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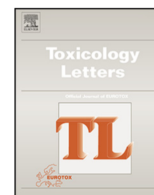

Kinetics of chemotaxis, cytokine, and chemokine release of NR8383 macrophages after exposure to inflammatory and inert granular insoluble particles

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HIGHLIGHTS

- We describe an *in vitro* model for the particle induced inflammation of the lung.
- Strong, dose dependent, and clearly differentiated chemotaxis in response to particles occurs.
- Particle induced transcription of inflammatory mediators is associated with chemotaxis *in vitro*.

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ABSTRACT

Accumulation of macrophages and neutrophil granulocytes in the lung are key events in the inflammatory response to inhaled particles. The present study aims at the time course of chemotaxis *in vitro* in response to the challenge of various biopersistent particles and its functional relation to the transcription of inflammatory mediators.

NR8383 rat alveolar macrophages were challenged with particles of coarse quartz, barium sulfate, and nanosized silica for one, four, and 16 h and with coarse and nanosized titanium dioxide particles (rutile and anatase) for 16 h only. The cell supernatants were used to investigate the chemotaxis of unexposed NR8383 macrophages. The transcription of inflammatory mediators in cells exposed to quartz, silica, and barium sulfate was analyzed by quantitative real-time PCR.

Challenge with quartz, silica, and rutile particles induced significant chemotaxis of unexposed NR8383 macrophages. Chemotaxis caused by quartz and silica was accompanied by an elevated transcription of CCL3, CCL4, CXCL1, CXCL3, and TNFα. Quartz exposure showed an earlier onset of both effects compared to the nanosized silica. The strength of this response roughly paralleled the cytotoxic effects. Barium sulfate and anatase did not induce chemotaxis and barium sulfate as well caused no elevated transcription.

In conclusion, NR8383 macrophages respond to the challenge with inflammatory particles with the release of chemotactic compounds that act on unexposed macrophages. The kinetics of the response differs between the various particles.

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Abbreviations: BET, brunauer, emmett, teller; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; dHL-60, differentiated human leukemia cells; DLS, dynamic light scattering; FCS, fetal calf serum; IC_{50}, half maximal inhibitory concentration; PBS, phosphate buffered saline; PICMA, particle-induced cell migration assay; PMN, polymorphonuclear neutrophils; TNFα, tumor necrosis factor α.

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1. Introduction

Inflammation of the lung is the main toxic effect of particle exposure (Donaldson et al., 2008; Klein et al., 2012): In the first line of defense particles are phagocytized by alveolar macrophages. Exceeding of the macrophage clearance leads to accumulation of additional macrophages and finally to the attraction of polymorphonuclear neutrophils (PMN). Activated PMN act toxic due to the myeloperoxidase mediated release of hypochlorous acid and reactive oxygen species (Parker et al., 2012). Prolonged particle overload leads by this way to chronic inflammation, tissue damage, and severe diseases such as fibrosis or lung cancer (Oberdörster, 1995; IARC, 1997, 2009).

Although accumulation of inflammatory cells is a key event in particle induced inflammation of the lung, only few attempts were made to use this key pathogenetic step as a functional toxicological endpoint *in vitro*. We recently used NR8383 macrophages and differentiated human leukemia cells (dHL-60 cells) to model the accumulation of inflammatory cells in the lung: The supernatants of particle-challenged NR8383 alveolar macrophages strongly attracted dHL-60 in a dose-dependent and reproducible way (Westphal et al., 2015).

While the activation of neutrophils can lead to severe lung damage, accumulation of alveolar macrophages is rather regarded as an adaptive response. Therefore, NR8383 macrophages and dHL-60 might respond differently to particles. NR8383 cells are especially suited for the investigation of particle induced chemotaxis, since they were derived from healthy primary rat alveolar macrophages (Helmke et al., 1987). In contrast, tumor-derived cell lines can compromise chemotaxis assays by acting chemotactic by themselves – without particle challenge or any other activation (Dumitru et al., 2013). Moreover, NR8383 cells preserved basic functions of physiological alveolar macrophages, especially phagocytosis (Helmke et al., 1987, 1989) and the release of inflammatory mediators such as tumor necrosis factor (TNF-α). Phagocytosis of particles was for example shown for the fcgammaII-receptor (FcγRII) mediated uptake of quartz particles (DQ10) (Haberzettl et al., 2008). Uptake of DQ12 and ultrafine titanium dioxide (TiO_2, 80% anatase and 20% rutile) – but not of fine pure anatase – was accompanied by TNF-α induction and DQ12 additionally triggered interleukin (IL) 1-β release (Scherbart et al., 2011).

