COARSE ($PM_{2.5-10}$), FINE ($PM_{2.5}$), AND ULTRAFINE AIR POLLUTION PARTICLES INDUCE/INCREASE IMMUNE COSTIMULATORY RECEPTORS ON HUMAN BLOOD-DERIVED MONOCYTES BUT NOT ON ALVEOLAR MACROPHAGES

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Diesel particles have been shown to possess adjuvant activity and influence the development of allergic sensitization. Also, more heterogeneous mixtures of pollution particles have been shown to affect host defenses and development of immunity in animal models. In the present study it was determined whether freshly collected particulate matter ($PM_{10}$) in the size ranges $2.5–10 \, \mu m$ ($PM_{2.5-10}$, coarse), $0.1–2.5 \, \mu m$ ($PM_{2.5}$, fine), and $\leq 0.1 \, \mu m$ (ultrafine) in diameter affected the development of antigen presenting cells by evaluating the expression of surface receptors involved in T-cell interaction on both human alveolar macrophages (AM) and blood-derived monocytes (Mo). A Mo–AM coculture was exposed to $50 \, \mu g/ml$ of particles and expression of HLA-DR, CD40, CD80, and CD86 on each cell type was assessed by flow cytometry. Mo upregulated the expression of all four receptors in response to each of the particle fractions, while expression was unaffected in AM. The cells were also exposed to two model air pollution particles, diesel dust and volcanic ash, neither of which affected receptor expression. Furthermore, Mo and AM were separately exposed to the three PM size fractions and supernatants assessed for the T-helper ($CD4^+$) lymphocyte chemoattractant interleukin-16 (IL-16). AM, but not Mo, produced IL-16, and this chemoattractant was released only in response to $PM_{2.5-10}$. These data suggest that a wide size range of pollution particles contain materials that may promote antigen presentation by Mo, while the capability to specifically recruit $CD4^+$ lymphocytes is contained in AM stimulated with the coarse PM fraction.

Air pollution particles are associated with asthma exacerbation and emergency room visits due to respiratory disease (Schwartz, 1996; Delfino et al., 1997; Atkinson et al., 1999; Petroeschevsky et al., 2001; Vichit-Vadakan et al., 2001). A key player in these outcomes may be the airway macrophages (AM), which

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phagocytize and remove inhaled particles but also release mediators, which produce airways inflammation and modulation of immunity (Ward, 1997; Monton & Torres, 1998). Monocytes (Mo) are likely to be recruited to the lung following release of chemoattractants by particle-exposed AM (Driscoll et al., 1993; Soukup & Becker, 2001). The fate of these inflammatory cells may be apoptosis, maturation into lung macrophages, or development into more effective antigen-presenting cells (Holian et al., 1998; Sebring & Lehnert, 1992; Randolph et al., 1999; de Boer et al., 2000).

Constitutively or upon stimulation with antigen, Mo and AM may express several surface receptors that interact with ligands on immune lymphocytes, to present foreign antigen or to enhance proliferative responses. These include the antigens B7-1 (CD80) and B7-2 (CD86), which interact with the T-cell receptor costimulatory molecules CD28 and CTLA-4 (Slavik et al., 1999; Salazar-Fontana & Bierer, 2001; Carreno & Collins, 2002) as well as CD40, which interacts with CD40 ligand on T cells (Grewal & Flavell, 1997). The processed foreign antigen is presented in context of histocompatibility antigens, including the HLA-DR molecules (Guagliardi et al., 1990). Increased expression of these receptors often results in more effective presentation of antigen. Exposure to an antigen in the right cytokine environment results in a highly increased capacity to stimulate immune responses (Jonuleit et al., 1996; Holt & Stumbles, 2000).

Pollution particles may contain components that affect development of immune responses and have been shown to have adjuvant activity (Granum, Gaarder & Lorik, 2001; Granum, Gaarder, Groeng et al., 2001; Lambert et al., 1999). This outcome may be the result of increased efficiency of antigen presentation. Although dendritic cells are more effective antigen-presenting cells than mononuclear phagocytes, some antigens can induce increases in CD80 and CD86 receptor expression on Mo, which promote their antigen-presenting capability (Blazevic et al., 2000; Goxe et al., 1998). A study in B7-1 and B7-2 knockout mice found these molecules were determinants in allergic pulmonary inflammation (Mark et al., 2000). Previously, blockade of CD80 and CD86 inhibited production of interleukin (IL)-4 and IL-2 and enhanced interferon (IFN)-gamma production (Mark et al., 1998). In an investigation of asthmatic subjects, both CD80 and CD86 on alveolar macrophages were required to stimulate cytokine production by allergen primed autologous T cells. CD86 but not CD80 was increased following allergen challenge (Balbo et al., 2001).

