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Diesel exhaust particulate associated chemicals attenuate expression of CXCL10 in human primary bronchial epithelial cells

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ABSTRACT

Air pollution affects a large proportion of the population particularly in urban areas, with diesel particulates recognised as particular causes for concern in respiratory conditions such as asthma. In this study we examined the response of human primary airway epithelial cells to diesel particulate chemical extracts (DE) and characterised gene expression alterations using RNA-SEQ. Using the antagonist CH223191, DE induced CYP1A1 and attenuation of CXCL10 among other genes were observed to be aryl hydrocarbon receptor dependent. Basal and toll like receptor dependent protein levels for CXCL10 were markedly reduced. Investigation of similar regulation in plasmacytoid dendritic GEN2.2 cells did not show DE dependent regulation of CXCL10. Instillation of DE into mice to recapitulate airway epithelial exposure to chemical extracts in an *in vivo* setting failed to demonstrate a reduction in CXCL10. There was however an increase in the Th2 type epithelial cell derived inflammatory mediators TSLP and SERPINB2. We also observed an increased macrophages and a decrease in the proportion of lymphocytes in bronchoalveolar lavage fluid. CXCL10 can play a role in allergic airway disease through recruitment of Th1 type CD4 + T-cells, which can act to counterbalance Th2 type allergic responses. Modulation of such chemokines within the airway epithelium may represent a mechanism through which pollutant material can modify respiratory conditions such as allergic asthma.

1. Introduction

Diesel exhaust particulates (DEP) are an important contributor to the adverse health effects associated with inhalation of pollutant material especially in urban areas (Jang et al., 2016; Manzetti and Andersen, 2016). In terms of respiratory impact, they have been linked to exacerbation of asthma with evidence also suggesting some contribution to the development of sensitisation and respiratory effects in early life (Codispoti et al., 2015; Brandt et al., 2015; Diaz-Sanchez et al., 1999; Brandt et al., 2013).

Allergic airway disease is the most common form of asthma and typically involves an adaptive immune response mediated through allergen specific activation of type 2 CD4 + T helper cells (Th2). These cells produce cytokines, which increase IgE production from plasma cells, recruit eosinophils and induce mucus production from airway epithelial cells, all contributing to airway restriction (Lambrecht and Hammad, 2015). The airway epithelium is the primary contact point for inhaled material and plays a role in the development of sensitisation through the release of Th2 type immune signals cytokines such as TSLP (Hammad and Lambrecht, 2015). Recruitment of Th2 lymphocytes and other immune cells to the airways occurs in response to the release of chemokines. Th2 chemokines include CCL17, CCL22, which bind to CCR4 + cells and CCL11, CCL24 and CCL26, which bind to CCR3 + cells (Bisset and Schmid-Grendelmeier, 2005). Th1 type immunity, which involves mainly cell-mediated adaptive immune responses and anti-viral activity, is controlled through type 1 CD4 + T helper cells. The recruitment of these cells to injured or infected tissues is primarily under the influence of chemokine ligands for CXCR3 including CXCL9, CXCL10 and CXCL11 (Griffith et al., 2014). Th1 and Th2 type immune signals can have modulatory effects on the others functionality, and alterations in the balance of these and other types of T helper cell subpopulations are suggested to underlie many of the pathological responses in allergic airway disease (Berker et al., 2016; Abrahamsson et al., 2011).

