In vitro antimicrobial assay of metHb-mediated ROS production using mammalian RBC

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Introduction

We hypothesized that upon systemic infection, the hemolysin-producing microbe lyses the erythrocyte and releases hemoglobin. Then microbial proteases and PAMPs enhance the ROS production by the metHb. Here, we used the mammalian (rabbit or human) red blood cell (RBC) to evaluate the antimicrobial activity of the metHb-mediated ROS production.

Procedure

1. Collect the RBCs into heparinized tubes.
2. Wash the RBCs two times by gentle resuspension with 10 volumes of pyrogen free saline (0.9% NaCl), and centrifuge at 1000 x g for 10 min at room temperature.
3. Gently resuspend the RBC pellet with pyrogen-free saline and dilute the RBC to 0.8% (v/v).

5. Set up the reaction mixture using the bacteria and the washed RBC. The final concentration of RBC in the reaction is 0.4% (v/v).
6. A mixture of the bacteria and pyrogen-free saline is used as a negative control.
7. Incubate the reaction mixture at 37 °C with gentle rotation at 90 rpm.
8. Quantify the bacterial population at certain time point of incubation. To this end, remove 20 µl from the reaction mixture and carry out 10 times serial dilutions with pyrogen-free saline until 10^{-5}. Then apply 100 µl of the 10^{-5}, 10^{-4}, and 10^{-3}dilutions (in triplicates) to nutrient agar plates and incubate at 37 °C overnight.
9. To investigate the necessity of microbial protease in triggering the ROS production, if any, add protease inhibitor Mix G (Serva Chemical Co., Westbury, NY, which have been tested not to substantially affect the bacterial growth on their own) at 1% (v/v) in the reaction mixture.

10. To confirm that the ROS produced is superoxide anion, add superoxide dismutase from bovine erythrocyte (Sigma, St Louis, MO, USA), at 10 Units/ml in the reaction mixture.

Anticipated Results

As shown in figure 6, the V8 protease-producing *S. aureus* is killed significantly whereas the protease-inactive strain remained viable. Addition of either SOD or protease inhibitor, Mix G, to the incubation mixture significantly reduced the antibacterial activity against the protease-producing strains.

Figures

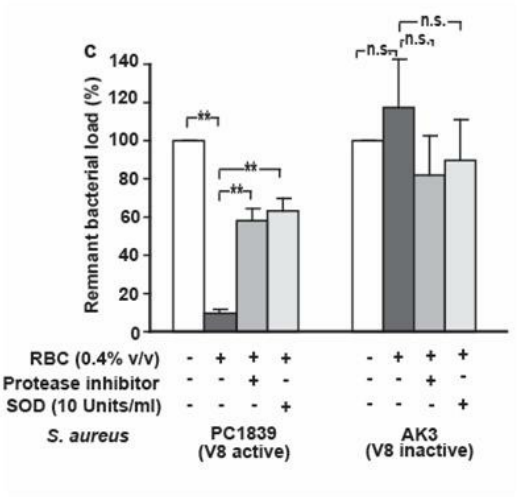

Figure 1

Antimicrobial activity against _S. aureus_ elicited by the rabbit RBC. Within 10 min, >80% of the protease-producing S. aureus was killed whereas the protease-negative strain remained viable. Addition of either SOD or protease inhibitor, Mix G, to the incubation mixture significantly reduced the antibacterial activity against the protease-producing strains.

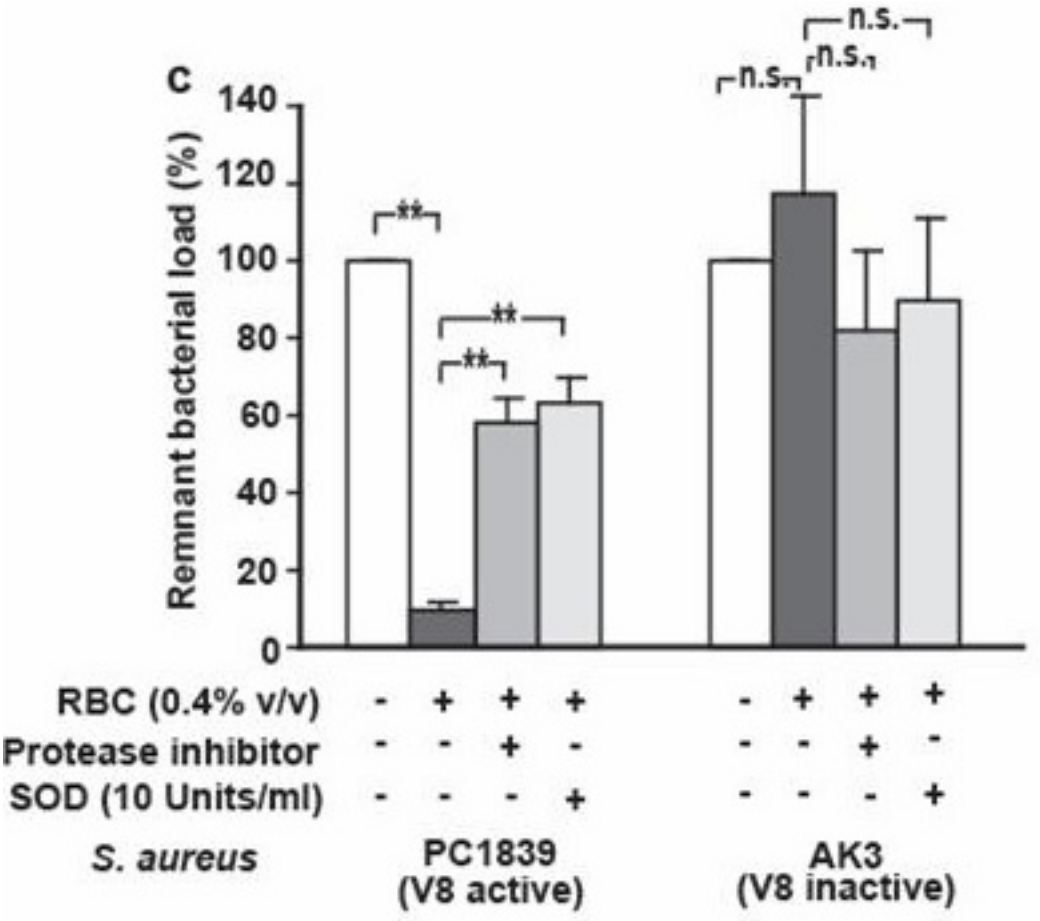

Figure 2

Respiratory protein–generated reactive oxygen species as an antimicrobial strategy

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