of the antilysozyme and anticomplement activities (3.9%) in providing the survival of *E. coli* in the presence of the serum. Hence, the total reliably measured intensity of the effects of the studied properties of *E. coli* on BES resistance was as high as 31% (Fig. 1, *a*).

Two-hour incubation of *S. aureus* 209P with native human blood led to the death of 86.4±2.1% of these bacteria. For the studied samplings of *S. aureus*

and *S. epidermidis* this value was 77.9±3.4 and 42.5± ±7.2%, respectively. It is noteworthy that the representatives of *S. aureus* were 1.82±0.28 times and those of *S. epidermidis* 3.83±0.49 times more resistant to BEB than strain 209P.

The variability of RIR-BEB of *S. aureus* was characterized by a statistically significant ($p<0.05$) dependence on the variability of anti-BCI, the anticomplement and antilysozyme characteristics, and the production of protein A (Fig. 1, *b*). The corresponding intensities of the effects of the characteristics were 20.1, 19.5, 10.2, and 4.5%, respectively. None of the possible combinations of characteristics exerted a potentiating effect on RIR-BEB, and the share of the variability of the relative resistance of *S. aureus* to BEB attributable to the sum of their effects was 54.3%.

In the group of *S. epidermidis* strains reliable relationships with BEB resistance were established for anti-BCI and antilysozyme activity (Fig. 1, *c*). The corresponding intensities of the effect were 48 and 12%, respectively; no combined effect of the characteristics on RIR-BEB was observed. The total share of RIR-BEB variability determined by the studied properties of *S. epidermidis* was as high as 60%.

Hence, the capacity of staphylococci to put out of commission the leukocytic factors (lysozyme and the antibacterial component of the interferon preparation) mediating the intracellular inactivation of microorganisms is a common factor in staphylococcal resistance to the bactericidal action of whole blood. On the other hand, *S. aureus* was found to contribute to BEB resistance and to inactivate the humoral factors (complement and immunoglobulins), which may be due to previously discovered role of these factors in interfering with the opsonic cooperation process [3].

These results permit us to regard the survival of microorganisms in the presence of bactericidal systems of the blood as a phenomenon largely regulated by their capacity to inactivate the natural antiinfectious resistance factors. The antilysozyme and anticomplement activities and anti-BCI and protein A (for *S. aureus*) are responsible for 31 to 60% of the total resistance of staphylococci and *E. coli* to the bactericidal effect of the blood (serum). This goes a long way toward explaining the high incidence of the properties of interest in persisting pathogens [1], which are thus endowed with selective advantages during survival in contact with the defense systems of the host.

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