Experimental modelling has suggested that the detrimental effects of DEP in allergic airway disease may be linked to an enhancement of adaptive immunity T helper cell driven responses (Provoost et al., 2012; Diaz-Sanchez et al., 1997). Knockout studies in mice have refined this mechanistic understanding and attributed the effect of diesel exhaust material on allergic airway disease to the regulation of type 2 immunity

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and T helper cell populations (De Grove et al., 2017). Furthermore, alterations in the levels of Th1 and Th2 chemoattractants favouring a Th2 cell response has been observed upon exposure of blood cells from allergic patients to diesel exhaust material (Fahy et al., 2002). Importantly, organic chemicals associated with diesel particles have been observed to increase the production of Th2 chemokines while reducing the Th1 chemokine CXCL10 in blood cells from non-atopic donors (Chang et al., 2006). Moreover, the organic chemical fraction of diesel exhaust particulates enhanced allergic airway inflammation including Th2 cytokine and chemokine production, while the carbonaceous core of these particulates devoid of chemicals did not and instead induced a profile of inflammation consistent with enhanced Th1 type inflammation (Yanagisawa et al., 2006). The concept that chemicals associated with diesel and other exhaust particulates are major contributors, responsible for allergic disease modification through modulation of T-cell responses is widely considered and further supported by studies including those that attribute pro-oxidative activity with an inhibition of Th1 promoting responses (Chan et al., 2006).

Despite this however, there is little information on how the airway epithelium may handle such exposures. Given that these cells are frontline sensors for inhaled material and have a proximal role in directing relevant immune responses (Hammad and Lambrecht, 2015) it was a primary aim of this study to examine how diesel associated chemical extracts alter human primary bronchial epithelial gene expression. In addition to airway epithelial cells, other cells within the lung come into contact with inhaled material and may be responsible for directing biological responses. Dendritic cells are professional antigen presenting cells and are located throughout the, trachea, bronchia and bronchioles as well as the alveolar regions of the normal human lung (Sertl et al., 1986). The number of these cells within the bronchial epithelium of patients with asthma are increased compared to normal volunteers (Tunon-De-Lara et al., 1996; Moller et al., 1996; Jahnsen et al., 2001). Recent characterisation of dendritic cell types within the normal human bronchial epithelium has identified both conventional and plasmacytoid dendritic cells (pDC) (Baharom et al., 2017; Baharom et al., 2016). pDC, which are primarily involved in mounting anti-viral activity, through type I interferon production (*e.g.* IFNA2) and directing adaptive immunity towards a Th1 type response through production of factors such as CXCL10 (Swiecki and Colonna, 2015). They have also been suggested to control Th2 responses within the lung, dysregulation of which has been suggested as a mechanism for asthma exacerbations (Pritchard et al., 2012). Similar to investigation into airway epithelial cell responses, there is a distinct lack of information on how pDC respond to diesel exhaust particulate associated chemicals. An additional aim of this study was therefore to examine how these cells respond to such exposures.

This type of mechanistic investigation coupled with exploration of acute responses within an *in vivo* murine exposure model within this current study, provide important evidence as to how we should consider chemical constituents of exhaust material for their ability to influence the pathogenesis of respiratory inflammatory conditions such as asthma.

2. Materials and methods

2.1. Chemicals and reagents

A dichloromethane extract of diesel particulate matter (DE) (https://www-s.nist.gov/srmors/certificates/2975.pdf) collected from an industrial diesel engine powered forklift truck collected under specifically designed heavy-duty conditions (SRM 1975), was obtained from the National Institute of Standards and Technology of the US. The extracted material represents 2.7% of the original particulate mass and has been extensively characterised for the presence of selected PAHs and nitro-substituted PAHs https://www-s.nist.gov/m-srmors/certificates/1975.pdf. Dichloromethane was exchanged for DMSO prior to cell treatment and stock preparation (20 mg/ml). CH223191 obtained from Sigma-Aldrich (Gillingham, Dorset, UK). Treatments were carried out using a minimum of 1:1000 dilution with DMSO control for *in vitro* exposures. A Toll like receptor (TLR 1–9) ligand cocktail from Invivogen (# tlrl-kit1hw; Toulouse, France) was used at final concentrations for *in vitro* exposures of Pam3CSK4 (100 ng/ml), HKLM (10^7 cells/ml), Poly(I:C) (1 μg/ml), Poly(I:C)-LMW (1 μg/ml), LPS (100 ng/ml), Flagellin (100 ng/ml), FSL1 (100 ng/ml), Imiquimod (100 ng/ml), ssRNA40 (100 ng/ml) and ODN2006 (0.5 μM). LPS alone was also used for selected treatments. All other reagents were obtained from Sigma-Aldrich unless otherwise stated.

