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Differences in Antiopsonic Effects of the Extracellular Products of *S. aureus* and *N. gonorrhoeae*

D. G. Deryabin, Yu. A. Brudastov, N. R. Akhunova, O. S. Zhurlov, and O. V. Bukharin

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Extracellular products of *S. aureus* and *N. gonorrhoeae* decrease the efficacy of opsonization of these bacteria by blood serum. Antiopsonic activity of *S. aureus* exometabolites is exhibited predominantly during their contact with serum components bound to bacterial surface, which disturbed the reactions between opsonines and neutrophils, as evidenced by decreased chemiluminescent signal during phagocytosis. With gonococci, this effect was observed predominantly during preliminary contact of their extracellular products with the serum, which attenuated the intensity of opsonization. Partial parallelism between changes in the neutrophil-stimulating activity of bacterial cultures and modification of their hydrophobic properties under the effect of the studied factors cannot be regarded as an absolute relationship.

Key Words: *Staphylococcus aureus; Neisseria gonorrhoeae; complement; opsonization; hydrophobic properties; chemiluminescence*

The reactions between microorganisms and neutrophilic phagocytes are a key component in the pathogenesis of staphylococcal and neisserial infections. Along with the resistance to the intracellular killing, the avoidance of the phagocytic reaction of a host organism is an important factor [8,13]. We have demonstrated this reaction in *Staphylococcus aureus* [3]: the efficacy of opsonization of bacterial cells by serum components was reduced by the extracellular protein A that shielded the Fc fragments of bound immunoglobulins and by an anticomplementary factor. The ability of *Neisseria gonorrhoeae* to inactivate the complement (mainly its C1 and C3 components) by the extracellular products [1] prompted us to investigate the probable antiopsonic effect and analyze the similarities and differences in the mechanisms of antiopsonic effects of staphylococcal and gonococcal products.

We also studied modifications in the hydrophopic properties of staphylococci and gonococci caused by fixation of serum components. The need in this comparison is explained by important role of physical properties of bacterial surface for adhesion to eukaryotic cells, including the opsonization [14] and other processes associated with specific adhesion mechanisms [9]. It should be noted that the relationship between the hydrophobic properties of bacterial surface and its phagocyte-stimulating ability is poorly understood.

MATERIALS AND METHODS

Twelve *S. aureus* strains isolated from patients with pyoinflammatory diseases of staphylococcal etiology and identified by biological and biochemical (Staphy-

Institute of Cellular and Extracellular Symbiosis, Ural Branch of the Russian Academy of Sciences, Orenburg

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TEST, Lachema) tests were studied. Fourteen strains of *N. gonorrhoeae* isolated from the urethral discharge of patients with urogenital gonorrhea and identified with the use a NeisseriaTEST kit (Lachema). These strains possessed high inhibitory activity of the extracellular products toward the complement [2].

Bacterial cells were opsonized by the commercial reagents R1, R3, and R5 (Reakom, Kirov, Russia) that represent C1, C3, and C5-deficient sera and allow isolated reproduction of the alternative and classical pathways of the complement activation; their combined effects rule out the formation of the membrane-attacking C5b-C9 complex. This is of the key importance in the opsonization studies, because *N. gonorrhoeae* are sensitive to the bactericidal effect of serum. Bacterial opsonization (10^9 CFU/ml) was carried out for 30 min at 37°C using the above-listed reagents in a final dilution of 1:4 for and was followed by three washings. The following opsonization protocols were used (Table 1): 1) opsonization with intact reagents (OP_1); 2) opsonization with reagents preincubated for 30 min at 37°C with homologous supernatants (the preincubation protocol) (OP_2); and 3) opsonization with intact reagents followed by cell incubation in homologous supernatants for 30 min at 37°C (the post-incubation protocol) (OP_3). Intact bacterial cells served as the control (OP_0).

The hydrophobic properties of the bacteria were studied by the method of separation (in a two-phase system) of water solutions of polyethylene glycol (PEG 6000, Sigma) and dextran (T500, Austranal) with final concentrations of 6.2 and 4.5 weight percent, respectively [10]. The degree of bacterial surface hydrophobia was expressed as the hydrophilic-lipophilic balance (HLB) equal to the logarithms of the ratio of light absorbances of the upper (enriched with PEG 6000) and lower (dextran T500) phases measured after stratification of the suspension [6].

