The Role of Opsonization of *Staphylococcus aureus* in the Development of Local Inflammation and System Response

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 9, pp. 298-300, September, 1996
Original article submitted January 19, 1996

The *in vitro* neutrophil-stimulating activities of two *S. aureus* strains are compared with their *in vivo* cytotoxic activities, including the use of intact heterologous neutrophils. After opsonization with normal autologous serum, clinical isolates of *S. aureus* differ in the ability to induce luminol-dependent chemiluminescence of guinea pig peritoneal neutrophils. After opsonization, the opsonin-dependent strain markedly stimulates chemiluminescence in comparison with the opsonin-independent strain. The local inflammation induced in guinea pig by intracutaneous administration of the opsonized opsonin-dependent strain is more intense than that induced by the opsonin-independent strain. Intramuscular administration of opsonin-dependent *S. aureus* strain increases mortality in mice from 10 to 46%, while the addition of normal guinea pig neutrophils to the inoculate has no effect on this process. Opsonization of opsonin-independent strain decreased mortality from 78 to 40%, the effect being potentiated by the addition of neutrophils to inoculate (mortality 14%). Presumably, the opsonin dependence of *S. aureus* manifested *in vitro* is associated with its pathogenicity *in vivo*, which may be caused by intense stimulation of the respiratory burst in neutrophils.

Key Words: *chemiluminescence; inflammation; opsonization*

Serum factors play an important role in phagocytic response to bacteria. Opsonization stimulates internalization and killing of bacteria by neutrophils (NP) and macrophages, for example, due to production of oxygen metabolites [8]. Meanwhile, impaired opsonization is linked with reduced resistance to a number of infections [18]. However, opsonized microorganisms can stimulate NP-mediated cell damage in tissue cultures [5,11,13,15] and increase mortality among animals [4]. This double function of NP prompted us to study a possible dependence between the mechanism of NP action and the ability of a microorganism to activate NP.

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MATERIALS AND METHODS

Experiments were performed on male albino Hardly guinea pigs weighing 300-350 g and ICR mice weighing 15-17 g.

In mice, the suspension of *Staphylococcus aureus* was injected into deep muscles of the fore and rear limb (two doses of 10^9 microbial bodies in 0.1 ml phosphate-buffered saline (PBS, pH 7.3). Some animals were injected the same dose of *S. aureus* with 10^6 NP in a total volume of 0.1 ml.

Clinical isolates of *S. aureus* (17-h cultures) were adjusted to a concentration of 10^{10} microbial bodies/ml and stored at -70°C. The microorganisms were opsonized by incubation with an equal volume of guinea pig serum for 30 min at 37°C and washed with buffer. Final concentration of microorganisms

0007-4888/96/0009-0912$15.00

TABLE 1. Properties of OD and OI *S. aureus* Strains *In Vitro* and *In Vivo (M±m)*

Parameter	OD strain		OI strain	
	intact	opsonized	intact	opsonized
Index of CL stimulation	2.64 (0.2)	6.04 (0.22)**	3.65 (0.45)**	5.03 (0.23)**
n	12	9	6	6
Mortality: ratio between the number of dead mice and the total number in the group				
without NP	2/20	7/15*	15/19	9/22*
with NP	2/13	3/16	12/23	4/27*

Note. *$p<0.05$, **$p<0.01$ compared with intact microorganisms; *$p<0.01$ compared with opsonized OD strain, **$p<0.1$ compared with intact OD strain.

in *in vitro* and *in vivo* experiments was 10^9 microbial bodies/ml.

Peritoneal NP were obtained from guinea pig 17 h after intraperitoneal administration of 30 ml normal saline. The Trypan blue exclusion test showed that 85-90% cells were viable. Final concentration of NP in *in vitro* and *in vivo* experiments was 10^6 cells/ml.