2.2. Cell culture and treatment protocols

Normal human primary bronchial epithelial cells (HPBEC) were obtained Epithelix Sàrl (Switzerland) at passage 1. Cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere. Cells were sub-cultured into 48 well plates using proprietary growth media (Cat# EP09AM). Prior to treatment, cells underwent differentiation using a modified airway epithelial culture media. This was composed of a 50:50 ratio of LHC-9 and DMEM (High Glucose) media and supplemented with bovine serum albumin 0.5 mg/ml, Bovine pituitary extract, 10 μg/ml, Insulin 1 μg/ml, Transferrin 0.125 μM, Hydrocortisone 0.1 μM, Triiodothyronine 0.01 μM, Epinephrine 2.7 μM, Epidermal growth factor 0.5 ng/ml, Retinoic acid 5 × 10–8 M, Phosphorylethanolamine 0.5 μM, Ethanolamine 0.5 μM, Zinc sulphate 3.0 μM, Penicillin G sulphate 100 U/m, Streptomycin sulphate 100 μg/ml, $CaCl_2$ 1.0 mM and other components as described elsewhere (Fulcher and Randell, 2013). Differentiation was initiated after cells reached confluence (at Day 7 of culture) and continued for a minimum of 12 days, prior to cell treatment. Media replacement was carried out every 2–3 days.

Plasmacytoid dendritic GEN2.2 cells were obtained from the French Collection of National Microorganism Cultures (Institut Pasteur, Paris, France) and characterised as described previously (Chaperot et al., 2006). Cells were cultured in a 75-cm^2 culture flask (Nunc, Cat# NC-156499) on top of an 80% confluent adherent MS-5 feeder cell layer in RPMI 1640 GlutaMAX media containing 2 mM L-glutamine, 10%(v/v) foetal bovine serum, 100 μM non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin G sulphate and 100 μg/ml streptomycin sulphate. Gen2.2 cells (4×10^6 cells/75 cm^2 flask) were passaged every third day into a new 75-cm^2 culture flask containing a fresh 80% confluent MS-5 cell flask. MS-5 cells (Cat# ACC 441) were obtained from DSMZ (Braunschweig, GERMANY) and maintained in 90% alpha-MEM + 10% (v/v) foetal bovine serum, 2 mM L-glutamine and 2 mM sodium pyruvate. Cells were sub-cultured every 3 days and seeded at a concentration of 1–2 × 10^6 cells/75 cm^2 flask.

HPBEC cells were exposed to treatments in the 48 well plates in which they were differentiated with 250 μl of cell culture differentiation media per well. GEN2.2 cells were removed from the feeder cell flask and transferred to 24 well plates at a concentration of 1 × 10^6 cells/ml (500 μl per well) prior to their treatment. For HPBEC, exposures were carried out between passage 2–4, while for pDC exposures were carried out between P12 and p18. HPBEC (3–8 different donors) were treated with diesel exhaust particle extract (DE) (0–20 μg/ml) in the absence or presence of the AHR inhibitor CH223191 (10 μM) (CH) for 24 h. HPBEC were also treated with DE in combination with a toll like receptor ligand cocktail (TLR) (Concentrations described in Section 2.1) 3 times over a 7 day period to examine chemokine production. GEN2.2 cells were treated with DE (5 μg/ml) in the absence or presence of the same toll like receptor ligand cocktail (TLR) for 24 h.

2.3. In vivo exposure and bronchoalveolar lavage analysis

Female Balb/c mice between 6 and 8 weeks were anaesthetised with 5% isoflurane in oxygen using a precision vaporizer and intranasally instilled with 25 μl of diesel exhaust particulate extracts (SRM 1975) at