In the present study we investigated whether pollution particles promote increased expression of receptors involved in antigen presentation on AM and blood-derived Mo. These cells may then stimulate immune responses in the airways and promote allergic sensitization in susceptible individuals. The release of the T-cell chemoattractant IL-16 was also investigated as this factor has been implicated in respiratory diseases such as asthma and COPD (Cruikshank et al., 2000; Conti et al., 2002), possibly recruiting immune T cells to the site of antigen exposure and presentation.
MATERIALS AND METHODS

Collection of Air Pollution Particles

Ambient air particles were collected in a ChemVol model 2400 high-volume cascade impactor (Rupprecht & Patashnick Co., developed by Harvard School of Public Health), approximately 40 ft above ground level at the EPA, Human Studies facility in Chapel Hill, NC. The impactor draws ambient air at a flow rate of 900 L/min through a series of impaction stages with preset slits for collection of particles of various sizes. Coarse and fine-mode pollution particles were deposited on polyurethane foam (McMaster-Carr, Atlanta, GA). The ultrafine particles were collected onto HEPA air filtration filter material (grade 5300; Monandock, Mt. Pocono, PA).

Recovery of Particles

Foam and filters with particles, as well as control supports, were aseptically handled and cut into approximately 1-cm² slices and placed in a 50-ml polypropylene tube with 30 ml sterile water. Particles were then removed from the foam by sonication with a probe (Brinkmann Instruments, Westbury, NY) for 10 min followed by placing the tube for 1 h in a sonicating water bath (SF60, Fisher Scientific, Atlanta, GA), at room temperature. The foam pieces were squeezed with sterile forceps, removed, and then discarded, while suspended particles were centrifuged for 90 min at 1000 × g. The pellet of particles was lyophilized and weighed. The recovery was 70–75% of the collected weight from the coarse particle foams, 50% from the fine particle foams, and approximately 30% from the ultrafine filters. The particles were stored frozen at −80°C at 2 mg/ml in water.

Isolation of Alveolar Macrophages and Peripheral Blood Monocytes

Human alveolar macrophages were obtained by bronchoalveolar lavage from nonsmoking healthy male and female subjects, 20–35 yr of age, by a previously detailed procedure (Ghio et al., 1998), approved by the Committee on the Protection of Human Subjects at University of North Carolina, Chapel Hill, NC. Human peripheral blood mononuclear cells were obtained by density-gradient centrifugation on lymphocyte separation medium (Organon Teknika, West Chester, PA) as per manufacturer’s instructions, followed by (35/51%) density-gradient separation steps with Percoll (Sigma Chemical Co., St. Louis, MO) to separate monocytes from lymphocytes.

BEAS-2B Cultures

The human bronchial epithelial cell line BEAS-2B subclone S6 was maintained in serum-free growth medium (KGM, Clonetics, San Diego, CA). Cells were plated at 1 × 10⁵ cell/well of a 12-well tissue culture plate (Costar, Cambridge, MA) in 1 ml KGM and incubated at 37°C and 5% CO₂ for 72 h when they were used in experiments.
Cocultures

Mo were cultured with and without AM at a 1:1 ratio in 17 x 100 mm round-bottom polypropylene tubes (Co-Star, Cambridge, MA) using RPMI 1640 (Life Tech., Rockville, MD) supplemented with 5% fetal bovine serum (FBS). PM$_{2.5-10}$ at 50µg/ml was added and the cultures were incubated at 37°C/5% CO$_2$ for 1, 2, or 3 d. Cells were removed for cell surface analysis on each of the days. Mo or AM were also preincubated with PM for 2 h at 37°C/5% CO$_2$ before addition to BEAS-2B monolayer cultures. The final culture medium was 3 parts KGM and 1 part RPMI 1640 + 5% FBS. Cocultures were incubated overnight and cells removed for cell surface marker analysis by gentle pipetting, as Mo and AM did not adhere to the monolayers. At least 90% of the phagocytes were recovered as determined by cell count. Furthermore, visual inspection of the BEAS-2B monolayers showed very few residual Mo or AM.

Analysis of Cell Surface Markers

Monoclonal antibodies to cell surface receptors CD40, CD80, CD86, HLA-DR, CD16, and CD14 were obtained as direct conjugates to fluorescein (FITC) or phycoerythrin (PE) (Becton Dickinson, San Jose, CA; Coulter Corp., Hialeah, FL). Cells were reacted with the antibodies for 45 min according to manufacturer’s recommendation, whereafter they were washed and fixed with 1% paraformaldehyde for analysis using a Becton Dickinson FACSORT. Cells reacted with conjugated irrelevant antibodies of the same isotype as the receptor antibodies were used as controls to establish gates for positive analysis.

