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Differential cellular responses in healthy mice and in mice with established airway inflammation when exposed to hematite nanoparticles

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ABSTRACT

The aim of this study was to investigate the inflammatory and immunological responses in airways and lung-draining lymph nodes (LDLNs), following lung exposure to iron oxide (hematite) nanoparticles (NPs). The responses to the hematite NPs were evaluated in both healthy non-sensitized mice, and in sensitized mice with an established allergic airway disease. The mice were exposed intratracheally to either hematite NPs or to vehicle (PBS) and the cellular responses were evaluated on days 1, 2, and 7, post-exposure.

Exposure to hematite NPs increased the numbers of neutrophils, eosinophils, and lymphocytes in the airways of non-sensitized mice on days 1 and 2 post-exposure; at these time points the number of lymphocytes was also elevated in the LDLNs. In contrast, exposing sensitized mice to hematite NPs induced a rapid and unspecific cellular reduction in the alveolar space on day 1 post-exposure; a similar decrease of lymphocytes was also observed in the LDLN. The results indicate that cells in the airways and in the LDLN of individuals with established airway inflammation undergo cell death when exposed to hematite NPs. A possible explanation for this toxic response is the extensive generation of reactive oxygen species (ROS) in the pro-oxidative environment of inflamed airways. This study demonstrates how sensitized and non-sensitized mice respond differently to hematite NP exposure, and it highlights the importance of including individuals with respiratory disorders when evaluating health effects of inhaled nanomaterials.

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1. Introduction

Exposure to air pollution has long been associated with impaired health (Bernstein et al., 2004; Brunekreef and Holgate, 2002), and particulate matter is known to induce airway inflammation (Salvi et al., 1999; Ghio et al., 2012), influence allergic sensitization (Granum et al., 2001; Gilmour, 2012), and exacerbate allergic airway disease (Kim et al., 2011; Arantes-Costa et al., 2008). With this knowledge and with the increasing use of potentially toxic engineered nanoparticles (NPs), there is an emerging concern about how these NPs might affect health. The risk of being exposed to NPs is particularly high for those working with the production of engineered NPs (European Union, SWD (2012) 288 final). Recently, it has been shown that exposure to engineered NPs can induce airway inflammation in animals (Gustafsson et al., 2011; Rossi et al., 2010; Roursgaard et al., 2011), induce adverse health effects in sensitized mice during the development of eosinophilic airway inflammation (Jonasson et al., 2013), and also induce lung tissue fibrosis in mice (Ryman-Rasmussen et al., 2009).

Nano materials that are made of transition metal oxides are widely used in technological applications, and some of these transition metal oxides are known to induce both pro-inflammatory and excessive pro-oxidative activity (Gustafsson et al., 2011; Ekstrand-Hammarström et al., 2012; Andersson et al., 2011; Wilkinson et al., 2012). Iron oxides are particularly interesting to study due to their ubiquitous nature and their role as a catalyst in the generation of reactive oxygen species (ROS) (Ghio, 2009; Turi et al., 2004). Iron oxide NPs are naturally present in urban air but they are also emitted from e.g. building materials and vehicles, such as cars and train brakes (Wilkinson et al., 2012; Gasser et al., 2009; Wahlström et al., 2010). Furthermore, engineered iron oxide NPs are widely used in energy and medical technology applications due to their magnetic, electrical, and catalytic properties (Huber, 2005; Mohapatra and Anand, 2010). The Organization for Economic Cooperation and Development (OECD) considers iron oxide NPs important to

Abbreviations: LDLN, lung draining lymph nodes; BALF, bronchoalveolar lavage fluid; NPs, nanoparticles; OVA, Ovalbumin; PBS, phosphate-buffered saline; ROS, reactive oxygen species; ECP, eosinophilic cationic protein.

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study to determine any potential adverse health effects (OECD, 2010). Previous studies on the effects of iron oxide NPs on healthy mice indicate an acute response with pro-inflammatory mediators, neutrophils, and lymphocytes being recruited to the airways (Cho et al., 2009; Park et al., 2010). It has also been shown that intratracheal instillation of iron oxide NPs in mice that are undergoing allergic sensitization modulates the immune response to the allergen by either suppressing or enhancing the allergic response, depending on the dose and the size of the particles (Ban et al., 2013).

Individuals with allergic airway disease are particularly sensitive to the inhalation of particulate matter, such as diesel exhaust (Di Giampaolo et al., 2011; Holgate et al., 2003; Nordenhäll et al., 2001), and it is of high relevance to determine whether sensitized allergic individuals are also particularly sensitive to NPs (Savolainen et al., 2013).

The aim of this study was to compare the cellular and the immune responses in the airways and in the mediastinal lung-draining lymph nodes (LDLNs) following intratracheal instillation of engineered hematite NPs. Non-sensitized mice and sensitized mice with an established eosinophilic airway inflammation were exposed to the same dose of NPs and were evaluated at the same time points in order to establish a comparison between their responses to the exposure.

2. Material and methods

2.1. Animals

Female Balb/c mice were purchased from animal breeder Harlan Laboratories, Netherlands, and were 8–10 weeks old at the beginning of the experiment. The mice were housed in plastic cages and maintained in a controlled atmosphere where they had access to food (R 36, Lantmännen Lantbruk, Lidköping, Sweden) and water ad libitum. The temperature was set at 22 °C, the humidity at 50–60%, and the light was on a 12-h on/off cycle. The mice were allowed to acclimatize to their environment for at least 7 days. The animal experimental procedures were performed according to guidelines provided by the Swedish Department of Agriculture and had been approved by the Animal Research Ethical Committee in Umeå, Sweden.

