

# Priming and Oxidative Burst Induction in Human Peripheral Blood Neutrophils by Treatment with Cigarette Smoke

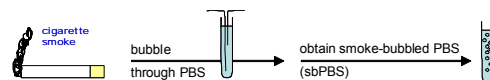
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## Objective

In order to investigate the mechanism of neutrophil activation by cigarette smoke, neutrophils isolated from peripheral blood of non-smokers were exposed *in vitro* either directly with cigarette smoke-bubbled phosphate-buffered saline (sbPBS) or indirectly with supernatants from sbPBS-treated Monomac-6 (MM6) cells. Changes in expression of the surface markers CD11b, CD66b, and CD62L; release of the granule proteins matrix metalloproteinase (MMP)-9, MMP-8, and lactoferrin; and oxidative burst induction as measured by N-formyl-methionyl-leucyl-phenylalanine (fMLP)-mediated superoxide release were determined.

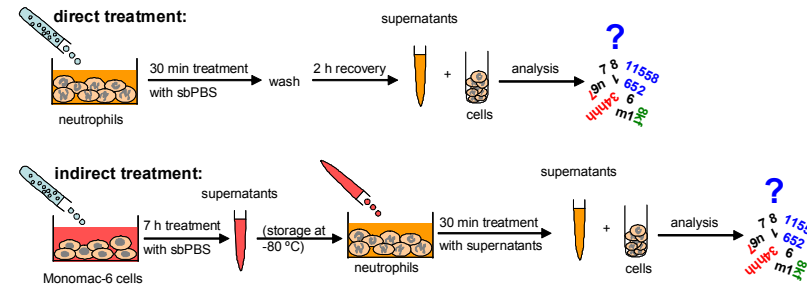
## Smoke Preparation

80 puffs of mainstream smoke from the Reference Cigarette 3R4F was bubbled through 32 ml Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free PBS at RT (= 2.5 puffs/ml). sbPBS was freshly prepared and used within 15 min.



## Isolation and Treatment of Neutrophils

Neutrophils were isolated from ACD-anticoagulated whole blood from healthy non-smokers by density gradient centrifugation using Polymorphprep™ medium. 2.5 x 10<sup>5</sup> cells were treated with sbPBS either directly or indirectly using supernatants from sbPBS-exposed MM6 cells.



## Detection of Neutrophil Priming

Staining with fluorochrome-conjugated antibodies against CD16, CD11b, CD66b, and CD62L followed by flow cytometry (LSRII). Detection of proteins in the supernatants: multiplex bead arrays or ELISA.

## Measurement of Neutrophil Oxidative Burst

Treated neutrophils were stimulated with 5 μM fMLP for 10 min followed by immediate analysis of superoxide release using the Superoxide Anion Detection Kit.