The efficacy of opsonization was evaluated from the intensity of induced luminol-dependent chemiluminescence (CL) of human peripheral blood neutrophils isolated in a Ficoll:Urograffin gradient (1.077: 1.119) and suspended to a concentration of 10^6 cell/ml. Chemiluminescence was recorded with a BHL-06 biochemiluminometer (Biofarmavtomatika, Nizhnii Novgorod, Russia) in a permanent mode for 10 min after the addition of opsonized bacteria to the neutrophil suspension (1:10); the background CL values were deduced [3]. The intensity of CL (ICL) was expressed as the ratio of the signal from opsonized bacteria to the signal of intact bacterial cells. The results were processed by the variation statistics methods [5].

RESULTS

Opsonization of *S. aureus* and *N. gonorrhoeae* in the regimens activating the complement system by the classical (R3 reagent) or alternative (R1 reagent) pathway and by both pathways without the formation of the membrane-attacking C5b-9 complex (R5 reagent) led to modification of the surface properties of bacterial cells and alteration of their activating effect toward neutrophils (Table 2).

The maximum increase of bacterial cell HLB for both *S. aureus* and *N. gonorrhoeae* was observed after opsonization with C5-deficient serum (R5):

$HLB_{SA}OP_1[R5] = 0.453 \pm 0.061$

vs. $HLB_{SA}OP_0 = 0.120 \pm 0.084$ ($p<0.01$)

and $HLB_{NG}OP_1[R5] = 0.553 \pm 0.122$

vs. $HLB_{NG}OP_0 = 0.161 \pm 0.138$ ($p<0.01$).

Less pronounced changes in this parameter were observed when the complement was activated by the alternative pathway: $HLB_{SA}OP_1[R1] = 0.342 \pm 0.049$ ($p<0.05$) and $HLB_{NG}OP_1[R1] = 0.422 \pm 0.101$ ($p<0.01$). The minimum changes in HLB were observed after cell opsonization with C3-deficient serum (R3):

$HLB_{SA}OP_1[R3] = 0.161 \pm 0.037$ ($p>0.05$)

and $HLB_{NG}OP_1[R3] = 0.422 \pm 0.101$ ($p<0.05$),

which in staphylococci did not significantly modify the hydrophobic properties of the bacteria.

Changes in the neutrophil-activating ability of *S. aureus* cells opsonized by different reagents were heterogeneous, but in general corresponded to the shifts in hydrophobic properties described previously, reaching the maximum after incubation with R5 reagent ($ICL_{SA}OP_1[R5] = 1.61 \pm 0.10$, $p<0.01$). The relative increase in

TABLE 1. The Order of Regimens of Opsonin Reaction with Extracellular Products of Staphylococci and Gonococci

Stage	OP_0	OP_1	OP_2	OP_3
Contact of reagents with bacterial culture supernatants	—	—	+	—
Addition of bacteria	Bacterial suspension, 10^9 CFU/ml			
Bacterial opsonization	—	+	+	+
Contact of opsonized bacteria with homologous culture supernatants	—	+	+	+
Changes in ICL and HLB	+	+	+	+

Note. "—" not performed (0.15 M NaCl was used); "+" the stage was performed.

TABLE 2. ICL in Phagocytosis and Absolute Shifts of HLB in Opsonization of Staphylococci and Gonococci by C1, C3, and C5-Deficient Sera (R1, R3, and R5) in Different Modes of Opsonin Reaction with Bacterial Extracellular Products ($M \pm m$)

Opsonization regimens		*S. aureus*		*N. gonorrhoeae*	
		ICL	ΔHLB	ICL	ΔHLB
OP_1	R1	1.25±0.10	0.22±0.10	1.58±0.14	0.26±0.08
	R3	1.14±0.11	0.04±0.08	1.43±0.18	0.16±0.07
	R5	1.61±0.10	0.33±0.08	6.18±1.56	0.39±0.09
OP_2	R1	1.15±0.13	0.12±0.07	0.53±0.10	0.15±0.07
	R3	1.08±0.13	0.06±0.09	1.05±0.15	0.28±0.07
	R5	1.25±0.18	0.37±0.11	3.48±1.49	0.14±0.08
OP_3	R1	0.98±0.09	0.07±0.10	1.37±0.17	0.34±0.09
	R3	1.24±0.10	0.08±0.14	1.27±0.14	0.39±0.08
	R5	0.72±0.08	0.14±0.06	4.86±1.12	0.26±0.08

ICL was minimal and statistically negligible after opsonization with R1 and R3 ($ICL_{SA}OP_1[R1]=1.25\pm0.10$, $ICL_{SA}OP_1[R3]=1.14\pm0.11$). The studied sample of *N. gonorrhoeae* was more homogeneous and showed a higher neutrophil-stimulating activity with the maximum with R5 ($ICL_{NG}OP_1[R5]=6.18\pm1.56$, $p<0.01$) and minimum with R3 ($ICL_{NG}OP_1[R3]=1.43\pm0.18$, $p>0.05$).

