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Effects of serum opsonins and intravenous immunoglobulin on phagocytosis and intracellular killing of *Staphylococcus saprophyticus* by human granulocytes

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Accumulated evidence suggests that one species of coagulase-negative staphylococci, *S. saprophyticus* is one of the most common cause of urinary tract infections in both males and females [1, 5]. In spite of its clinical significance our knowledge concerning host defense mechanisms against *S. saprophyticus* is incomplate, but it can be assumed that granulocytes and antibacterial serum factors are important in dealing with these microbes. In this paper we present results of our recent study that was designed to identify the requirements of extracellular opsonins for the phagocytosis and subsequent killing of *S. saprophyticus* by human peripheral blood granulocytes.

Materials and Methods

Bacterial strains and growth conditions

Three clinical isolates of *S. saprophyticus* identified at the Department of Microbiology, Debrecen University School of Medicine were tested. Bacteria were cultured overnight at 37 °C in Nutrient broth (Oxoid, London), harvested by centrifugation at 1500 × g for 10 min, washed twice in Krebs-Ringer phosphate buffer containing 5% glucose (KRPD), and resuspended to a final concentration of 10^7 microorganisms/ml.

Opsonins

Normal human serum (NHS) was prepared from the blood of healthy adult donors. The blood was allowed to clot for 1 hr, at room temperature and was then centrifuged for 20 min at 1200 ×g after which small aliquots of serum were stored at −40 °C. Heat inactivated serum (IS) was prepared by heating serum for 30 min at 56 °C. In a few experiments pH-4 treated intravenous immunoglobulin (IVIG) concentrate (Sandoz AG, Basel) was used as a source of opsonins.

Granulocytes

Blood granulocytes were isolated from healthy adult donors by dextran sedimentation of the erythrocytes as described elsewhere [3], and resuspended in KRPD to a final concentration of 10^7/ml.

Determination of phagocytosis of S. saprophyticus

Phagocytosis of bacteria was determined as a decrease in the number of viable extracellular microorganisms in the phagocytic mixture. In short, granulocytes and bacteria were incubated in the absence or in the presence of opsonins at 37 °C, for 60 min under slow rotation. At 0 and 60

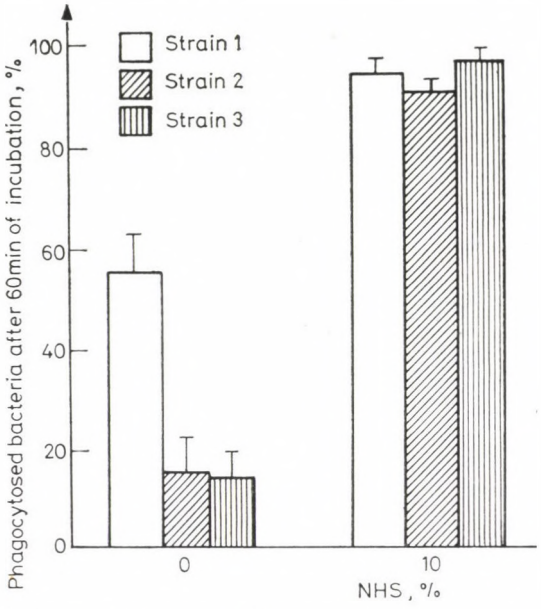

FIG. 1. Phagocytosis of *S. saprophyticus* strains by granulocytes in the absence of opsonins and in the presence of normal human serum (NHS)

min time points aliquots of the mixture were removed and the number of bacteria determined microbiologically [3].

Determination of bacterial killing

Killing of *S. saprophyticus* by granulocytes was determined as a decrease in the number of viable microorganisms in the phagocytic mixture. Aliquots taken at 0 and 60 min were added to destilled water to lyse the granulocytes and the total number of bacteria determined by colony counts.

RESULTS

Uptake of S. saprophyticus by granulocytes in the presence of NHS

When granulocytes were incubated together with various strains of *S. saprophyticus* in the presence of 10% NHS, a high rate of phagocytosis was reached and the number of viable extracellular bacteria decreased by more than 90% after 60 min (Fig. 1). Decrease in the number of viable extracellular bacteria could also be detected in the absence of any opsonins. The rate of phagocytosis of Strain-1 was significantly higher than that of Strain-2 and Strain-3 (Fig. 1).

Uptake of S. saprophyticus in the presence of IS or IVIG

When heat-inactivated serum (serum treated for 30 min at 56 °C) was used rather than fresh serum, the rate of phagocytosis was lower than that found with the similar concentration of fresh serum.

