Some Microorganisms Inactivate Thrombocytic Cationic Protein (β-Lysin)

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A new biological property of microorganisms realized through inactivation of a natural immunity factor, thrombocytic cationic protein, is described. This property may be associated with bacterial persistence. Species-specific differences in the incidence and degree of this potency in microorganisms were revealed.

Key Words: *platelets; antibacterial cationic proteins; mechanisms of persistence*

Adaptation of a pathogen to protective and regulatory systems of the body realized through inhibition of factors of cell-mediated and humoral immunity [2] plays a significant role in the parasite-host interactions.

Thrombocytic cationic protein (TCP) is a factor retaining and maintaining natural resistance of the body [3]. This protein is known as β-lysin. TCP belongs to acute-phase proteins whose levels in biological fluids are sharply increased in stress, infectious diseases, and neoplastic processes [2]. Modulatory action of TCP on the immune phagocytic cells was described [3]. TCP exerts bactericidal effects on various microorganisms (for the major part, in relation to gram-positive microorganisms) [2,3,8]. The data suggest that TCP is the factor of nonspecific resistance of the body.

Bacteria inactivate the components of nonspecific immunity (lysozyme, complement, etc.) providing persistance in the host organism [1].

The resistance of bacteria to TCP was described [9]. However, the problem of inhibition of TCP by microorganisms remains unsolved. The resolution of this issue wold allow us to reveal new mechanisms of bacterial resistance to the of effector systems of a macroorganism.

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This work was designed to study the inhibition of microbicidal effects of TCP by extracellular metabolic products of microorganisms.

MATERIALS AND METHODS

Eighty strains of microorganisms were used: *St. aureus* (n=10), *St. epidermidis* (n=20), other strains of coagulase-negative staphylococci (CNS, n=13), *E. coli* (n=12), *Corynebacterium spp.* (n=15), and *Enterococcus faecalis* (n=10). These strains were isolated from staphylococcal carriers and patients with urogenital diseases.

The ability of microorganisms to inactivate TCP was determined by analyzing inhibition of bactericidal effects of TCP in relation to a highly sensitive strain of *Bacillus subtilis* (ATSS 6633) [8]. The dilution of TCP that provided a 50% inhibition of the strain was assumed as the activity titer. TCP (0.3 ml, dilution rate $^1/_3$ of the activity titer) was added to 0.6 ml of sterile culture conditioned by the studied microorganism and incubated at 37°C for 40-60 min. The mixture of TCP and nutrient broth, as well as the mixture of 0.85% NaCl and nutrient broth served as control I and control II, respectively. After incubation, the suspension of *B. subtilis* (0.1 ml) was added to experimental and control samples. The suspension was prepared from a 16-18-h agar culture on 0.85% NaCl. The optical den-

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sity of the suspension was adjusted to 0.27 (SF-46, λ=650 nm). This suspension was then diluted (1:1000). The mixtures obtained were incubated at 37°C for 40-60 min. Nutrient agar (1.5%) was inoculated with these mixtures (0.2 ml). After a 24-h incubation in a thermostat, *B. subtilis* colonies were calculated on experimental and control plates. Inactivation of TCP by microorganisms was analyzed using the following formula:

$$\frac{N_O\text{-}Nk_I}{Nk_{II}\text{-}Nk_I}\times 100\%,$$

where N_o, Nk_I, and Nk_{II} are the numbers of *B. subtilis* colonies under various conditions (experiment, control I, and control II, respectively).

Results were analyzed statistically [5].

RESULTS

The ability to inactivate TCP was shown to be typical of microorganisms of various groups isolated from the body (Fig. 1). However, there was a considerable species-specific variability of this potency (Fig. 2).

The ability to inactivate TCP was revealed in most strains of staphylococci studied. This potency was more pronounced in CNS than in *St. aureus*. Extracellular products of *E. coli* decreased bactericidal activity of TCP by 69.74±34.93% (in 91.7% of tests). More than a half of corynebacterium strains inactivated TCP. However, the average level of expressivity in these bacteria was minimal compared with that in other microorganisms. Enterococci were characterized by the most pronounced variability of the ability to inactivate TCP.

Thus, extracellular bacterial products inhibit microbicidal effect of TCP. This ability may be examined by analyzing protective effects in relation to the *B. subtilis* strain.

The variability of the incidence and degree of this potency in microorganisms of various groups can be employed for differentiating normal and pathological variants of bacteria (relative to inactivation of TCP). The results obtained allow us to consider the ability to inactivate TCP as the specific feature characterizing microbial resistance to cationic peptides with antibacterial activities [7]. The data suggest that inactivation of defensins and similar peptides [3,6] in the body induced by bacteria should be considered as the property of a pathogen providing its survival in the parasite-host system [1].

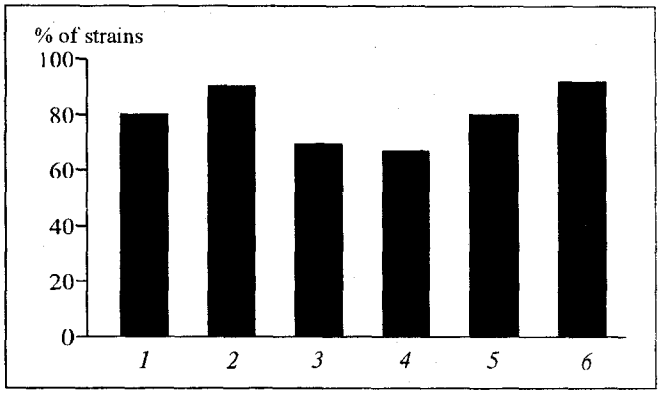

Fig. 1. The occurrence of the ability of various microorganisms to inactivate thrombocytic cationic protein. Here and in Fig. 2: (*1*) *St. aureus*, (*2*) *St. epidermidis*, (*3*) other strains of coagulase-negative staphylococci, (*4*) *Corynebacterium spp.*, (*5*) *E. faecalis*, and (*6*) *E. coli*.

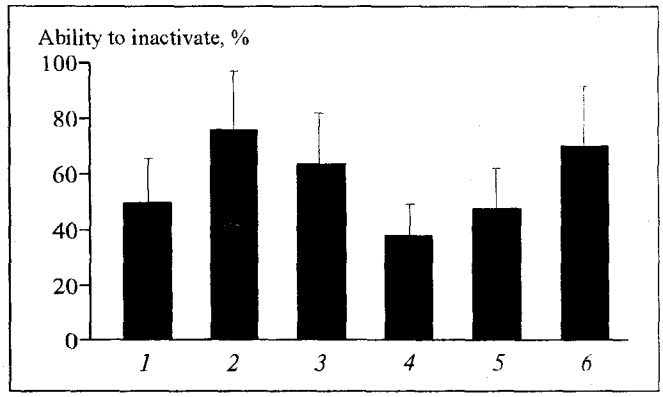

Fig. 2. The ability of various microorganisms to inactivate thrombocytic cationic protein.

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