Real time *in vivo* investigation early alveolar neutrophil dynamics during ventilator-assisted nanoparticle inhalation

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Inhalation of nanoparticles (NPs) can induce a pro-inflammatory response of the lung, characterized by the influx of neutrophilic granulocytes into the airspace. However, the spatio-temporal events taking place in the early phase of NP induced neutrophil recruitment from the pulmonary microvasculature to the alveolar compartment remain largely elusive.

To visualize and measure in real-time the cellular pulmonary innate immune response simultaneously with NP dynamics, we apply state of the art intravital microscopy (IVM) on the alveolar region of the murine lung, in combination with ventilator-assisted inhalation of nebulized NP aerosols.

This novel approach enables the study of (sub-)cellular dynamic events, which were inaccessible up to now.

Carboxyl Quantum-Dots (cQDs, 20 nm diameter) - which served as fluorescent model NPs - became visible within seconds after the onset of inhalation of 2.8 µm cQD suspension droplets and accumulated as distinct fluorescent spots at the alveolar walls. Already at 45 min after inhalation, a deposited NP dose of 16 cm^2/g (geom. surface area of NPs / mass-lung), determined by quantitative fluorescence measurements, elicited an increase in neutrophil numbers (immunolabeled with fluorescent anti-Ly6-G) in the area of observation, as compared to the control group. Neutrophils preferentially arrested in microvessels in close proximity to the NPs, where they exhibited a probing/crawling behavior. The number of alveolar localized neutrophils, i.e. after trans-endothelial and trans-epithelial migration, was significantly increased 60 min upon NP inhalation, compared to the control group, receiving vehicle. Interestingly, we frequently observed alveolar localized neutrophils with ingested NPs. This observation may point towards a contribution of neutrophils in the alveolar clearance of NPs. In addition, our results suggest a specific immune function of cells of the alveolar walls in response to NPs, including crosstalk with microvascular endothelium, facilitating rapid and site-specific recruitment of neutrophils.