

Increased HLA-DR expression after exposure of human monocytic cells to air particulates

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Summary

Background The expression of HLA-DR on the cell membrane of antigen-presenting cells is of major importance for the induction of an allergic response in the airways. Environmental particulates are thought to play an important role in inducing or enhancing allergic sensitization, possibly by increasing the expression of HLA-DR on the cell membrane of antigen-presenting cells. In addition, these particulates may synergize with common sensitizing agents in inducing or enhancing HLA-DR and thus antigen presentation.

Objective In this study, we investigated the potential of three particle types, namely carbon black, diesel exhaust particles and urban air particulates (0.1–1000 ng/cm²), to induce the expression of HLA-DR on differentiated THP-1 cells, taken as a model for alveolar macrophages. We also assessed the ‘adjuvant’ potential of the particles on interferon (IFN)- γ , a known enhancer of HLA-DR.

Results By themselves, the particles (0.1–1000 ng/cm²) were not able to induce HLA-DR on the THP-1 cells after an incubation of 48 h. However, even at very low concentrations, carbon black (from 1 ng/cm² on) and diesel exhaust particles (from 0.1 ng/cm² on), interacted with IFN- γ (100 U/mL) to enhance HLA-DR expression (up to 2.5-fold increase).

Conclusion This finding may reflect *in vitro* one of the mechanisms by which pollutant particles exert an ‘adjuvant’ activity and may partially explain how exposure to particles can be related to the enhancement of allergic sensitization.

Keywords alveolar macrophages, antigen presentation, HLA-DR, environmental particles, IFN- γ , adjuvant activity

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Introduction

Over the past decade, environmental exposure to airborne particulates has been the subject of several epidemiological studies. Overall, these studies indicate that particulate air pollution is associated with increased mortality and morbidity among the general population [1–3]. PM₁₀ (particulate matter with a cut-off diameter of 10 μ m) is a complex mixture consisting of elemental and organic carbon, metals, sulphates, nitrates, pollen and microbial contaminants [4]. In areas with heavy traffic density, diesel exhaust particulates (DEP) are an important constituent of PM₁₀. They consist of an inert carbon core containing unburned fuel compounds such as polycyclic aromatic hydrocarbons (PAHs) [5]. PM₁₀ and especially DEP are thought to play an important role in inducing and enhancing allergic inflammation in the airways [4–6], possibly by exerting an ‘adjuvant’ activity on common sensitizing agents [7].

When inhaled particulates reach the alveoli, they encounter alveolar macrophages, which are the most abundant cells in the airway lumen [8]. These alveolar macrophages can

initiate immune-inflammatory processes by producing pro-inflammatory cytokines such as interleukin (IL)- β and tumour necrosis factor (TNF)- α [5, 6]. Besides releasing these mediators, the alveolar macrophages may also play a role in antigen presentation [5], in addition to the pulmonary dendritic cells. Antigen presentation is regulated by major histocompatibility class (MHC) II human leucocyte antigen-DR (HLA-DR) molecules [9]. The density of HLA-DR molecules on the cell membrane of the antigen-presenting cell determines the efficiency of antigen presentation [10]. Although alveolar macrophages are known to be poor accessory cells, enhancement of the role of alveolar macrophages as antigen-presenting cells is thought to be a potential mechanism by which particles exert an adjuvant activity on allergic inflammation [5].

In addition, these environmental particles may synergize with common sensitizing agents in inducing or enhancing HLA-DR expression and thus possibly antigen presentation [5, 7].

The goals of the present study were (1) to investigate the antigen presentation-inducing potential of three pDocument types of different origins, namely carbon black (CB), diesel exhaust particles (DEP) and urban particulate matter (UPM), all tested at low concentrations (≤ 1000 ng/cm²) and (2) to investigate the ‘adjuvant’ potential of these particulates. We studied this in THP-1 cells, a human monocytic cell line,

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which can be differentiated into mature macrophages *in vitro*, and we measured the expression of HLA-DR molecules on the cell membrane. To assess the 'adjuvant' potential of the particulates, the cells were simultaneously exposed to the particles and IFN- γ , which is a known enhancer of HLA-DR expression on THP-1 cells [10,11].