Chemiluminescence (CL) of NP was measured after mixing NP with the suspension of *S. aureus* in 2 ml PBS containing 5 mM glucose and 10 mM luminol (Aldrich). The mixture was incubated at 37°C with periodical shaking. The reaction was read in a Packard Tri-Carb scintillation counter in a tritium channel at 10-min intervals. The results were expressed as the ratio between the peak response (cpm) after the addition of the microorganism and the nonstimulated response prior to the addition of the microorganism (stimulation index).

Local inflammation was induced in guinea pig by intradermal injection 0.1 ml of *S. aureus* suspension in the ear. The reaction was evaluated from the weight of the inflamed area.

Systemic response was assessed in a mouse model of intramuscular infection after injection of the *S. aureus* suspension with and without NP. The infection was assessed by a six-day mortality test.

The significance of differences between CL data and intensity of local reaction was evaluated by Student's *t* test. The mortality data were analyzed using the χ^2 test.

RESULTS

Stimulating activity of S. aureus strains. Peak CL of unstimulated NP (the baseline response) was not higher than 200 cpm. Two strains, opsonin-dependent (OD) and opsonin-independent (OI), were selected according to their ability to induce the CL response of NP after opsonization (Table 1). The CL-stimulating activity of nonopsonized strain (designated OD) was practically the same as that of intact OI strain. Opsonization of OD strain increased the stimulation index by 2.28±0.25 times *vs.* 1.36± 0.23 times for the OI strain ($p<0.02$).

Local inflammation. Opsonization had no effect on the intensity of inflammation induced by OD strain (the inflamed area weighed 99.3±8.6 mg *vs.* 90.0±9.0 mg for nonopsonized strain). By contrast, opsonization of OI strain reduced the intensity of inflammation: 112.5±15.8 *vs.* 69.0±12.0 mg after opsonization. When individual variations were taken into account, opsonization of OI strain proved to decrease the intensity of inflammation by 0.65±0.09 times in contrast to opsonization of OD strain, which resulted in a weak stimulation of the process: by 1.23±0.09 times ($p<0.001$).

Mortality among infected mice. Opsonization of OD strain increased mortality from 10 to 46% during a 6-day observation period (Table 1). The addition of normal guinea pig NP had no effect on mortality caused by opsonized or intact OD strain. By contrast, opsonization of OI strain decreased mortality from 78 to 40%, while the addition of NP potentiated this effect, decreasing mortality to 14% ($p=0.08$).

Activation of the respiration burst in phagocytes is thought to enhance their antibacterial activity [2,7,9,14,17]. However, phagocytes can damage the host tissues [5,13], which may be associated with increased oxygen production [3,7].

Opsonization of bacteria *in vitro* is the most potent activator of phagocyte metabolism and bacterial killing [7,14,16]. Analogously, activated NP can induce local damage to endothelial cells [15].

In the present study we investigated the CL response of NP induced by two strains of *S. aureus*. Opsonization of the OD strain but not of the OI strain by normal guinea pig serum stimulated the CL response. In order to assess the correlation between *in vivo* and *in vitro* data we used the same concentrations of both strains and NP.

It was demonstrated that the opsonized OD strain slightly increases the intensity of local inflammation, whereas the opsonized OI strain decreases it. The intact OD strain, which was less virulent for mice than the OI strain, acquired higher lethality after opsonization. The addition of NP to the inoculate had no protective effect. By contrast, opsonization of the OI strain reduced its lethality, while the addition of NP to the inoculate potentiated this effect. The ratio between the CL induced by the OD strain and that induced by the OI strain was 0.72±0.14 before opsonization and 1.2±0.09 after it ($p<0.02$). A similar effect was observed for mortality among mice inoculated with these strains before and after the addition of NP to the inoculate (Table 1).

Thus, an increased CL response induced by bacteria is not always protective. In fact, preopsonization of bacteria results both in increased mortality and local tissue damage [1,4].

The damaging effect is potentiated by reproduction of a microorganism in the primary focus of infection [6] irrespective of increased NP migration [4]. The ability of NP to respond by a more intense respiratory burst to stimulation by certain microorganisms correlates with the cytotoxic potential of these NP [10-12]. A more intense respiratory burst *in vitro* may be the precursor of local and systemic damaging effects.

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