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Titanium dioxide nanoparticles induce human eosinophil adhesion onto endothelial EA.hy926 cells via activation of phosphoinositide 3-kinase/Akt cell signalling pathway

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ABSTRACT

The use of nanoparticles (NPs) for developing new therapeutic strategies in a variety of diseases is gaining increasing attention. However, NPs could possess undesired effects, including pro-inflammatory activities. Despite the fact that several studies reported that NPs may induce or exacerbate eosinophilic inflammation in vivo in rodents, the information regarding the direct interaction between NPs and human eosinophils is lacking. In the present study, we test the possibility that NPs could alter the capacity of human eosinophils to adhere onto a cellular substratum. Using a panel of NPs, we found that several were able to increase the adhesion of human eosinophil onto endothelial EA.hy926 cells. Among them, TiO_2 NPs were the most potent and we therefore pursue this study with these NPs. TiO_2 NPs were found to increase the adhesion of eosinophils in a concentration dependent fashion. TiO_2 NPs did not alter the cell surface expression of a panel of cellular adhesion molecules, but CD29. Indeed, a weak to moderate, but significant, decrease of CD29 was observed after 30 min but returned to normal levels after 90 min. TiO_2 NPs were found to activate Akt, one important target of phosphoinositide 3-kinase (PI3K). However, despite the fact that cells were fully responsive to the cytokine GM-CSF activating both Akt and Erk-1/2, TiO_2 NPs did not activate Erk-1/2. Using a pharmacological approach with the PI3K/Akt inhibitor, wortmannin, the ability of TiO_2 NPs to activate Akt was drastically inhibited and, further, their capacity to increase adhesion of eosinophils was reversed. This study provides insights into the effects of NPs on the biology of human eosinophils indicating that as other agents, NPs, namely TiO_2 NPs, can induce intracellular events associated with a cellular function, adhesion.

1. Introduction

Nanoparticles (NPs) are excellent potential candidates to be used for medical diagnosis and therapeutic purposes and research in this area is very dynamic and intensive (Acharya et al., 2009; Allegra et al., 2011; Babu et al., 2010; Chandolu and Dass, 2013; Dolati et al., 2016; Lu et al., 2016). In the literature, an incredible number of studies reported or deal on the potential use of NPs for drug delivery in cancer therapies (Akhtar et al., 2012; Almeida et al., 2014; Ma and Yang, 2016). However, despite the fact that NPs are gaining increasing popularity and interest for developing nanotreatments and drug systems, there are some potential toxic risks. Indeed, cytotoxicity (Ahamed, 2013; Lin et al., 2007), oxidative stress (Ahamed, 2013; Alarifi et al., 2014), genotoxicity (Ahamed et al., 2013; AshaRani et al., 2009), and inflammation (Asharani et al., 2012) are the most common potential toxic effects reported in the literature. Since inhalation represents one of the major potential routes of human exposure to NPs, it is not surprising to note that several in vitro and in vivo studies were aimed at investigating the effects of NPs on pulmonary cells and/or lung inflammation (Inoue and Takano, 2011; Lu et al., 2014; Mohamud et al., 2014). Although some studies reported data promoting the use of NPs for the treatment of lung diseases (Barreto et al., 2015; Kim et al., 2014), several others demonstrate that NPs could induce/aggravate lung inflammation in vivo as evidenced by an increased number of neutrophil or eosinophil granulocytes in the bronchoalveolar lavages and lungs (Anderson et al., 2014; Brandenberger et al., 2013; Chen et al., 2006; Cho et al., 2012; Haberl et al., 2013; Huang et al., 2015).