Individual compounds – such as IL-1β – act quite weakly in *in vitro* chemotaxis assays. Though IL-1β is able to induce leukocyte recruitment *in vivo*, it fails to stimulate cell migration *in vitro*. It was therefore proposed that cell migration *in vivo* is induced by cooperative acting of chemotactic compounds such as the acute phase protein serum amyloid 1 (SAA1) and MIP-1α/CCL3 (Macrophage Inflammatory Protein-α synonym to Chemokine (C-C motif) ligand 3) as well as IL-8/CXCL8 (IL-8 synonym to chemokine (C-X-C motif) ligand 8) that further enhance and prolong leukocyte recruitment to the inflammatory site (Gouwy et al., 2015). The comparably strong particle induced migration of dHL-60 cells in response to particle challenge (Westphal et al., 2015) might accordingly have been caused by the induction of cooperatively acting chemoattractants.

The coordinated inflammatory response is thus tightly controlled by the stepwise activation of pro- and anti-inflammatory mediators, and particles of different toxicity can differ in the strength and presumably in the time course of this response. Although the importance of kinetics in particle toxicity *in vitro* is appreciated (Sanchez et al., 2011), kinetic data of particle effects *in vitro* are rare. Certainly, the knowledge of the time course of the inflammatory events is a prerequisite for the sampling of functionally related inflammatory mediators. A comparison of the time course of the effects might allow an assignment of inflammatory mediators by the temporal relationship of induction and chemotaxis.

Therefore, this study aims on the identification of inflammatory mediators that are temporally associated with cell migration. For this purpose, we investigated migration of unexposed NR8383 cells in response to particle challenged NR8383 cells and the transcription of inflammatory mediators following challenge with quartz, silica, and barium sulfate particles at three different incubation times. The investigated particles were selected according the amount of toxicological data and the availability of the particles. In order comprehend the experiments, the particles should be easily available. Furthermore, it is needed to investigate toxicologically well characterized compounds, since otherwise the data that are generated with the new assay cannot be assessed. In addition we aimed on particles showing differentiated effects (no, weak, medium and strong toxicity) and a widest possible distribution of the particles size or surface respectively. In addition different crystallinity was of interest (anatas v. rutile). Barium sulfate served as inert control. Barium sulfate induced no, if any very weak inflammogenic effects *in vivo* (Klein et al., 2012; Tran et al., 2000; Cullen et al., 2000). The direct functional assignment of particle induced chemotaxis and the transcription of inflammatory mediators in response to granular particles of various toxicity and physicochemical characteristics can help to optimize research strategies concerning toxic particle effects. Notably the methods applied in our study avoid particle interference that is one of the major problems in the *in vitro* testing of particles (Kroll et al., 2012; Stone et al., 2009).

2. Material and methods

2.1. Particles

The studies were performed with commercially available SiO_2 particles (nanosized silica, Sigma-Aldrich, Steinheim, Germany) and TiO_2 particles (rutile and anatase), with purities between 99.5% (nanosized rutile) and 99.9% (coarse rutile). Barium sulfate particles were kindly provided by Christian Monz (BG RCI – Institut für Gefahrstoff-Forschung, Bochum, Germany). All particles were characterized with respect to chemical nature, crystallinity, size in the dry state, and size distribution in the culture medium. The data concerning particle characterization and the methods used were reported previously (Westphal et al., 2015).

Additionally, the specific surface area of the particles was measured by nitrogen adsorption (BET). Samples with a mass of approximately 2 g were degassed with nitrogen and dried for 2 h under vacuum at 200 °C (SmartPrep, Micrometrics, Norcross, GA, USA) to remove adsorbed water. The measurements were conducted under cooling with liquid nitrogen at 77 K, with a mixture of nitrogen and helium in a Tristar 3000 (Micrometrics, Norcross, GA, USA). Eleven different adsorption steps in the range of relative pressures $p_r = p/p_0$ from 0.05–0.3 were conducted. For the calculation of the specific surface area, the equation of Brunauer, Emmet, and Teller (BET-equation) (Brunauer et al., 1938) was applied.

2.2. Cell lines

NR8383 cells were obtained from ATCC via LGC Standards GmbH (Wesel, Germany). They were cultivated at 37 °C, 100% humidity and 5% CO_2 in Ham's F12 + 15% FCS (fetal calf serum, Biochrom KG, Berlin, Germany), 2 mM L-glutamine, 100 $\mu g\,mL^{-1}$ penicillin, and 100 $U\,mL^{-1}$ streptomycin. Approximately 1.2×10^6

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