Studies of the antiopsonic properties of extracellular products of *S. aureus* and *N. gonorrhoeae* showed that the effects of serum factors on the efficiency of opsonization were more complex and consisted not only in modification of the surface properties of a bacterial cell. A significant increase in HLB after R3 opsonization of *N. gonorrhoeae* (preincubation with extracellular products of gonococci, $ICL_{NG}OP_2[R3]=0.437\pm0.112$, $p<0.01$) led to a statistically significant increase in ICL ($ICL_{NG}OP_2[R3]=1.05\pm0.15$, $p>0.05$). For *N. gonorrhoeae* this phenomenon can be explained by the protective effect of C1q component [11] which prevents activation of the humoral and cellular bactericidal mechanism after its fixation on the cell wall. After opsonization of *S. aureus* with C3-deficient serum, these parameters approximated the levels recorded in opsonization with the native R3 reagent.

Another pattern of changes was observed when R1 was used for opsonization in different protocols of its contact with extracellular bacterial products. The hydrophobic properties and the phagocyte-stimulating activity of *S. aureus* decreased in comparison with the control in the following series of opsonization protocols: intact reagent>preincubation>postincubation ($HLB_{SA}OP_2[R1]=0.239\pm0.061$, $HLB_{SA}OP_3[R1]=0.188\pm0.097$, $p>0.05$ and $ICL_{SA}OP_2[R1]=1.15\pm0.13$, $ICL_{SA}OP_3[R1]=0.98\pm0.09$, $p>0.05$, respectively). This parallelism is probably due to the presence of a specific staphylococcal protease [7] that destroys both free and bound C3 component (C3b), thus decreasing the efficacy of the reaction between opsonized bacteria and C3b-receptors of phagocytes [4]. In opsonization of *N. gonorrhoeae* by R1, a decrease in HLB and ICL was observed only after preincubation ($HLB_{NG}OP_2[R1]=0.315\pm0.114$, $ICL_{NG}OP_2[R1\}=0.53\pm0.10$, $p<0.05$), while after postincubation these parameters were higher than after opsonization with intact reagent ($HLB_{NG}OP3[R1]=0.496\pm0.086$, $ICL_{NG}OP_3[R1]=4.86\pm1.12$, $p>0.05$). This can be regarded as the evidence that gonococci possess no anticomplement proteases.

Assessment of the alternative and classical activation of the complement by R5 showed that preincubation with the reagent did not modify the HLB and ICL ($HLB_{SA}OP_2[R5]=0.487\pm0.091$, $ICL_{SA}OP_2[R5]=1.25\pm0.18$, $p>0.05$), while postincubation inhibited the opsonization ($HLB_{SA}OP_3[R5]=0.264\pm0.051$, $ICL_{SA}OP_3[R5]=0.72\pm0.08$, $p<0.05$). The fact that the degree of the neutrophil activation after R5 opsonization in the postincubation regimen was lower than ICL after opsonization with intact serum and ICL of intact bacteria, is principally important.

Study of the effect of extracellular products on the efficacy of opsonization in *N. gonorrhoeae* showed a different distribution: postincubation affected this process negligibly ($ICL_{NG}OP_3[R5]=4.86\pm1.12$, $p>0.05$), while preincubation markedly decreased it: $ICL_{NG}OP_2[R5]=3.48\pm1.49$, $p<0.05$). This phenomenon may be due to extracellular inactivation of the complement by the lipopolysaccharide components of the *N. gonorrhoeae* cell wall which accumulate in the medium during culturing. This mechanism of escaping the bactericidal effect of the complement is probable *in vivo*, where it may lead to a local decrease in the activity of the complement system in a focus of inflammation [12].

Study of the effects of extracellular products of *S. aureus* and *N. gonorrhoeae* on the efficacy of these bacteria opsonization by serum components showed

different patterns of antiopsonic effects. On the one hand, antiopsonic effect of *S. aureus* extracellular products is stronger after modification of a surface pretreated with serum components, which may be due to shielding of immunoglobulin Fc-fragments and modification of C3b. On the other hand, the antiopsonic effect of *N. gonorrhoeae* extracellular products develops after the contact with the complement, decreasing the efficacy of subsequent opsonization. Modifications of bacterial surface HLB in general correspond to changes in its phagocyte stimulating ability. This parallelism is the best pronounced in opsonization unrelated to the interference of bacterial exometabolites, which confirms the need for further studies of their effects on the effectiveness of the host immune reactions.

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