Materials and methods

RPMI 1640 with 25mM Hepes and glutamax™ I (446mg/L), penicillin-streptomycin (P/S; 5000µg/mL) and phosphate buffered saline (PBS) $\pm$ Ca/Mg were obtained from Gibco-BRL (N.V. Life Technologies, Merelbeke, Belgium); fetal bovine serum (FBS) from Hyclone (Bornem, Belgium); IFN- γ from Biosource SA (Nivelles, Belgium); carbon black (CB) Vulcan M from Cabot (Leiden, the Netherlands); diesel exhaust particles (DEP, standard reference material 1650) and urban particulate matter (UPM, standard reference material 1648) from the National Institute for Standards and Technology (NIST, Gaithersburg, MD, USA); Tween-80 and acetone from Merck (Overijse, Belgium); methanol, trypan blue and phorbol myristate acetate (PMA) from Sigma (Bornem, Belgium); human anti-HLA-DR monoclonal antibody and goat anti-mouse polyclonal antibody conjugated with FITC (GAM) from Becton Dickinson (Erembodegem, Belgium).

Preparation of CB, DEP and UPM particle suspensions and IFN- γ

CB, DEP and UPM suspensions were all prepared using the same procedure. Initially, 8mg of each pDocument type was suspended in 5mL serum-free tissue culture medium (99% RPMI 1640 + 1% P/S), supplemented with 0.1% Tween-80. This mixture was sonicated for 1 min on ice, using an ultrasonic disrupter (Branson Sonifer 250, at 80 W). This suspension was further diluted in serum-free tissue culture medium to 1600, 160, 16, 1.6 and 0.16ng/mL, which corresponds to 1000, 100, 10, 1 and 0.1 ng/cm² for a 96-well plate. In a previous study [12], the mean diameter of the particles in tissue culture medium was characterized using laser diffraction (Coulter LS230) and the mean diameters were 100nm for CB, 400nm for DEP and 2µm for UPM. IFN- γ was diluted to 1000U/mL in (PBS-Ca/Mg + 6% FBS).

Cell culture

THP-1 (human monocytic cell line) was obtained from the European Collection of Animal Cell Cultures (ECACC) (cat. nr. 88081201). The cell line was cultured in RPMI 1640 with 25mM Hepes and glutamax™ I, supplemented with 10% FBS and 1% P/S (= full tissue culture medium) and was kept in a humidified atmosphere at 37°C and 5% CO₂. The cells were subcultured every 3–4 days. Prior to use, the proportion of living cells was determined by trypan blue staining.

Exposure

Cells were seeded in 96-well plates at 175000 cells/mL complete tissue culture medium (200µL/well). To allow adherence and differentiation into mature macrophages [13], the cells were incubated with 0.1µg/mL PMA for 48h before exposure to the test substance. From here on the differentiated cells are referred to as 'THP-1 cells'. After differentiation the cells were exposed

for 48h to (1) tissue culture medium (normal control cells), (2) tissue culture medium supplemented with 10% [PBS-Ca/Mg + 6% FBS] (vehicle control), (3) 100U/mL tissue culture medium IFN- γ (IFN- γ -exposed control cells), (4) CB, DEP and UPM (1000, 100, 10, 1, 0.1 ng/cm² tissue culture plate), or (5) CB, DEP and UPM (at the same concentrations as above) plus IFN- γ (100U/mL). All exposures were performed in serum-free tissue culture medium (99% RPMI 1640 + 1% P/S). For each exposure condition, at least four replicate wells were used. As a control for a possible background fluorescence signal of the antibodies, four replicate wells without cells were incubated simultaneously with tissue culture medium.

HLA-DR assay

After 48h of incubation with IFN- γ and/or the particles, the tissue culture medium was removed and the cells were washed three times with 200µL PBS-Ca/Mg. 200µL acetone/methanol (1:1) were added to each well for 7 min at room temperature (21°C). After removal of the acetone/methanol, the cells were washed three times with 200µL PBS-Ca/Mg. For each exposure condition three replicate wells were incubated with 50µL anti-HLA-DR (1:50 in RPMI 1640) during 1h at 4°C. The remaining wells were incubated with 50µL RPMI 1640 alone (isotype control).

