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Induction of cytokine expression by engineered nanoparticles in a lung epithelia and macrophages coculture model

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Introduction

Inflammatory cytokines play an important role in the intercellular signalling cascade leading to tissue recruitment of leukocytes challenged by a pathogen or by particulate matter. In order to study possible inflammatory effects of engineered nano particles, we established a coculture system of different cell lines representing lung tissue and cells of the immune system and exposed the test system to nanoparticles provided by NanoCare partners. Here we employed the coculture model of type II pneumocyte-like A549 and the monocyte/macrophage-like line Mono-Mac-6 (MM6) cell lines. This system provides a relatively simple model of the alveolar response to nanoparticle exposure either with or without simulating an existing inflammatory state in the coculture model by LPS-prestimulation of the monocytes/macrophages. Additionally, two new categories of positive controls were introduced into the coculture system: nanoparticles added to a cell free system spiked with recombinant IL-8 protein, and IL-8 added to the different cell and culture types.

Summary & Conclusion

In our studies, coculture enhanced the particle induced production of IL-8 compared to the two respective monocultures. LPS-prestimulated MM6 showed a reduced response to additional LPS stimulation, but not to nanoparticles. Of the engineered nanoparticles tested, zinc oxide induced the highest significant concentration-dependant increase in production of IL-8 at 10 µg per square cm using different cell combinations, with the highest induction triggered by nanoparticulate zinc oxide in LPS-prestimulated A549 and MM6 cocultures. ZnO powder, TiO₂ P25 and Carbon Black showed a smaller significant release of IL-8, while three different CeO₂ displayed only a minimal, insignificant increase at the higher concentration. BaSO₄ and TiO₂ (stabilized, ITN) induced no additional IL-8 expression. In conclusion, the test system proved to be useful in studying the inflammatory effects of several nanoparticles in regard to chemokine induction. Only a few nanoparticle types were able to elicit a significant proinflammatory response, especially in prestimulated cocultures.

Results

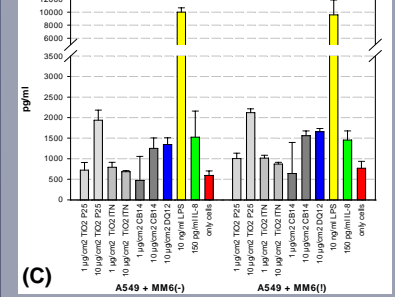
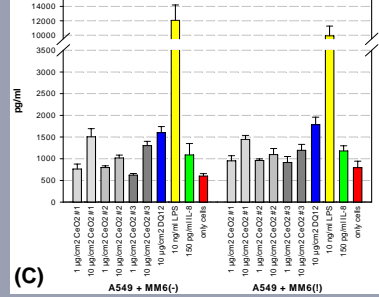
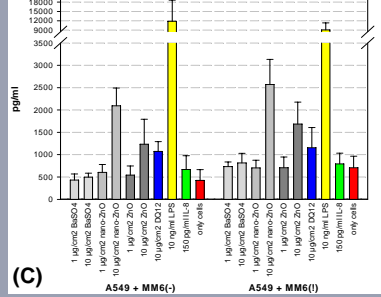
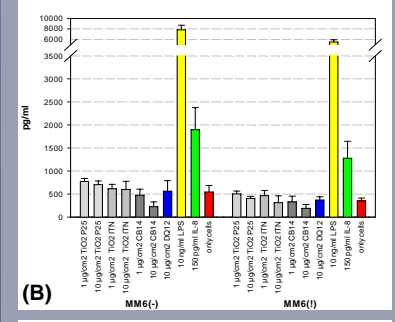
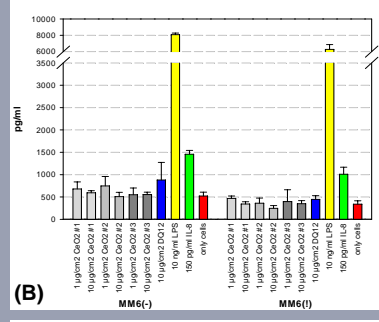
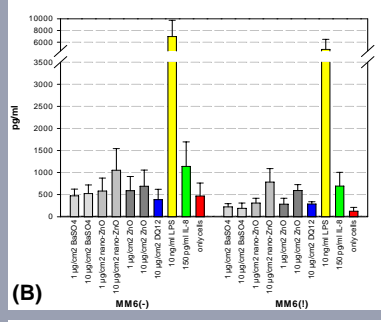
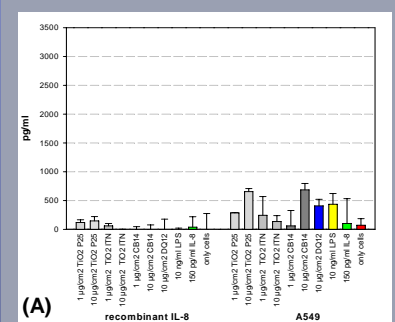
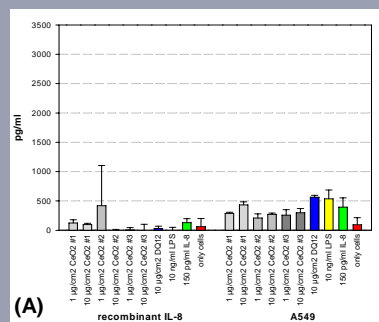
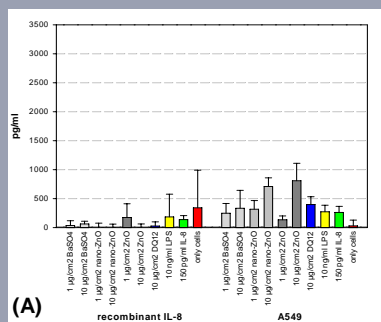


