Phagosomal granulocytic ROS in septic patients induces the bacterial SOS response

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Background
Immune dysregulation is a hallmark of sepsis and relates directly to prognosis. Granulocytes use ROS in the phagosome to mediate pathogen killing through protein and DNA oxidation. However, low concentrations of ROS may activate bacterial genetic networks like the SOS response, responsible for DNA repair and bacterial adaptation. We hypothesized that during adaptive immune response in sepsis, the production of ROS by Mature Granulocytes (MG) and Immature Granulocytes (IG) differentially induce bacterial SOS response.

Methods
We developed an ex vivo model of Granulocyte/E. coli co-culture to assess granulocytes’ phagocytosis, ROS production, and bacterial SOS induction by flow cytometry and microscopy. Tested Granulocytes were recovered from healthy volunteers (n=10), hematopoietic stem cell donors treated with G-CSF (n=5), patients with a diagnosis of septic shock (n=5), and one patient with Chronic Granulomatous Disease. An E. coli strain constitutively expressing mCherry was used to follow phagocytosis. A plasmid carrying a transcriptional fusion of the promoter of sfiA, an inducible gene of E. coli’s SOS regulon, and GFP was used to indicate SOS induction. Granulocyte subsets were sorted and incubated with the reporter strain for 10, 30, 40, 60, and 90 minutes.

Results
By 60 minutes, more than half of phagocytic granulocytes contained bacteria with induced SOS response (59±12% Healthy-MG; 58±21% Healthy-IG; 50±20% Septic-IG; 11% G-CSF-MG). Healthy-IG induced the SOS response more rapidly, concordance with its lower phagocytosis and ROS production. Septic-IG had a continued increase in the proportion of SOS-induced bacteria that reached the highest rate (83±6%) compared to the other granulocytic subsets (59±12% Healthy-MG; 58±21%; 25% GCSF-MG).

Conclusions
We demonstrate that the immune response alone can activate bacterial genetic adaptive mechanisms. The SOS response is involved in the expression/acquisition of antimicrobial resistance, potentially impacting antimicrobial resistance in septic patients.