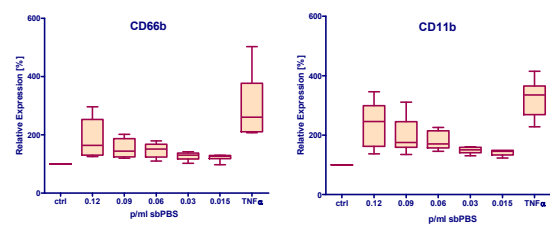
## Abbreviations

MFI = median fluorescence units  
RLU = relative luminescence units

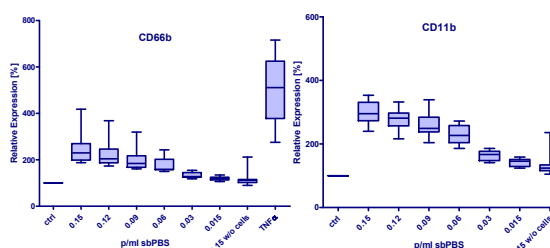
## Results: Neutrophil Priming

### Surface Markers

#### Direct Treatment

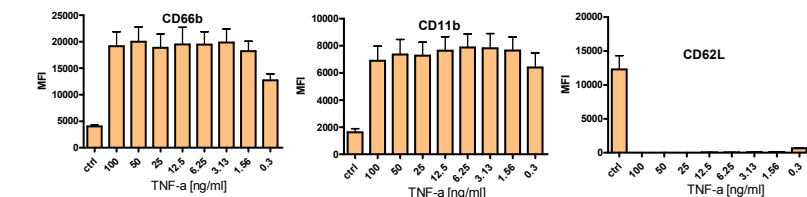


#### Indirect Treatment

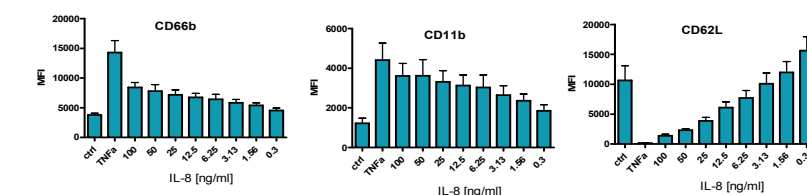


### Cytokine-Induced Priming

#### TNF-α

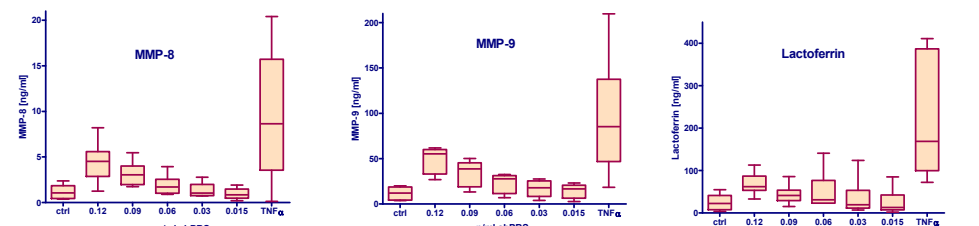


#### IL-8

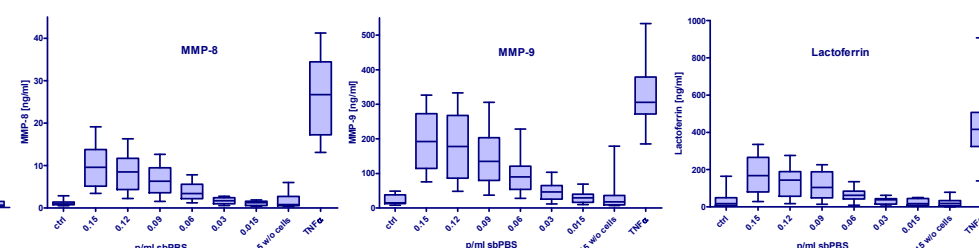


### Mediator Release

#### Direct Treatment



#### Indirect Treatment



## Conclusion

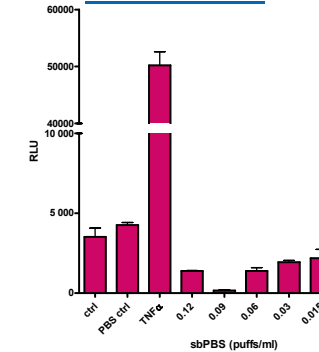
- Direct treatment with sbPBS induced neutrophil priming, but did not increase the oxidative burst response of human peripheral blood neutrophils.
- Indirect treatment with sbPBS resulted in the priming and an increased oxidative burst response of neutrophils.
- Only TNF-α seemed to be involved in the increased oxidative burst response of neutrophils induced by MM6 supernatants.
- A similar activation of lung neutrophils by pro-inflammatory mediators, e.g., TNF-α, released from other inflammatory or structural cells residing in the vicinity of neutrophils can be assumed to take place in the inflamed lung as a result of chronic cigarette smoking.

These results suggest that cigarette smoking might lead to a continuous activation of neutrophils in the lung tissue, which perpetuates the chronic lung inflammation seen in COPD patients.

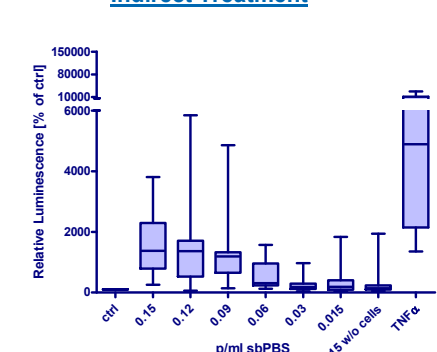
## Materials and Methods

## Results: Neutrophil Oxidative Burst

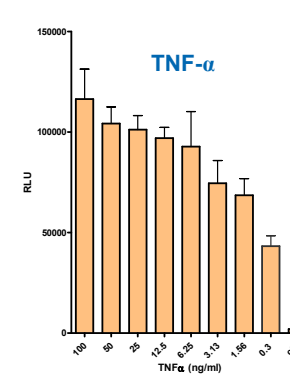
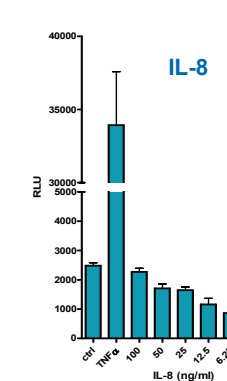
### Direct Treatment



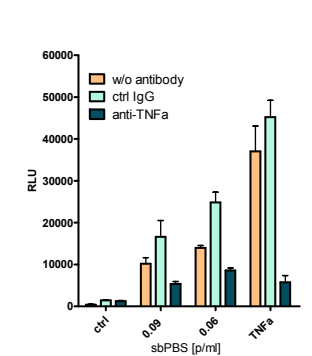
### Indirect Treatment



### Cytokine-Induced Oxidative Burst



### Effect of Anti-TNF-α Antibody – Indirect Treatment



### IL-8 and TNF-α Content of MM6 Cell Supernatants

