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Role of GM-CSF in neutrophil survival and neutrophil-mediated diseases

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Failure of neutrophil apoptosis is involved in immune-mediated diseases. The aims of this study were to define the role of GM-CSF and CAM3001 in human neutrophil survival.

Human neutrophils were purified using Dextran sedimentation and plasma:Percoll density gradients and cells incubated for 20 hrs in 10% autologous serum; apoptosis was assessed by morphology and PI/An-V-FITC staining; inflammatory cytokines produced by cultured neutrophils were detected by protein arrays; receptor levels were quantified by 5 microsphere populations labelled with increasing amount of PE-mouse-anti-human-CD116 Ab.

The main findings were: GM-CSF caused a concentration-dependent inhibition of neutrophil apoptosis (An-V-FITC positive cells at 20 hrs: control 65.7 ± 1.9%, GM-CSF 1 ng/ml 31.8 ± 4.9%, $P<0.01$, n=9) with an EC_{50} of 30 ± 5 pg/ml; that the EC_{50} for this effect varied within healthy individuals by >1 log order; this marked pro-survival effect of GM-CSF was abrogated by pre-treatment of cells with CAM3001 (1 μM) (95.3% inhibition; IC_{50} 0.11 ± 0.04 μM) and was even effective when added up to 6 hrs post GM-CSF; LPS, IL-1β, C5a and mononuclear cell-mediated neutrophil survival was unaffected by CAM3001 and not associated with GM-CSF secretion; GM-CSF induced loss of GM-CSFRα expression, which was maximal by 45 mins (control 7,079.7 ± 558.2 GM-CSFRαs/neutrophil, 45 mins-GM-CSF 1 ng/ml 1,434.2 ± 160.2, $P<0.01$).

These data indicate that GM-CSF causes aberrant neutrophil activation and survival *in vitro*. Those aspects are under investigation in inflammatory (COPD and ALI) neutrophils. Furthermore, CAM3003 post-insult administration effect will be tested *in vivo* in LPS-dependent lung inflammation models.