After another washing cycle all the wells were incubated with 40µL GAM – FITC (1:2 in RPMI 1640) for 1h at 4°C.

Finally, the cells were washed three times with 200µL PBS-Ca/Mg and 50µL of PBS+Ca/Mg were added to each well. Fluorescence was measured using a cytofluoroscrophotometer (Millipore 2340; ex. 485nm, em. 530nm, sensitivity 6).

For each exposure condition the mean value of three replicate measurements of absolute units of fluorescence (UOF), i.e. the measured fluorescence signal minus the isotype control and background signal, was calculated. Each experiment was repeated six times independently.

Statistics

Statistics were performed using a repeated measures ANOVA ($P < 0.05$) on the absolute UOF values of the six independent HLA-DR experiments. If $P < 0.05$, a Dunnett's multiple comparison post-test was performed to assess significant differences ($P < 0.05$) between control cells (non-exposed or IFN- γ -exposed) and particles exposed cells. These tests were performed using Graphpad Prism 2.01.

Results

Expression of HLA-DR molecules

No HLA-DR molecules were detected on non-exposed control cells, nor could the different particle types induce HLA-DR expression by themselves. However, IFN- γ , which we used as an *in vitro* stimulating agent, was able to induce expression of HLA-DR, as shown in Figs 1, 2 and 3 (0-value on the x-axis). When the cells were simultaneously exposed to IFN- γ and either CB or DEP, there was a significant enhancement of HLA-DR expression when compared to the IFN- γ control cells (Figs 1 and 2). The increase in HLA-DR expression occurred already at the lowest concentrations – from 1ng/cm² and 0.1ng/cm² onwards for CB and DEP, respectively. The degree of expression increased with particle concentration up

Fig. 1. HLA-DR expression on THP-1 cells after 48 h exposure to carbon black (CB) and IFN- γ 100U/mL (0 value); individual experiments are shown as individual data-points; the mean HLA-DR expression is shown by the line graph; mean control value of six experiments $\pm$ 95% CI (dotted line) is shown; * P < 0.01 when compared to IFN- γ control cells (=0).

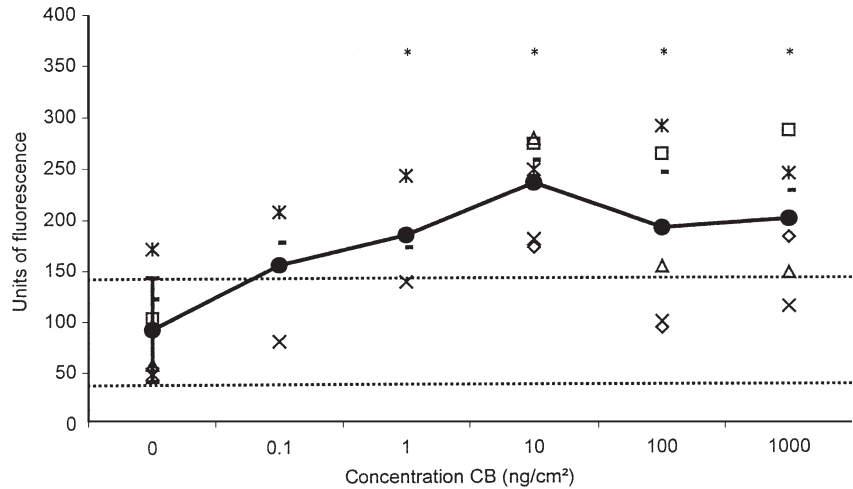


Fig. 2. HLA-DR expression on THP-1 cells after 48 h exposure to diesel exhaust particles (DEP) and IFN- γ 100U/mL (0 value); individual experiments are shown as individual data-points; the mean HLA-DR expression is shown by the line graph; mean control value of six experiments $\pm$ 95% CI (dotted line) is shown; * P < 0.01 when compared to IFN- γ control cells (=0).