Fig. 1: Interleukin-8 levels in culture supernatants after 24 h exposition to BaSO₄ and nano-ZnO nanoparticles as well as ZnO powder. (A) Recombinant IL-8 and epithelial cell controls; (B) monocyte-macrophage controls; (C) cocultures.

Fig. 2: Interleukin-8 levels in culture supernatants after 24 h exposition to CeO₂-A, CeO₂-B and CeO₂-C nanoparticles. (A) Recombinant IL-8 and epithelial cell controls; (B) monocyte-macrophage controls; (C) cocultures.

Fig. 3: Interleukin-8 levels in culture supernatants after 24 h exposition to TiO₂ (P25), TiO₂ (stabilized, ITN) and Carbon Black nanoparticles. (A) Recombinant IL-8 and epithelial cell controls; (B) monocyte-macrophage controls; (C) cocultures.

Methods

The cell line A549 was obtained from ATCC and the Mono-Mac-6 line from DSMZ. 20,000 lung epithelial cells (up to passage 20) per well were seeded in a 96 well plate and incubated overnight. Two different dilutions (for final assay concentrations of 1.0 and 10.0 µg/cm²) of industrial nanoparticles plus pure medium and controls were stirred for 24 h at RT and 900 rpm according to the NanoCare dispersion protocol (BASF 2007). On the same day, MM6 were evenly splitted into two cultures; LPS was added to one culture to a final concentration of 1 ng/ml, the other one received an equal amount of vehicle medium as control. After change of culture medium in the A549 well plates on the next day, 3,000 leucocytes were added to the appropriate wells. After 2 hours, nanoparticle dilutions were dispensed onto the cells and the plates were incubated for 24 h. Culture conditions were 37 °C with 5% CO₂. final assay volume was 200 µl. After centrifugation the supernatants were removed from the assay plates, diluted 20-fold in DMEM + 10% serum and stored at -20 °C. Determination of cytokine levels in the diluted samples was performed using the BD Biosciences Human IL-8 OptEIA™-ELISA-Kit according to manufacturer's protocol. Data presented here are from three separate experiments with each sample present on three individual cell culture assay plates.