Despite the above observations, the direct interaction between a given NP and human granulocytes has not been investigated deeply. However, the effects of some NPs on the biology of human neutrophils

Abbreviations: NP, nanoparticle; PI3K, phosphoinositide 3-kinase; VAA-I, viscum album agglutinin-I; DLS, dynamic light scattering; GM-CSF, granulocyte macrophage-colony stimulating factor

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have been reported, mostly from our laboratory (Babin et al., 2013; Goncalves et al., 2010; Goncalves and Girard, 2014; Liz et al., 2015; Noel et al., 2016; Poirier et al., 2014, 2015) and others (Abrikossova et al., 2012; Couto et al., 2014; Dianzani et al., 2006; Paino and Zucolotto, 2015; Papatheofanis and Barmada, 1991; Soares et al., 2016). In contrast, the literature regarding the direct interaction with NPs and human eosinophils is curiously presently lacking. Therefore, it is completely unclear how NPs can alter the biology of human eosinophils.

Eosinophils represent only less than ~5% of total blood leukocytes in humans by opposition to up to ~70% for neutrophils. However, eosinophils can accumulate in tissues and airways under certain pathological conditions, especially in asthma and allergic diseases where they are known to play important roles (Gleich, 2000; Ilmarinen and Kankaanranta, 2014; Shamri et al., 2011). Eosinophils are known to undergo spontaneous apoptosis and their elimination by professional phagocytes (efferocytosis) is of major importance for the resolution of eosinophilic inflammation, including in the airways occurring in asthma (Ilmarinen and Kankaanranta, 2014). During an inflammatory response, eosinophils have to leave blood circulation to migrate to the inflamed site and one of the first major steps involved is this situation is adhesion. Indeed, for example, eosinophils are known to adhere onto airway epithelial cells during the development of asthma and are also known to adhere onto endothelial cells to quite the blood circulation (Johansson, 2014).

Since NPs were previously found to alter the biology of human neutrophils, including their adhesion onto human umbilical vein endothelial cells (Dianzani et al., 2006), we have decided to investigate here how different engineered NPs will alter the ability of human eosinophils to adhere onto endothelial cells. Among them, titanium dioxide (TiO_2) NPs were found to be the most potent for increasing eosinophil adhesion. Further, we show that this occurs via phosphoinositide 3-kinases/Akt activation, a cell pathway involved in several granulocyte functions.

2. Materials and methods

2.1. Chemicals

The nanoparticles titanium dioxide (−) (TiO_2), gold$^+$ amine-functionalized (Au^+), gold$^-$ carboxy-functionalized (Au^-), zinc oxide (ZnO), iron oxide or magnetite (Fe_3O_4), cerium (IV) oxide (CeO_2), carboxy-functionalized palladium (Pd), carboxy-functionalized platinum (Pt) were purchased from Sciventions Inc. (Toronto, ON). Aluminium oxide (Al_2O_3) and gold of 70 nm (Au_{70}) were from Nanostructured & Amorphous Materials, Inc. (Houston, TX) and Ted Pella Inc. (Redding, CA), respectively. Recombinant human (rh) granulocyte macrophage-colony stimulating factor (GM-CSF) was purchased from Peprotech Inc. (Rocky Hill, NJ, USA). The plant lectin *Viscum album* agglutinin-I (VAA-I), trypan blue, bovine serum albumin (BSA), phosphate buffered saline solution (PBS), rhTNF-α, and dextran were from Sigma Aldrich Ltd. (St. Louis, MO, USA). The mouse monoclonal anti-GAPDH (FL-335), anti-p38 (C-20), anti-CD62L (L-selectin)-FITC, anti-CD11b- FITC, anti-CD11-FITC, anti-ICAM-3-FITC, anti-IgG2a FITC (normal mouse) and anti-IgG1 FITC (normal mouse) were from Santa Cruz Biotechnology Inc. (Santa cruz, CA) and anti- Phospho-Akt (D9E), anti-Phospho-Erk-1/2 (D13.14.4E), anti-Phospho-p38 (D3F9), anti-Erk-1/2 (137F5) were purchased from Cell Signaling Technology (Danvers, MA). The mouse monoclonal anti-CD49d-PE, anti-CD18-PE, anti-CD54-PE, anti-IgG1-PE and anti-CD29-PE were obtained from BD Pharmingen (Mississauga, ON). Ficoll-Hypaque was purchased from GE Healthcare (Uppsala, Sweden). Roswell Park Memorial Institute (RPMI-1640), HEPES, penicillin, streptomycin, Annexin-V- FITC and Hank's Balanced Salt Solution (HBSS) were from Life Technologies (Grand Island, NY, USA). HRP-labeled goat anti-mouse IgG antibody, HRP-labeled goat anti-rabbit IgG antibody and HRP-labeled rabbit anti-goat IgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). The 30% acrylamide/BIS solution, polyvinylidenedifluoride (PVDF) membrane and Clarity TM Western ECL substrate were purchased for Bio-Rad. Otherwise specified, all other chemicals were purchased from Sigma-Aldrich.