Cytokine ELISAs

IL-16 kits (enzyme-linked immunosorbent assay, ELISA) were purchased from R&D Systems (Minneapolis, MN), and each assay was run according to the manufacturer’s directions on supernatants obtained from overnight cultures. The lower limit of detectability was 3.13 pg/ml.

Statistical Analysis

Statistically significant differences between unexposed and particle-exposed cells were assessed by Student’s paired t-test (two-tailed). Data obtained comparing cells stimulated with the three particle fractions was analyzed by one-way analysis of variance (ANOVA) with Dunnett’s post hoc test for multigroup comparisons. A p value <.05 was considered significant.

RESULTS

Effects of Particles on Mo and AM Cell Surface Receptors Involved in Antigen Presentation

To better mimic the interaction of cell types in the lung, Mo and AM exposed to PM were cocultured in polypropylene tubes, or Mo were plated
onto monolayers of BEAS-2B, in the presence or absence of AM. In parallel, both cell types were stimulated alone in polypropylene tubes. Particles were toxic to Mo when cultured alone in polypropylene tubes for more than 24 h, while plating on epithelial cells retained their viability. The Mo also remained viable when cocultured with AM (not shown). The Mo on epithelial cells or in coculture with AM showed a similar baseline receptor expression as well as response to particles to be described later. The AM showed no apparent toxicity when exposed to 50 μg/ml particles in polypropylene tubes and expressed a similar level of receptors in cocultures with Mo, on epithelial cells or alone. A summary of experiments (n=9) showing the response to particles in Mo–AM cocultures and Mo exposed on epithelial cells is depicted in Figure 1. The cells were stimulated with 50 μg/ml coarse, fine, or ultrafine PM for 24 h. The three particle fractions all induced increased expression of HLA-DR, CD40, and CD86 on Mo (Figure 1A). There was a significant increase in CD80 expression in the Mo stimulated with coarse PM but not by fine and ultrafine PM. Baseline expression of the receptors in AM was not affected by any of the three size fractions of PM (Figure 1B), neither when cultured alone, on epithelial cells, nor in coculture with Mo. CD16, a low-affinity Fc-receptor on Mo and AM that interacts with CD11b in phagocytosis (Wolf et al., 1988), was downregulated by particles in both Mo and AM.

The development of receptor expression was followed for 3 d in cocultures of Mo and AM (Figure 2). The cells were exposed to 50 μg/ml PM_{2.5-10}. Uptake of PM was effective in both populations, as shown in the shift in side-scatter characteristics in each cell type (not shown). HLA-DR and CD86 peaked at 48 h in Mo, while CD80 expression was further increased at 72 h. No change in receptor expression on AM was seen at any time point. Furthermore, Mo decreased expression of CD14, a receptor recognizing microbial antigens including lipopolysaccharide (LPS), while no such effect was seen in the macrophage population (Figure 3). Mo and AM were also stimulated with diesel particles or with volcanic ash. Receptor expression on neither Mo (Figure 4) nor AM (not shown) was affected by these particles.

Previous studies have shown that both AM and Mo stimulated with PM_{2.5-10} release a variety of cytokines potentially attracting lymphocytes (such as MIP-1α and MIP-1β) and modulating immune responses (such as IL-1, IL-6) (Soukup & Becker, 2001). Here, studies determined whether PM-exposed phagocytes produced IL-16, a chemoattractant specific for CD4+ lymphocytes. AM stimulated with the coarse PM fraction produced IL-16, while particle-stimulated Mo did not (Figure 5). The fine and ultrafine PM did not significantly induce IL-16 in the AM.

**DISCUSSION**

Previous studies have shown that surface receptors and phagocytosis are selectively downmodulated on AM exposed to air pollution particles (Lundborg et al., 2001; Becker & Soukup, 1998; Soukup & Becker, 1999). The receptor
FIGURE 1. Coarse, fine, and ultrafine PM increase on markers of dendritic cell maturation on monocytes but not on alveolar macrophages. Cells were exposed to the various size fractions of PM, all at 50 µg/ml for 24 h, and then analyzed for expression of cell surface markers by flow cytometry. The bars represent the mean ± SEM of nine experiments. Asterisk indicates a significant difference, *p* < .05, between receptor expression on untreated and particle-treated cells as determined by ANOVA and Dunnett’s post hoc test.
most sensitive to exposure is CD11b involved in phagocytosis of opsonized microorganisms, and also recognizing yeast wall glucans and LPS (Thornton et al., 1996; Wright et al., 1989). In the present study, both Mo and AM were exposed to PM and expression of markers involved in antigen presentation investigated.

