



Figure 1. Neutrophils release an increasing amount of NETs when incubated with increasing concentrations of PMA. Human neutrophils were stimulated with various concentrations of PMA for 1, 2, 3 hours. NET formation was quantified with 1 μ M Sytox Green. a) The lines represent the mean \pm SEM normalised (to 60 nM PMA) NET formation of three individual donors. b) NET release upon various concentrations of PMA after 4 hours. The line represents the mean \pm SEM NET formation of one individual donor measured in triplicate.

Supplementary

Note 1

Human neutrophil isolation

Blood from healthy volunteers was collected in lithium-heparin tubes (BD Vacutainer). The neutrophils were purified using a discontinuous Percoll density gradient. From the whole blood, 2 ml was layered on top of two times 3 ml layers of 1.079 g/ml and 1.098 g/ml Percoll in 0.15 M NaCl, followed by centrifugation at 150 x g for 8 minutes without brakes. Subsequently the tubes were centrifuged a second time at 12000 x g for 10 min without brakes. The neutrophil layer was isolated and red blood cells were lysed in lysis buffer, containing 155 mM NH₄Cl, 10 mM KHCO₃, 110 μM Na₂-EDTA and 2.5% (w/v) bovine serum albumin. neutrophils were washed in RPMI-1640 and resuspended in phenol red-free RPMI-1640 supplemented with L-glutamine (2 mM), streptomycin (100 μg/ml) and penicillin (100 U/ml). Cell purity was ~98% or higher.

Note 2

FISH Immunohistochemistry

Isolated neutrophils (0.2×10^6) were placed onto Ø 22 mm glass slides and stimulated with 50 nM PMA. After 30 minutes, *E.coli* JM109 bacteria at an MOI of 6 were added and incubated at 37 °C. After three hours, cells and bacteria were fixed in 4% paraformaldehyde. The slides were stained with 16S-Alexa488 probe. The 16S probe (1.0 μg) is diluted in 100 μl hybridisation buffer (20 mM TRIS-HCL, pH 7.4, 0.9 M NaCl, 0.1% (w/v) SDS) and added to every slide. The slides were incubated for four hours at 50°C, washed in FISH washing buffer (20 mM TRIS-HCL, pH 7.4, 0.9 M NaCl) for 20 minutes at 50°C. Three washing steps with PBS were performed, after which the slides were stained with 0.5 μM Sytox orange for 5 minutes and embedded in VectaShield. Images were obtained with a Zeiss 510 CLSM.