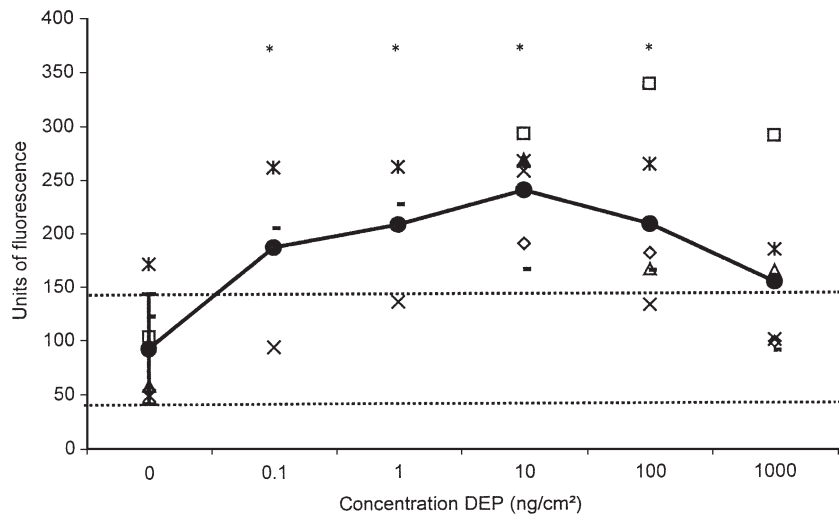
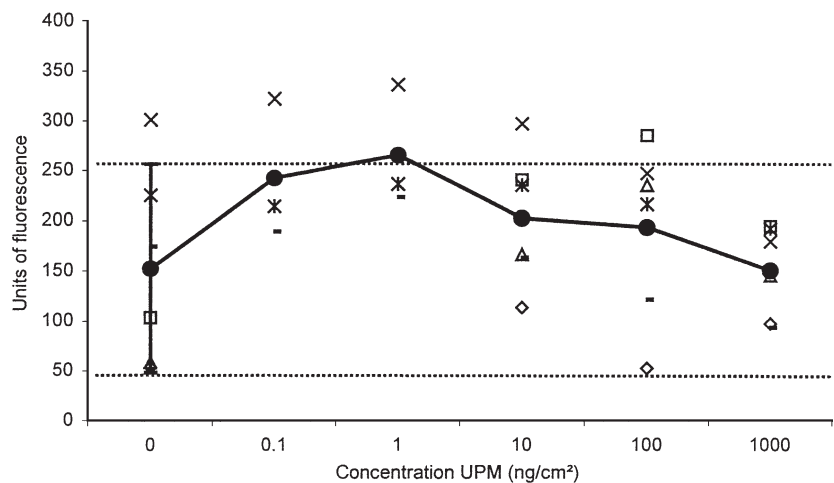


Fig. 3. HLA-DR expression on THP-1 cells after 48 h exposure to urban particulate matter (UPM) and IFN- γ 100U/mL (0 value); individual experiments are shown as individual data-points; the mean HLA-DR expression is shown by the line graph; mean control value of six experiments $\pm$ 95% CI (dotted line) is shown; control cells are IFN- γ exposed cells (=0).



to 10ng/cm² for both types of particles, when more than a doubling of the expression was reached compared to IFN- γ alone, and then expression tended to decrease again. Figure 3 shows that UPM did not significantly enhance the IFN-

γ -induced expression of HLA-DR. There was a trend to higher expression at the lower concentrations of particles, but this was not significant because the dispersion in the control values was rather high in these experiments.

Discussion

We have investigated the potential of three different types of pollutant particles (CB, DEP and UPM) to influence the expression of HLA-DR on differentiated THP-1 cells, taken as a model of alveolar macrophages. By themselves, none of the pDocument types were able to induce HLA-DR at the concentrations tested. However, we also assessed the possible 'adjuvant' effect of the particles on a relevant stimulating agent. For this purpose, we used IFN- γ , which, as expected [10], was able to induce HLA-DR expression at 100 U/mL after 48 h of exposure.

