NEUTROPHILS RELEASE OXIDANTS THAT STIMULATE MACROPHAGES TO RELEASE VASCULAR ENDOTHELIAL GROWTH FACTOR.

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Purpose: Macrophages and neutrophils interact during the initial phases of wound healing as they accumulate at the wound site. Neutrophils are stimulated to oxidative burst activity, and release oxidants; some of which reach the extracellular space. We hypothesize that 1)exogenous oxidants and 2)oxidant-secreting neutrophils stimulate macrophages to release vascular endothelial growth factor (VEGF). By eliciting this stimulatory pathway, we expect to delineate the role of neutrophils and neutrophilic oxidants in wound angiogenesis.

Methods: Tumor monocytic cells (U937) were exposed to phorbol ester and differentiated into adherent macrophages. Macrophages were exposed to varying concentrations of H2O2, with or without catalase and Acetaminophen D in culture media for 30 min and then maintained in H2O2-free media for 16 h. The media were assayed for VEGF by ELISA.

Next, human venous neutrophils were exposed to optimized zymosan to stimulate respiratory burst. These neutrophils were cocultured with differentiated U937 macrophages in a 4:1 cell ratio for 10 h. The experiment was repeated with a 0.45 micron filter separating the two populations to prevent cell to cell interactions. VEGF was assayed by ELISA.

Results: Oxidant exposed macrophages secreted 33.3% more VEGF at 0.5mM H2O2 vs control (p<0.05). The addition of the antioxidant, catalase, abolished the increase in VEGF. Acetaminophen D inhibited VEGF secretion by 90% indicating that H2O2 increased VEGF synthesis (p<0.05).

Oxidant-releasing neutrophils stimulated macrophages to release 25% more VEGF when cocultured in a 4:1 cell ratio vs control (p<0.05). Neutrophils separated from macrophages by a micropore filter similarly stimulated VEGF release by 22% (p<0.05).

Conclusions: These results support the hypothesis that oxidants can potentiate the release of newly synthesized VEGF from macrophages. Furthermore, antioxidants block this stimulated release. Finally, activated neutrophils secrete soluble factors, putatively oxidants, which stimulate macrophages.

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