2.2. Characterization of NPs by dynamic light scattering

The size distribution and zeta potential of the NPs used in this study were determined by dynamic light scattering (DLS) using a Zetasizer Nano-ZS (model ZEN3600) from Malvern Instruments Inc. (Westborough, MA). Measurements were performed with NPs in suspension in RPMI-1640 + 10% fetal calf serum, according to the tested functions, at a concentration of 50 μg/ml and at 37 °C, the temperature at which the experiments were performed. The concentration was selected according to previous publications performed with neutrophil granulocytes (Babin et al., 2013; Goncalves et al., 2010; Goncalves and Girard, 2014; Noel et al., 2016; Poirier et al., 2014).

2.3. Eosinophil isolation

Blood donations were obtained from informed and consenting individuals according to our institutionally approved procedures. Granulocytes were isolated from venous blood of healthy volunteers by dextran sedimentation followed by centrifugation over Ficoll-Hypaque, to obtain neutrophils and eosinophils as previously described (Lavastre et al., 2005). When > 5% of eosinophils were detected in granulocyte enriched preparations, as determined by cytology from cytocentrifuged preparations colored by the Hema 3 Stain Set (Biochemical Sciences Inc., Swedesboro, NJ), eosinophils were separated from neutrophils by negative immunomagnetic selection using anti-human CD16-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's recommendations. Purity after eosinophil isolation was routinely > 96% and cell viability was always greater than 98%, as determined by trypan blue exclusion before and after treatment with NPs.

2.4. EA.hy926 cell culture

The human umbilical vein cell line, EA.hy926 (ATCC® CRL-2922™) was purchased from American Type Culture Collection (Manassas, VA), and was grown in RPMI-1640 supplemented with 10% fetal calf serum and antibiotics. Cell viability was systematically evaluated before and after each treatment, and mortality never exceeded 5%.

2.5. Cell adhesion

Eosinophils were treated with buffer (Ctrl) or the indicated agonists for 30 min and then labeled for 30 min with 5 μM calcein-AM (Molecular Probes, Inc., Eugene, OR, USA). Eosinophil adhesion onto EA.hy926 cells was measured essentially as we have previously published using human A549 cells as substrate (Pelletier and Girard, 2005). The number of adherent eosinophils was calculated by counting the number of fluorescent cells from five randomly selected high-power fields (× 400) observed with a photomicroscope Leica DMRE equipped with an ebq 100 dc epifluorescent condenser. Images were taken with a Cooke Sensicam High performance camera coupled to the Image Proplus® (version 4·0) program.

2.6. Assessment of eosinophil apoptosis

Apoptosis was evaluated by cytology and by flow cytometry. Eosinophils (5×10^5 cells/ml in RPMI-1640 + antibiotics + 10% fetal calf serum) were incubated at 37 °C in 5% CO_2 in 48-well plates for 24 h in the presence of buffer (Ctrl), the pro-apoptotic plant lectin VAA-I (1 μg/ml) or the indicated concentrations of TiO_2 NPs. After the