We found that CB was able to increase the effect of IFN- γ on HLA-DR expression, from 1 ng/cm² onwards, and that DEP was also able to do so, from 0.1 ng/cm² on. UPM in combination with IFN- γ could not enhance HLA-DR expression. We have no explanation for the latter, but apart from technical factors (larger variation in the control data), this is presumably related to the size and the composition of the particles. Based on the same mass concentration, the CB and DEP samples contain much more particles, since the mass of a 1- μ m particle ($\sim$ UPM sample) equals the mass of 1000 0.1- μ m particles ($\sim$ CB and DEP samples) [14]. Due to the larger specific surface, these smaller particles are able to carry more toxic substances and thus may have a higher capacity to cause effects [14].

Despite large differences among independent experiments, the observed increases in HLA-DR expression found with CB and DEP were consistent and statistically significant, as shown by repeated measures ANOVA. The quantitative variation between experiments may be due to differences in the passage number of the cells, differences in cell cycle status or changes in the differentiation status.

The IFN- γ -related increased induction of HLA-DR on THP-1 cells is consistent with earlier findings [10]. In the study by Portillo et al. [10], IFN- γ was the only cytokine that could significantly induce HLA-DR expression on non-differentiated THP-1 cells, at concentrations (0.1–100 U/mL) and exposure times similar to our conditions.

To our knowledge, the issue of possible pollutant particle-induced HLA-DR expression has not been investigated until now and we have found no articles in the literature addressing this specific topic. There are a number of *in vivo* studies, reviewed by Nel et al. [5], in which the allergy inducing potential of diesel particles was studied by assessing IgE production. Overall, diesel particles or organic extracts thereof are able to enhance IgE production in exposed animals or in the provoked human upper respiratory tract. It is not clear, however, if this is due to a direct effect on B cells or if other cells, such as antigen-presenting cells, are critically involved in this process.

The major novel finding of our study is the potentiating effect of pollutant particles on IFN- γ on the expression of HLA-DR. This observation may reflect *in vitro* the mechanism by which particles exert an 'adjuvant' activity and may partially explain how exposure to particles can enhance allergic sensitization [5]. Although we have exposed the THP-1 cells simultaneously to the particles and IFN- γ , we believe that the particles potentiate

IFN- γ , and not the other way round, because the particles augment the effect of IFN- γ , without causing detectable effects by themselves.

We are also in the process of studying this with primary human alveolar macrophages to see whether pollutant particles switch these cells from being poor accessory cells to becoming more effective antigen-presenting cells.

It is important to realize that the concentrations of CB and DEP used here are considerably lower than in most other *in vitro* studies, in which, for example, cytokine production was measured [4,6]. We have estimated that the concentrations of the particles used in our *in vitro* experiments (0.1 ng/cm²–1 μ g/cm²) correspond to a 48-h exposure to particulate concentrations in air of 5 μ g/m³ to 50 mg/m³, if we consider a total lung surface of approximately 100 m² and an inhaled air volume of approximately 20 m³ per day [15], and if we assume that all inhaled particles reach the alveoli and are retained in the lung. Such extrapolations are, of course, not entirely correct, but they are useful to put our *in vitro* data in some perspective. Since we demonstrated significant effects on HLA-DR expression at particle concentrations as low as 1 ng/cm², we propose, with the necessary caution, that our findings are relevant to current levels of environmental pollution. One could object that we have only found effects if the THP-1 cells had been previously 'primed' by incubation with IFN- γ , but this cytokine is a common and an important player in the pathogenesis of allergic inflammation [8]. Our experiments are therefore likely to apply to situations of pre-existing pulmonary inflammation, such as asthma. In future studies, we will test whether factors other than IFN- γ , e.g. bacterial endotoxins, exert a similar synergy with pollutant particles.

The results obtained in our study show an increased expression of HLA-DR on the cell membrane of differentiated THP-1 cells, however, the functional antigen-presenting capacity of the cells in our *in vitro* culture system was not studied. This could also be the subject of future research.

