Antiopsonizing Effect of Extracellular Staphylococcal Products

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The ability to inhibit the opsonizing activity of immunoglobulins and the C3 component of the complement is demonstrated for *Staphylococcus aureus*. This ability is due to the presence of extracellular products such as the anticomplement factor and protein A. An independent and statistically significant determination of the antiopsonizing effect of these extracellular products by the given parameters is established.

Key Words: *staphylococci; opsonization; chemiluminescence*

The ability to suppress natural resistance of the host organism is important for prolonged survival (persistence) of microorganisms [2]. In the case of staphylococci this is achieved through disorders in opsonizing cooperation [6] that lower the effectiveness of the interaction between phagocytes and bacterial cells. The factors determining this phenomenon remain unknown. However, the ability of *St. aureus* to inhibit the complement system [1] in line with the evidence on a possible alternative Fc-receptor binding of immunoglobulins by protein A of these microorganisms [4] raises the question about the role of these factors in the generation of the antiopsonizing effect.

Our aim was to study the mechanisms responsible for the ability of staphylococci to protect themselves against the opsonizing activity of blood serum; this protection has been related to the anticomplement factor and protein A. For this purpose we employed the method of luminol-dependent chemiluminescence (CL) of macrophages [9].

MATERIALS AND METHODS

When the C3-mediated opsonizing effects were examined, CL was induced with zymosan (10 mg/ml) preincubated with pooled normal blood sera (NBS) obtained from 15-20 donors [9]; native zymosan was used as the control. The opsonizing effect of immunoglobulins was evaluated by inducing CL with heat-inactivated staphylococci (strain 209P, 1 bln. cells per ml); intact microorganisms were used as the control, and those treated with commercial preparation of human antistaphylococcal immunoglobulin (10 IU/ml) were used in the experiment. Staphylococci treated with NBS (the same object of phagocytosis) were used to reproduce the opsonizing reactions mediated by the complex of the above-mentioned factors [8]. The effect of the extracellular staphylococcal products on the effectiveness of opsonization was evaluated after contact of the supernatants of *St. aureus* cultures grown in liquid media with CL inducers followed by washing (3 times with a 0.85% NaCl solution) with standardization to the initial optical density. The studied group of staphylococci included 54 strains which manifested either the anticomplement or protein A activity or varied degrees of both activities. Peritoneal macrophages were obtained by perfusing the abdominal cavity of $(CBA \times C57B1/6)F_1$ mice with 0.85% NaCl and purified by adhesion on glass. The cells were suspended to a final concentration of 0.5 mln. cells/ml in Eagle's culture medium supplemented with 0.2% bovine serum albumin and 0.02 M luminol.

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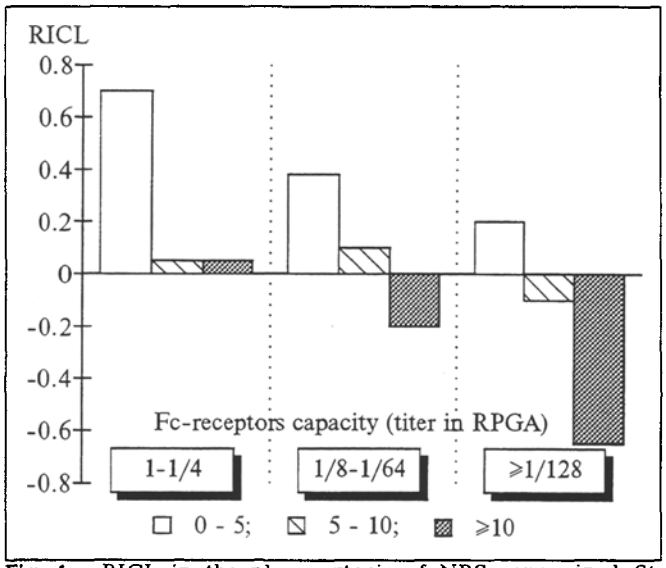

Fig. 1. RICL in the phagocytosis of NBS–opsonized *St. aureus* 209P treated by extracellular staphylococcal products with different levels of anticomplement activity and Fc–receptor capacity.

Chemiluminescence was measured in a BHL-06 biochemiluminometer (Biofarmavtomatika Conglomerate, Nizhnii Novgorod) in a continuous signal recording regime for 10 min after the addition of the phagocytosis objects to the macrophage suspension; the background CL values were subtracted. The relative intensity of chemiluminescence (RICL) was calculated from the following formula:

$$RICL = (A\text{-}C)/(B\text{-}C),$$

where C and B are the CL levels upon phagocytosis of intact and opsonized particles, respectively; A is the CL level upon phagocytosis of opsonized particles incubated with the supernatants of the tested *St. aureus* cultures. The results were evaluated using Student's t test and correlation and variance analyses [5].

