

## ACCUMULATION OF NEUTROPHILS AND TNF- $\alpha$ SYNTHESIS DURING WOUND HEALING PROCESS IN THE SKIN: EFFECT OF INOCULATION OF PSEUDOMONAS AERUGINOSA

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Neutrophils are the first inflammatory cells recruiting at the wound site and play a role in killing bacteria and wound debridement. Recent investigations have reported that these cells are involved in promoting the healing process, although the mechanism remains to be clearly understood. In the present study, we examined the kinetics of neutrophilic inflammatory responses during wound healing and the effect of inoculation of *Pseudomonas aeruginosa*.

Deep partial thickness wounds were created on the backs of SD rats. *P. aeruginosa*, PAO1, was inoculated at the wound surface. The wound tissues were harvested at 1, 3, 6, 12 and 24 hours and 3, 5 and 7 days for analysis of re-epithelialization, accumulation of neutrophils and synthesis of inflammatory cytokines (TNF- $\alpha$ , TGF- $\beta$ , IL-1 $\alpha$ , IL-1 $\beta$  and IL-6). Inflammatory cells were prepared from the wound tissues by treatment with hyaluronidase and DNase followed by density-gradient centrifugation.

In rats received *P. aeruginosa* inoculation, re-epithelialization was accelerated at the early stage, and neutrophilic inflammatory responses were promoted, and TNF- $\alpha$  synthesis was induced at mRNA and protein levels, detected by real time RT-PCR and ELISA, in the wound tissues, when compared with in uninoculated groups. TNF- $\alpha$  was detected in neutrophils by flow cytometry and immunohistochemical analyses. Similar findings were obtained in other inflammatory cytokines at mRNA level.

Our results indicate that TNF- $\alpha$  was produced by accumulating neutrophils during the wound healing process in the skin, which was accelerated by inoculation of *P. aeruginosa*, and suggest that TNF- $\alpha$  may play an important role in these responses.