Interestingly, the effects found on HLA-DR expression exhibited a maximum at 10 ng/cm² and then tended to decrease at higher concentrations. A previous study [12] with the same particles and cells showed no cytotoxicity, assessed by the AlamarBlue™ assay, of the particles at these higher concentrations. However, we did find significant DNA damage at the higher concentrations tested, as assessed using the Comet assay. This DNA damage may be an early sign of toxicity and may be responsible for the observed decrease of HLA-DR expression. On the other hand, it is possible that certain responses only occur at low concentrations or within a relatively narrow window of exposure and they are possibly offset at higher concentrations. This notion is important in the field of risk assessment and environmental pollution where simple dose–response relationships are often assumed to exist.

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References

- 1 Koren HS, Utell MJ. Asthma and the environment. *Environ Health Perspect* 1997; 105:534–7.
- 2 Pope III CA, Thun MJ, Namboodiri MM et al. Particulate air pollution as a predictor of mortality in a prospective study of U. S. adults. *Am J Respir Crit Care Med* 1995; 151:669–74.
- 3 Spurny KR. Chemical mixtures in atmospheric aerosols and their correlation to lung diseases and lung cancer occurrence in the general population. *Toxicol Lett* 1996; 88:271–7.
- 4 Becker S, Soukup JM, Gilmour MI, Devlin RB. Stimulation of human and rat alveolar macrophages by urban air particulates: effects on oxidant radical generation and cytokine production. *Toxicol Appl Pharmacol* 1996; 141:637–48.
- 5 Nel AE, Diaz-Sanchez D, Ng D, Hiura T, Saxon A. Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. *J Allergy Clin Immunol* 1998; 102:539–54.
- 6 Yang HM, Ma JYC, Castranova V, Ma JKH. Effects of diesel exhaust particles on the release of interleukin-1 and tumor necrosis factor- α from rat alveolar macrophages. *Exp Lung Res* 1997; 23:269–84.
- 7 Ormstad H, Johansen BV, Gaarder PI. Airborne house dust particles as allergen carriers. *Clin Exp Allergy* 1998; 28:702–8.
- 8 Déry RE, Bissonnette EY. IFN γ stimulates the release of TNF α and MIP-1 α by alveolar macrophages during allergic reactions. *Am J Respir Cell Mol Biol* 1999; 20:407–12.
- 9 Bühling F, Ittenson A, Kaiser D et al. MRP8/MRP14, CD11b and HLA-DR expression of alveolar macrophages in pneumonia. *Immunol Lett* 2000; 71:185–90.
- 10 Portillo AJ, Turner M, Chantry D, Feldmann M. Effect of cytokines on HLA-DR and II–1 production by a monocytic tumour, THP-1. *Immunology* 1989; 66:170–5.
- 11 Arenzana-Seisdedos F, Mogensen SC, Vuillier F, Fiers W, Virelizier JL. Autocrine secretion of tumor necrosis factor under the influence of interferon- γ amplifies HLA-DR gene induction in human monocytes. *Proc Natl Acad Sci USA* 1988; 85:6087–91.
- 12 Don Porto Carero A, Hoet PHM, Verschaeve L, Schoeters G, Nemery B. Genotoxic effects of carbon black particles, diesel exhaust particles and urban air particulates and their extracts on a human alveolar epithelial cell line (A549) and a human monocytic cell line (THP-1). *Environ Mol Mutagen* 2001; 37:155–63.
- 13 Auwerx J. The human leukemia cell line, THP-1, a multifaceted model for the study of monocyte-macrophage differentiation. *Experientia* 1991; 47:22–31.
- 14 Salvi S, Holgate ST. Mechanisms of particulate matter toxicity; *Clin Exp Allergy* 1999; 29:1187–94.
- 15 Gardner DE, Kennedy GL. Methodology and technology for animal inhalation toxicology studies. In: Gardner DE, Crapp JD, McClellan RO, eds. *Toxicology of the Lung*. New York: Raven Press, 1993:1–2.