

## IMMUNOGLOBULIN Fc RECEPTORS IN CLINICAL STRAINS OF *Staphylococcus aureus* DO NOT CONFER RESISTANCE TO PHAGOCYTOSIS IN AN *IN VITRO* ASSAY

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### SUMMARY

*Staphylococcus aureus* binds Immunoglobulin G (IgG) on its external surface due to the presence of specific receptors for the Fc domain of this immunoglobulin. This mechanism represents a kind of camouflage against phagocytic cells. In order to confirm that possibility an *in vitro* evaluation of the phagocytic activity of leukocytes polymorpho-nuclear (PMN) against strains of *Staphylococcus aureus* was done, comparing 18 strains isolated from clinical samples and 16 from healthy individuals. The presence of Fc receptors was evaluated by haemagglutination (HA) with erythrocytes group A after incubation of the strains with IgG anti blood group A. Phagocytosis of *S. aureus* was carried out by mixing live bacteria with a suspension of human PMN and incubating at 37 °C for 1 h; survivors were counted as colony forming units by plating. The strains from clinical specimens showed higher HA than those from healthy individuals ( $p = 0.01$ ); but the former were killed more efficiently than the latter (80-90% and 40%, respectively). It may be possible that *S. aureus* showed different behavior *in vivo*, where could express other virulence factors to prevent the action of phagocytes.

**KEYWORDS:** *Staphylococcus aureus*; Fc receptors and phagocytosis; Phagocytic evasion; Polymorphonuclear leukocytes; Haemagglutination.

### INTRODUCTION

One of the most important non-specific immune reactions of a host is the activity of phagocytes, which internalize bacteria to destroy them. It has been proposed that the presence of protein A on the cell wall of *S. aureus* would cause them to be covered with host immunoglobulins (Ig) bound to the bacterial cell through their Fc domains<sup>6</sup>. Due to its affinity to Ig Protein A has been called "the Fc receptor". Bacteria covered with host Ig would not be recognized by phagocytic cells, thus preventing their activation<sup>1,5,6</sup>.

Fc receptors on bacterial surfaces can be estimated using an assay developed by YAMADA *et al.* (1991)<sup>11</sup> and modified by HERNÁNDEZ & RIVERA (1998)<sup>3</sup>. Bacterial cells are incubated with IgG specific against group A red-blood cells. Fc-receptors on the surface of the bacterial cells bind IgG through their Fc domain, allowing IgG molecules to bind to group A red-blood cells, resulting in a haemagglutination reaction (HA). The titers of HA obtained in this manner provide indirect measurements of FC receptors on bacterial surfaces.

The overall aim of this study was to evaluate whether the presence of Fc receptors on the surface of *S. aureus* protects bacteria against phagocytosis and killing in an *in vitro* assay of phagocytosis.

### MATERIALS AND METHODS

**Bacteria:** A total of thirty-four strains of *S. aureus* were evaluated, 18 of them were isolated from abscesses or blood from patients at San Juan de Dios Hospital, San José, Costa Rica. The other 16 strains were from the skin of healthy high-school students. All strains were identified using a BACTEC 9050 (Becton Dickinson Diagnostic Instrument System).

**Haemagglutination (HA):** Bacteria were coated with human monoclonal anti (blood group) A IgG, by mixing a bacterial suspension (density equivalent to tube 1 of a MacFarland nephelometer -  $3 \times 10^8$  cells/mL), with the antibody and incubating 30 min at 37 °C. The bacterial cells were washed 3 times and aliquotes of 50 µL of this suspension were placed in U shaped microtiter wells, then 50 µL of a 5% suspension of blood A erythrocytes were added and the plate was incubated at 37 °C for 30 min and placed at 4 °C. The HA was evaluated at 4, 8 and 24 h. To estimate the density of IgG-receptors on the surface of bacteria, serial two-fold dilutions of the antibody were made and the process was followed as above described to obtain the end point for the HA<sup>3,11</sup>.

**Phagocytosis assay:** The method described by GADEBERG *et al.*<sup>2</sup> was used. Briefly, 500 µL of polymorphonuclear (PMN) leukocytes (5

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$\times 10^6$  cells/mL) suspended in phosphate buffered saline with 1 mM calcium and 5 mM glucose (PBS-Ca-glucose), concentrated according KALMAR *et al.*<sup>4</sup> were placed in Ependorff tubes. Each tube received a mixture of 300  $\mu$ L PBS-Ca-glucose, 100  $\mu$ L of serum from a healthy individual, and 100  $\mu$ L of a suspension of each bacterial strain (density equivalent to the tube number 2 of MacFarland nephelometer -  $6 \times 10^8$  cells/mL) incubation followed for 1 h at 37 °C, with mixing of contents every 10 min.

At the same time the phagocytosis assay was run, another control tube for each strain was prepared without PMNs to evaluate bacterial survival in the presence of serum. Into these tubes were added 100  $\mu$ L of serum, 100  $\mu$ L of the bacterial suspension, and 800  $\mu$ L of PBS-Ca-glucose, and were incubated at 37 °C. At time 0 and after 60 min of incubation, aliquots were taken and then serially diluted to  $10^{-7}$ , plated onto tripticase soy agar plates and incubated for 24 h at 35 °C, after which surviving bacteria (colony formed units, or CFU) were counted.

