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Flow cytometric characterisation of antigen presenting dendritic cells after in vitro exposure to diesel exhaust particles

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Abstract

The aim of this study was to obtain more insight into the effect of diesel exhaust particles (DEP) on the maturation of primary human dendritic cells. Monocyte-derived dendritic cells (Mo-DC) derived from seven different donors were exposed to different DEP concentrations (0.2, 2, 20, 200 and 2000 ng/ml) in the presence or absence of lipopolysaccharide (LPS), and changes in the surface expression of HLA-DR, CD86 and CD83 were examined. Exposure of Mo-DC to DEP alone did not alter expression levels of any of the markers. Treatment with LPS alone increased the expression levels of all three surface markers, although the levels were not significantly different compared to untreated DCs. The LPS-induced marker expression could be further enhanced by co-stimulation of the cells with DEP. Statistical significantly increased levels of CD83 expression were observed after exposure to 0.2 ($p = 0.018$), 20 ($p = 0.010$) and 200 ng/ml ($p = 0.047$) DEP combined with LPS in the group of responders. We conclude that DEP has an adjuvant effect on LPS-induced maturation of Mo-DC.

1. Introduction

The prevalence of respiratory allergy has increased during the last few decades in western countries (van Zijverden et al., 2000; Parnia et al., 2002). A variety of factors have been suggested to be involved, such as genetic factors, changes in allergen exposure and increased allergen loads. Genetic factors alone cannot explain the widespread increased incidence in respiratory allergy, because the increase has occurred only within one or two generations (Karol, 2002). Increased allergen loads and exposure can mainly be explained by changes in the environment and lifestyle, such as dietary changes, the microbial environment, and air pollution (Björkstén, 1999). Air pollution, especially fine particles, such as diesel exhaust particles (DEP), have been shown to contribute to various health outcome indices, including exacerbations of asthma, chronic bronchitis and respiratory tract infections in different geographical locations of the world (Salvi and Holgate, 1999).

Dendritic cells (DC) that reside in the pulmonary mucosal tissues have recently been implicated in allergic diseases of the respiratory tract. Here, they sample environmental antigens, take them up and start to mature. Matured DCs then migrate to regional lymph nodes where they present processed antigenic peptides to T cells. As such, they are fundamental to our ability to deal with foreign antigens and to generate an appropriate immune response (Upham and Stumbles, 2003).

The purpose of this study was to obtain more insight into the effects of DEP on the activation and maturation of primary DC. We have tested whether DEP exerts its effect either by direct stimulation of DC or indirectly by an adjuvant effect on other allergens.

Abbreviations: DEP, diesel exhaust particles; Mo-DC, monocyte-derived dendritic cells; LPS, lipopolysaccharide; SI, stimulation index.

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2. Materials and methods

2.1. Human cord blood

Detailed protocols concerning the isolation and cultivation of monocyte-derived dendritic cells (Mo-DC) can be found in De Smedt et al., 2002. Cord blood samples of seven women were collected from the umbilical vessels of placentas of normal, full-term infants. Collection of cord blood samples was approved by the ethical commission of the hospital (Heilig Hart Ziekenhuis, Mol, Belgium) and a signed informed consent was obtained from the mothers participating in this study.

2.2. Cell culture and exposure

Within 24 h, mononuclear cells (MNC) were separated from the diluted cord blood (1:2 in phosphate buffered saline (PBS)) by density gradient centrifugation (Ficoll Paque Plus, Amersham Biosciences, Uppsala, Sweden). MNC (10^6 cells per ml) were seeded in 6-well plates and incubated at 37 °C and 5% CO_2, allowing monocytes to adhere to the bottom. After at least 2 h, non-adherent cells were cautiously aspirated and the adherent cells were rinsed with warm medium, detached and cultured in 24-well plates (10^6 cells per ml) in complete medium [RPMI 1640 with 25 mM Hepes and glutaMAX I (GIBCO-BRL, Invitrogen, Merelbeke, Belgium), 1% penicillin/streptomycin (GIBCO-BRL) and 10% fetal calf serum (FCS; GIBCO-BRL)]. The medium was supplemented with 750 units/ml recombinant human granulocyte macrophage-colony stimulating factor (Novartis Pharma AG, Basel, Switzerland) and 500 units/ml recombinant human interleukin-4 (Endogen, Woburn, USA) to allow differentiation of the cells into Mo-DC. After three days, half the volume of medium was aspirated and two-third volume of freshly prepared complete medium with cytokines was added to the cultures. On day 6, the medium was substituted with freshly prepared medium containing the test substances. Cells were either left unexposed (control cells) or were exposed for 24 h to lipopolysaccharide (LPS *E. coli* O26:B6; Sigma, St. Louis, USA), an equivalent volume of PBS + Tween-80 (Merck, Brussels, Belgium) (solvent control), or different concentrations of DEP (preparation see below) in the presence or absence of LPS. After exposure, changes in phenotype of Mo-DC were investigated by flow cytometry. Immature Mo-DC in the unexposed control cultures were $CD1a^+$ (2.59%), $HLA\text{-}DR^+$ (18.2%), $CD86^+$ (2.16%), $CD83^+$ (0.15%) and $CD14^-$.

2.3. Preparation of DEP

We used standard DEP (standard reference material (SRM) 2975) obtained from the National Institute for Standards and Technology (NIST, Gaithersburg, USA). The material was collected from a filtering system designed specifically for diesel-powered forklifts. Its chemical composition is mentioned in the Certificate of Analysis from NIST. Ten milligrams of DEP were suspended in 5 ml tissue culture medium (RPMI 1640 + 1% penicillin/streptomycin), supplemented with 0.1% Tween-80. The mixture was sonicated for 1 min on ice using an ultrasonic disruptor (Bransor Sonifier 250). The suspension was diluted in tissue culture medium to the final concentrations required for exposure of the cells (2000–200–20–2–0.2 ng/ml), so that the final concentration of Tween-80 in medium did not exceed 0.01%.

2.4. Flow cytometry

After 24 h of exposure, the expression of different surface markers was analysed by flow cytometry. Cells were harvested from the cultures, counted and aliquots of 10^5 cells were made in PBS-Ca/Mg containing 10% FCS. Cells were incubated for 30 min at 4 °C with monoclonal antibody conjugated to either fluorescein isothiocyanate (FITC) or phycoerythrin (PE; CD1a-FITC, CD14-PE, CD86-PE, CD83-PE and HLA-DR-PE (BD Pharmingen™, Erembodegem, Belgium), and were washed twice. Non-reactive antibodies of the same isotype were used as controls: mouse IgG1-FITC, IgG1-PE and IgG2a-PE (Becton Dickinson). During flow cytometry dead cells were identified using propidium jodide (PI) staining and gated out by setting a threshold on the forward scatter. Ten thousand cells were acquired for each exposure condition and were analysed on a FACStar Plus, using CellQuest software (Becton Dickinson). The results of the phenotypic analysis were expressed as percentages of positive cells.

2.5. Statistics

Mean stimulation indices (SI) for the three surface markers were calculated as the mean ratio of the percentage of cells that expressed a particular marker after exposure, to the percentage of cells in the solvent control (SC) culture expressing the marker. SI, calculated on a defined number (n) of donors, and their respective standard deviations (SD) are given. The paired Student's t-test was used to determine whether surface marker expression after exposure was significantly different compared to solvent exposure. If p-values were less than 0.05, these differences were considered significant.

3. Results

Bacterial LPS is a known inducer of DC maturation (Lipscomb and Masten, 2002). To examine the potential adjuvant effect of DEP on the maturation process,