To determine the effect of a pH-4 treated IVIG concentrate on the phagocytosis of *S. saprophyticus* experi-

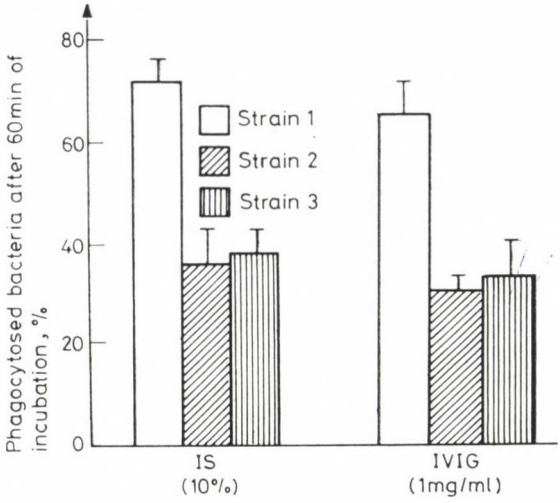

FIG. 2. Phagocytosis of *S. saprophyticus* strains by granulocytes in the presence of heat-inactivated serum (IS) or intravenous immunoglobulin concentrate (IVIG)

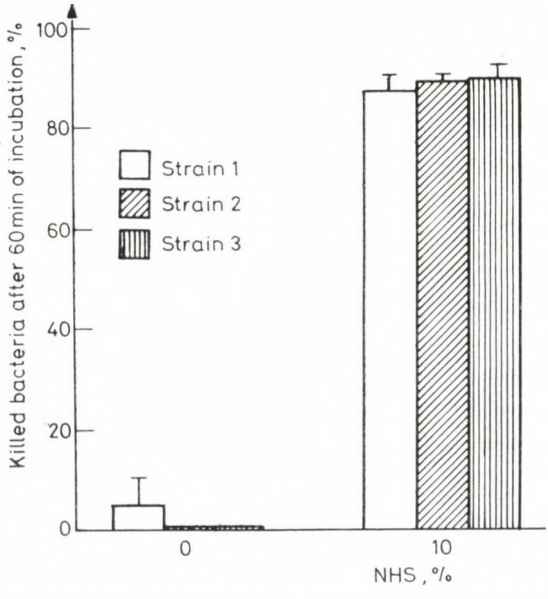

FIG. 3. Killing of *S. saprophyticus* strains by granulocytes in the absence of opsonins and in the presence of normal human serum (NHS)

ments were performed using 1 mg/ml concentration of immunoglobulin as a source of opsonins which is approximately equals to the concentration of IgG in 10% serum. After 60 min of incubation the percentage decrease in the number of extracellular bacteria was about the same as that found

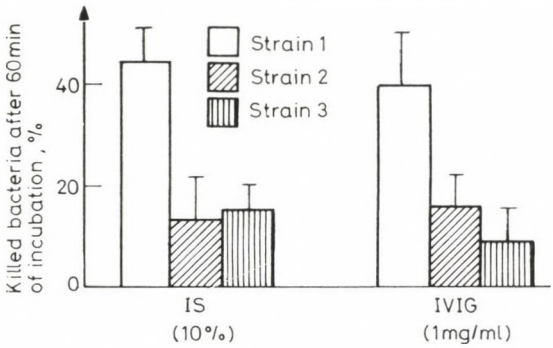

FIG. 4. Killing of *S. saprophyticus* strains by granulocytes in the presence of heat-inactivated serum (IS) or intravenous immunoglobulin concentrate (IVIG)

with IS (Fig. 2). The effect of IVIG to promote phagocytosis of *S. saprophyticus* differed significantly for Strain-1 compared to that for Strain-2 and Strain-3 (Fig. 2).

Killing of S. saprophyticus in the presence of NHS

The opsonic requirements for the killing of *S. saprophyticus* by granulocytes was investigated by incubating of cells and bacteria in the presence or absence of serum. The total number of viable bacteria determined after lysis of the phagocytes decreased rapidly in the presence of 10% NHS (Fig. 3). In the phagocytic mixture containing no serum decrease of the number of viable bacteria was not measured for Strain-2 and Strain-3, and 4% of Strain-1 was killed after 60 min.

Killing of S. saprophyticus in the presence of IS or IVIG

Heat inactivated serum prometed a lower rate of killing than fresh serum (Fig. 4).

After 60 min the percentage decrease of the total number of bacteria incubated in the presence of IVIG (1 mg/ml) was similar to that found with IS.

DISCUSSION

Phagocytosis of all of these strains proved to be a rapid process in the presence of NHS. To find out whether the high rate of ingestion of *S. saprophyticus* strain by granulocytes promoted by normal human serum could be attributed to immunoglobulins or was due to activities of both heat stable and heat lable opsonins, experiments were performed with heat inactivated serum and IVIG preparation. The results showed that IgG promoted a lower degree of ingestion and was not sufficed for optimal phagocytosis of these microorganisms. In this respect *S. saprophyticus* resembles *S. aureus* [2]. However some degree of uptake could also be detected in the absence of any opsonins. Such non-opsonic phagocytosis is never seen with *S. aureus* in suspension [2].

Opsono-phagocytosis of bacteria depends largely on surface properties of phagocytic cells and microorganisms. In this study strain differencies were observed in the uptake and subsequent killing of *S. saprophyticus* strains in the absence of complement or in the absence of any opsonins. This finding might be related to pathogenicity of these bacteria as etiologic agents of urinary tract infections and could be due to distinct differences in the adherence properties among individual strains tested. One of the properties that provide *S. saprophyticus* with mechanisms for persistance in the urinary tract is the capacity for adherence to the uroepithelial surface by using lectins [4]. Non-opsonic phagocytosis and subsequent killing of these microorganisms may also be related to bacterial lectin – phagocyte receptor association. Thus the capacity of certain strains of *S. saprophyticus* for adherence to surface receptors on uroepithelial cells is necessary for infection but the same property may enhance non-opsonic or IgG mediated phagocytosis.

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