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Title: Neutrophil dysfunction in respiratory infection: Impaired killing efficiency after successive phagocytic stimuli?

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Body: Background Neutrophils often fail to clear a single infection source such as pneumonia, but the effect of successive infective insults is unknown. Aim To investigate our hypothesis that neutrophil exposure to a single phagocytic stimulus or ‘primary meal’ impairs subsequent killing of a ‘secondary meal’ of live bacteria. Methods Healthy volunteer neutrophils were exposed to different primary stimuli: killed Staphylococcus aureus (SA), zymosan, live SA or Pseudomonas aeruginosa (PA), followed by a secondary stimulus of either live SA or PA. Phagocytosis and neutrophil viability were measured using flow cytometry; bacterial killing by serial dilution, plating and colony counting. Results Exposure to killed SA (at multiplicity of infection, MOI≥20:1) or zymosan (MOI≥4:1) impaired subsequent killing of both live SA and PA (both MOI~10:1) in suspension ($p<0.05$, n=5), but not in adherent neutrophils ($p=0.2$, n=6). Neutrophils in suspension exposed to live SA or PA (MOI~10:1) showed impaired subsequent killing of PA and SA (MOI~10:1), respectively ($p\leq0.05$, n=5). Neither a 2-hour recovery period between stimuli nor granulocyte-macrophage colony stimulating factor improved killing. Neutrophil apoptosis was ~5% after both inert or live primary stimuli but the necrosis rate rose to ~40% after PA exposure, ~20% after SA, and only ~10% after inert stimuli (n=4). Conclusions Our study supports our hypothesis that neutrophils lose killing efficiency after a single exposure to a phagocytic stimulus, in part due to neutrophil cell death. Potential additional factors, including competition for phagocytic receptors, excessive degranulation and oxidative burst, require further study.