**Lysostaphine assay:** Four strains of *S. aureus*, two isolated from clinical samples and another two from healthy individuals were subjected at the same process described above, with the exception that after 45 min into incubation period, 15  $\mu$ L of lysostaphine (Sigma 0761) in PBS-Ca-glucose (25 U/mL) were added to lyse all extracellular bacteria.

**Microscopic count of PMN with phagocytosed bacteria:** From each tube used for both, the phagocytosis assay count of CFU and lysostaphine, smears were prepared and stained with Leishman solution to count the number of PMN cells either with associated or internalized bacteria.

## RESULTS

*S. aureus* strains isolated from clinical specimens showed higher haemagglutination titers than those isolated from healthy individuals ( $X^2 = 5.81$ ,  $p = 0.01$ ). These results indicate that the former possess more surface Fc receptors than those isolated from healthy individuals (Table 1).

Surprisingly, when clinical strains were challenged with PMN leucocytes in a phagocytosis assay, bacterial killing was above 90%, whereas the range of bacterial killing was 40 to 50% in strains isolated from healthy individuals (Table 2).

Light microscopy assessment of phagocytosis at 60 min showed that control strains were not as frequently associated with leukocytes (40%) as clinical isolates (80-90%). Phagocytosis was assessed also in prepa-

**Table 1**

Haemagglutination of *Staphylococcus aureus* incubated with human IgG anti-A followed by group-A-erythrocytes

Bacterial source	Haemagglutination	Titer
	$\leq 1 : 2$	$\geq 1 : 8$
Healthy individuals	9	7
Clinical samples	3	15

$X^2 = 5.81$ ,  $p = 0.01$ .

**Table 2**  
Bacterial killing after phagocytosis of *Staphylococcus aureus*

Strain origin	Mortality	Mortality	Total
	< 50%	> 50%	
Healthy individuals	15	1	16
Clinical samples	1	17	18

$X^2 = 26.45$ ,  $p < 0.0001$ .

rations to which lysostaphin was added after 45 min to lyse all but intracellular bacteria in order to avoid mistaking externally associated bacteria with ingested ones. We thus corroborated that only 40% from non-clinical strains were killed, in comparison with 80-90% of bacteria from clinical isolates. These data support the finding that the latter are phagocytosed and killed to a greater extent.

## DISCUSSION

In previous work, we labeled Ig attached to Fc receptors on the bacterial surface of *S. aureus* with anti-human-IgG conjugated with colloidal gold particles, showing that strains with high HA titers exhibited higher number of surface gold particles, homogeneously distributed on bacterial surface, than strains with low HA titers. In these labeling experiments gold particles represent the distribution of Fc receptors<sup>5</sup>. Also, if the IgG attached on the bacteria by their Fc domains represent a kind of camouflage, we expected that strains with high concentration of Fc receptors could exhibit some antiphagocytic activity or survived to the phagocytosis more than strains with fewer Fc receptors. Nevertheless, our findings are unexpected and taken to indicate that the presence of Fc receptor in clinical *S. aureus* specimens does not offer protection against phagocytosis, at least in our *in vitro* assay.

This means the possible masking effect of serum IgG bound to the bacterial surface was not enough to prevent adherence and ingestion by phagocytes, and it somehow resulted in increased association and phagocytosis of *S. aureus*. It may be possible for this agent to behave differently *in vivo*, where expression of other virulence factors may come into play, such as coagulase production. Alternatively, host factors such as interleukins could increase the bactericidal activity of PMN<sup>8-10</sup>.

This data showed that under our experimental conditions, *S. aureus* strains which exhibited more Fc receptors were phagocytosed and killed in higher proportion than strains from healthy persons, which showed fewer Fc on their surface.

## RESUMEN

### Los receptores Fc para inmunoglobulinas en cepas clínicas de *Staphylococcus aureus* no confieren resistencia a la fagocitosis *in vitro*

*Staphylococcus aureus* liga inmunoglobulinas G (IgG) a su superficie externa debido a la presencia de receptores para el dominio Fc de esas inmunoglobulinas. Este mecanismo representa una clase de camuflaje contra células fagocíticas. Para confirmar tal posibilidad se realizó una evaluación *in vitro* de la actividad fagocítica de leucocitos polimorfonucleares (PMN) contra cepas de *Staphylococcus aureus*, comparando

18 cepas aisladas de casos clínicos y 16 de individuos sanos. La presencia de receptores fue evaluada por hemaglutinación (HA) con eritrocitos grupo A luego que las cepas fueron incubadas con IgG anti grupo sanguíneo A. La fagocitosis de *S. aureus* fue realizada mezclando células vivas con una suspensión de PMN e incubada a 37 °C por una hora; las bacterias sobrevivientes fueron contadas como unidades formadoras de colonias por plato. Las cepas provenientes de especímenes clínicos mostraron mayor HA que aquellas provenientes de individuos sanos ( $p = 0.01$ ); pero las primeras fueron eliminadas más eficientemente que las últimas (80-90% y 40%, respectivamente). Es posible que *S. aureus* muestre un comportamiento diferente *in vivo*, donde podría expresar otros factores de virulencia para prevenir la acción de los fagocitos.

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