2.2. Nanoparticle characterization

Nano-sized hematite (α-Fe_2O_3) particles, with a primary particle size of ~30 nm, were subsequently added to a phosphate-buffered saline solution (PBS, pH 7.4, Sigma Aldrich) according to a previously described procedure (Makie et al., 2011). The electrochemical potential (Zeta (ζ) potential) was measured with a Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK) at different pH in order to determine the isoelectric point. The agglomerate size in the solution was determined by photon cross-correlation spectroscopy (PCCS) (Nanophox, Sympatec GmbH, Clausthal-Zellerfeld, Germany) immediately following in-situ probe sonication of the solution for 1 min. Transmission electron microscopy (TEM) analysis was performed with a Jeol JEM 1230 microscope equipped with a digital multiscan camera (Gatan MSC 600CW). NPs samples were suspended in water and 5 μL of the suspension was added to a Formvar-coated 3-mm grid where it was dried prior to the analysis. Further details of the material characterization have been reported elsewhere (Makie et al., 2011). A summary of the particle characterization is outlined in Table 1. The size distribution of the hematite particles in PBS solution is shown in Fig. 1A and a TEM image of a few hematite NPs agglomerates is shown in Fig. 1B.

2.2.1. Endotoxin test

The hematite NPs were screened for Gram-negative bacterial endotoxin contamination with a Limulus amebocyte lysate test. The test was carried out with a chromogenic endotoxin quantification kit according to the manufacturer's directions (Thermo Scientific, Nordic Biolabs AB, Stockholm, Sweden).

Table 1
Physicochemical properties of hematite (α-Fe_2O_3) nanoparticles.

Measurement	Hematite (α-Fe_2O_3)
Crystalline structure	Sphere
Primary size (nm)	30.74[a], ~30[b]
Surface area ($m^2\ g^{-1}$)[c]	28.98
Mean diameter of α-Fe_2O_3 in PBS[d]	1.28 μm
ζ potential in PBS (pH 7.4)[e]	−29 mV
Size distribution[d]	Unimodal: 950–2000 nm
Concentration	3.33 mg/ml
Endotoxin[f]	Non-detectable

Method.
[a] Determined from Scherrer analysis of the <012>, <104>, <110> and <116> reflections XRD (results from Makie et al. 2011).
[b] Transmission electron microscopy (results from Makie et al. 2011).
[c] Brunauer–Emmett–Teller (results from Makie et al. 2011).
[d] Photon cross correlation spectroscopy.
[e] Malvern Zetasizer Nano ZS.
[f] Limulus amebocyte lysate Chromogenic Endotoxin Quantification Kit.

2.3. Intratracheal instillation

After being lightly anesthetized with a mixture of isoflurane (Abbot Scandinavia AB, Solna, Sweden) and oxygen, the mice were intratracheally instilled with either hematite NPs in PBS or with only PBS (vehicle group). Before the intratracheal instillation the NP solution was probe sonicated for 1 min to dissociate agglomerates.

2.4. Preliminary studies for setting the experimental design

In order to determine a suitable dose for the NP exposure in this study, the cellular response in mice was evaluated at three different doses of hematite NPs: 1.25, 2.5, and 5.0 mg/kg body weight (bw). The cellular responses were evaluated 24 h after the NP instillation and were compared with the response in vehicle-exposed control mice. The highest dose, 5 mg/kg bw, elicited the highest total leukocyte response and was thus chosen for the subsequent experiments. The dose 2.5 mg/kg was the lowest dose to elicit a neutrophil response and was used in a pilot time kinetic experiment to determine the time-kinetics of the inflammatory response. In the kinetic experiment the mice were evaluated at the time points 1, 2, 7, and 14 days post-exposure to hematite NPs and the cellular responses were compared with those in untreated (naïve) mice.

2.5. OVA-induced airway inflammation

Allergic airway inflammation was induced in mice with Ovalbumin (OVA) (Sigma-Aldrich, St. Louis, MO) during a 35-day-long protocol, in which day 0 refers to the day that the mice were exposed to either hematite NPs or to PBS. At days −34 and −20, some mice were sensitized (*sensitized mice*) with an intraperitoneal (i.p.) injection of 10 μg OVA emulsified in $Al(OH)_3$ (Alu–Gel–S suspension 1.3%, research grade sterile, cat no: 12261, SERVA electrophoresis, Thermo Fisher Scientific Inc., Waltham, MA). The sensitized mice were then challenged with aerosolized 1% OVA (diluted in H_2O) in nose-only exposures for 30 min on days −5, −2, and 0 (Fig. 2A). Other mice were not sensitized (*non-sensitized mice*), and they were challenged with aerosolized 1% OVA according to the protocol described above for the sensitized mice (Fig. 2B). On day 0, both the sensitized and the non-sensitized mice were intratracheally instilled with either hematite NPs (5.0 mg/kg bw) or PBS. The mice were sacrificed and analyzed, as described below (Section 2.6.), on days 1, 2, and 7 post-hematite NP exposure.

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