RESULTS

The interaction between macrophages and phagocytized particles was accompanied by the development of a luminol-dependent CL with two maxima; the first one occurred after 178±34 sec of incubation and was initiated by the ligand-receptor interaction between the surface of the phagocytized object and the macrophage membrane, and the second one, which was determined by the release of active oxygen into the phagosome with the subsequent production H_2O_2 [9], was recorded after 456±21 sec of the observation period. Opsonization of phagocytized particles increased the intensity of the "oxidative burst," the increase being the most pronounced for the first maximum: 2.73-fold upon interaction with C3-opsonized zymosan and 4.69-fold upon phagocytosis of Ig-opsonized staphylococcus strain 209P.

Preincubation of C3-opsonized zymosan with the supernatants of *St. aureus* grown in liquid medium decreased the total values of luminol-dependent CL (Table 1); the degree of CL inhibition by supernatants of individual strains proved to be directly proportional (r=0.67) to the level of their anticomplement activity determined by inhibition of the complement-dependent hemolysis in gel [1]. This phenomenon was not reproduced either with a purified protein A preparation or after incubation of *St. aureus* culture supernatants displaying no anticomplement activity. The degree of inhibition was somewhat greater for the first CL maximum (RICL=0.397±0.179; p<0.001), testifying to the predominant role of the anticomplement factor in the impairing of the ligand-receptor interaction between macrophage and phagocytized object. This fact could be explained by the breakdown of the bound C3 component of the complement by specific proteases, the presence of which has been demonstrated for microorganisms of other taxonomic groups [10-12] but so far not for *St. aureus*.

A more pronounced suppression of the initial steps of CL was observed when Ig-opsonized staphylococcus strain 209P preincubated with supernatants of *St. aureus* cultures were used as the objects of phagocytosis (Table 1). In this case negative values of RICL proved to be typical for the first maximum (-0.879±0.452; p<0.001), indicating not only an impaired interaction between macrophage receptors and Fc-fragment due to its alternative binding by protein A but also a less pronounced activating ability of Ig-opsonized particles treated by extracellular of staphylococcal products compared with intact particles. In the studied staphylococci the effect on the intensity of CL correlated with the Fc-receptor capacity of their supernatants (r=0.94) and the antiopsonizing effect was specific, since it was impossible to reproduce it with the cultures exhibiting anticomplement activity but lacking protein A. On the other hand, preincubation of phagocytized particles with protein A (commercial preparation) also resulted in a dose-dependent suppression of the CL response of macrophages.

In the study of the combined action of these factors in modeled phagocytosis of heat-inactivated *St. aureus* strain 209P opsonized by pooled NBS yielded negative *RICL* values were obtained in 1/3 of cases against the background of the above-mentioned regularities when supernatants with high levels of both anticomplement activity and Fc-receptor capacity were used (Fig. 1). Two-factor

TABLE 1. RICL of Macrophage in the Phagocytosis of C3 – Opsonized Zymosan and Ig – Opsonized *St. aureus* 209P Incubated with Extracellular Staphylococcal Products ($M \pm m$)

Phagocytized object	Relative intensity of CL		
	1st maximum	2nd maximum	total (10 min)
C3 – zymosan	0.397±0.179**	0.481±0.217*	0.411±0.245*
Ig – 209P	−0.879±0.452**	0.355±0.0409	0.129±0.356*

Note. One asterisk indicates $p<0.05$, two asterisks $p<0.001$.

analysis of variance confirmed the independence and significance of the influence of each of the studied properties on the intensity of CL. The share of the anticomplement activity in this influence was 24.7% of the final antiopsonizing effect of extracellular staphylococcal products and the share of Fc-receptor capacity was 27.2%. On the other hand, these effects are not due solely to the described factors. This may be explained by the participation of other serum proteins in opsonization [7] and the influence of other extracellular staphylococcal products which were not taken into account in this study.

Our results offer new viewpoints for the evaluation of the formation of a protein layer on the surface of a bacterial cell after it has invaded the host organism [3]. This process may be biologically favorable for the causative agent, since subsequent modification of the bound serum components by the anticomplement factor and protein A renders the agent into a weak "stimulus" for phagocytes: their response may be even less intense than in the case of nonopsonized staphylococci.

Thus, we have elucidated the mechanism responsible for the antiopsonizing activity of *St. aureus*, a mechanism which implies the ability of extracellular staphylococcal products to inactivate immunoglobulins and the C3 component of the complement. The disturbances in phagocytosis revealed by this study are evidently a pathogenetic link in the persistence of staphylococci.

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