Evidence suggests that air pollution particles can act as a stimulus that results in elevated expression of immune costimulatory molecules. Previous studies have shown that particles such as diesel and residual oil fly ash promote immunogenicity of antigens, especially in models of allergy and asthma (Granum, Gaarder & Lovik 2001; Granum, Gaarder, Groeng et al., 2001;
Lambert et al., 1999; Hamada et al., 1999). In addition to functioning as adjuvants as shown for diesel and oily fly ash components of PM, the particles themselves may also carry allergens/antigens into the lung (Williams et al., 1995; Knox et al., 1997; Ormstad, 2001). We found that pollution particles collected from the ambient air increased the expression of immune costimulatory receptors on Mo but not on AM. AM are in general considered poor antigen-presenting cells; a reason could be the inability to modulate receptor expression by antigen encounter. In contrast to size-fractionated ambient air PM, neither diesel soot nor volcanic ash affected receptor expression on Mo, suggesting that an antigenic component needs to be present in the particles to affect receptor modulation. Diesel particles co-instilled with allergen have been shown to induce/increase immunoglobulin E (IgE) responses in human nasal passages and animal models (Diaz-Sanchez et al., 1999; Granum, Gaarder & Lovik et al., 2001; Granum, Gaarder, Groeng et al., 2001). However, the adjuvant

FIGURE 3. Downregulation of CD14 on particle-exposed monocytes. Expression of CD14 was investigated in (A) Mo and (B) AM following exposure to coarse PM for 24, 48, and 72 h. The bars represent the mean ± SEM of three experiments with cells from different donors. Asterisk indicates a significant difference, *p < .05, between untreated and particle-treated cells at the same time point of analysis (t-test).
activity of diesel may occur by mechanisms other than direct effects of these particles on the antigen-presenting cells. In fact, Don Porto Carero et al. (2002) showed that diesel and carbon black exacerbated the effect of interferon-gamma–induced HLA-DR expression, suggesting that with the diesel component, immune modulation may occur by altered response to immune cytokines. High expression of HLA-DR and various receptors (CD40, CD80, CD86), interacting with ligands on T cells (T-cell receptor, CD40L, CD28), are hallmarks of effective antigen presenting cells (Salazar-Fontana & Bierer, 2001; Carreno & Collins, 2002). Blood Mo better than AM may serve as antigen-presenting cells in responses to recall antigens, while only dendritic cells are believed to be capable of stimulating naïve T cells (Mohamadzadeh et al., 2001). However, effective antigen presenting cells with dendritic cell phenotype develop in vitro by exposing monocytes to granulocyte-macrophage colony–stimulating factor (GM-CSF) and IL-4 (Chapuis et al., 1997; Gieseler et al., 1998), followed by stimulation with TNFα or LPS (Thurnher et al., 1997; Cochand et al., 1999; Spisek et al., 2001). Mo themselves have been shown to release products capable of inducing cells with a stable DC phenotype when stimulated appropriately (Romani et al., 1996). In an animal model of dendritic cell development from phagocytic monocytes, Randolph et al. (1999) showed that approximately
25% of phagocytic cells can migrate to the lymph node and develop into dendritic cells with high expression of costimulatory molecules. It is conceivable that Mo develop to effective antigen-presenting cells at sites of inflammation where the conditions favor release of mediators such as GM-CSF and IL-4/IL-13, as proposed in asthmatic airways. Therefore it was intriguing to find that Mo exposed to all three size fractions of PM increased expression of receptors involved in antigen presentation and development into dendritic cells, suggesting a mechanism whereby PM may affect asthmatic individuals.

AM but not Mo produced elevated levels of IL-16 when stimulated with PM$_{2.5-10}$. IL-16 is a cytokine specifically attracting CD4$^+$ lymphocytes (Center et al., 1997). In asthmatic airways the presence of CD4 positive lymphocytes has been shown to correlate with presence of IL-16 (Laberge et al., 2000). In vitro, lymphocyte chemoattraction to bronchoalveolar lavage (BAL) of allergen-challenged asthmatics was shown to be blocked by antibodies to IL-16 (Krug et al., 2000). It was interesting that only the coarse particle fraction, and not the fine and ultrafine PM, stimulated significant IL-16 release. In other studies it was found that the coarse fraction contains most of the stimulatory activity resulting in release of immunomodulatory and proinflammatory mediators (Soukup & Becker, 2001), among them MIP-1$\alpha$ and -1$\beta$. It is proposed that following recruitment of Mo to the airways by particle-exposed AM, the inflamed airways may be more responsive to provocation by allergens and
microbial antigens carried on the particles, as the Mo upregulate immune modulatory receptors in response to particles or a combination of particles and antigen. The concomitant recruitment of CD4+ cells may establish conditions for immune activation in the lung parenchyma and this may be a potential mechanism of asthma exacerbation by PM.

REFERENCES


