

Ambient particulate matter directs nonclassic dendritic cell activation and a mixed T_H1/T_H2-like cytokine response by naive CD4⁺ T cells

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Background: Dendritic cells (DCs) translate environmental cues into T-cell activating signals, and are centrally involved in allergic airway inflammation. Ambient particulate matter (APM) is ubiquitous and associated with allergic diseases, but it is unknown whether APM directly activates DCs.

Objective: To study comprehensively the effects of APM on myeloid DC phenotype and function.

Methods: Development of DC was modeled using human CD34⁺ progenitor cells. APM was collected from ambient outdoor air in Baltimore city. We studied the effects of APM on DC activation *in vitro*, compared with LPS.

Results: Ambient particulate matter enhanced DC expression of costimulatory receptors but suppressed the expression of both the endocytosis receptor CD206 and uptake of fluorescein isothiocyanate-conjugated dextran. The expression of the Toll-like pattern-recognition receptors Toll-like receptor 2 and Toll-like receptor 4 was also blunted. APM-exposed DCs secreted less IL-12 and IL-6 but exhibited increased secretion of IL-18 and IL-10 compared with LPS stimulation. A T_H2-like pattern of cytokine production was seen in cocultures of APM-stimulated DCs and alloreactive naive CD4⁺ T cells where the IL-13 to IFN- γ ratio was reversed. This contrasted with the T_H1 polarizing effects of LPS on DCs.

Conclusion: We report for the first time that APM-exposed DCs direct a complex T_H1/T_H2-like pattern of T-cell activation by mechanisms that involve nonclassic activation of DCs.

Clinical implications: Inhaled APM can act directly on DCs as a danger signal to direct a proallergic pattern of innate immune activation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, allergy, dendritic cell, Toll-like receptors, pollutants, cytokines

The increase in the morbidity and mortality associated with asthma is a concern especially in urban inner city populations. Factors that contribute to this are not entirely understood.¹ Ambient air pollution including airborne particulate matter (APM) may contribute to the increased incidence, morbidity, or mortality from asthma in adults and children in urban areas.² Studies support the link between exposures to air pollution and the induction or exacerbation of asthma.³⁻⁶ Increases in APM air pollution levels exacerbate existing airway diseases and are associated with the promotion of allergic airway diseases.^{7,8} Exposing mice to particulate matter (PM) induces asthmatic conditions, airway responsiveness, and inflammation.^{4,9,10}

To initiate differentiation into effector and memory cells, naive T cells require instruction from dendritic cells (DCs) and other antigen-presenting cells.¹¹ Immature DCs respond efficiently to danger signals (eg, endotoxin) that help direct a primary immune response. Danger signals are transduced in part by the Toll-like receptor (TLR) family of pattern recognition receptors, which are expressed on DCs and other cells of the immune system.¹² Occupation of TLR by ligand (eg, LPS binding to TLR4) instructs DCs to mature¹³ and promotes their T-cell activating functions.¹⁴⁻¹⁶ DCs respond to other environmental danger signals, but the effects of ambient PM on DC activation has not been well studied. DCs are located at surface epithelia of the lungs in an immature functional state. Immature DCs are highly effective at taking up exogenous antigen, yet they exhibit low T-cell stimulating ability.¹⁷ Both lung and skin DCs are repopulated from circulating precursors, a process that is enhanced by environmental stimuli.¹⁸

Current thinking is that allergic diseases are immune-mediated and directed by T cells against allergens and microbial byproducts.^{19,20} CD4⁺ T_H2 cells, which secrete IL-4 and IL-13, cause many of the pathological hallmarks of allergic inflammation including IgE synthesis, eosinophilia, and tissue remodeling.²⁰ Although characterized by a T_H2-bias, there is a growing appreciation of an IFN- γ or T_H1 component in asthma and other allergic diseases.²¹ It is unknown how DCs translate allergen

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Abbreviations used

APM: Ambient particulate matter
 CB: Carbon black particle
 DC: Dendritic cell
 FACS: Fluorescence-activated cell sorting
 FITC: Fluorescein isothiocyanate
 PBPC: Peripheral blood progenitor cell
 PM: Particulate matter
 TLR: Toll-like receptor

exposure into signals that initiate T_H2 differentiation, but in the setting of asthma, activated airway DCs may induce T_H2 polarization of allergen-specific T cells.²² We speculated that DCs may provide 1 link between PM exposure and a T_H2 -driven allergic immune response, consistent with the notion that DCs evolved to respond to diverse environmental cues.¹⁷ Here we provide a comprehensive analysis of DC functional activation after interaction with a relevant source of environmental PM.

METHODS**Reagents**

For a detailed description of the cytokines used for DC culture as well as all other reagents, see this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Collection, preparation, and use of Baltimore APM

A detailed description of this material can be found in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Preparation and enrichment of highly pure $CD4^+$ allogeneic and naive $CD4^+$ T cells

The procedures used to enrich for these cells has been previously described with some modifications.¹⁵ This is a standard procedure and is described in greater detail in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Generation, expansion, and stimulation of $CD34^+$ peripheral blood progenitor cells-derived DCs

Dendritic cells were generated from $CD34^+$ enriched peripheral blood progenitor cells (PBPCs) as previously described.²³ Complete details on this procedure can be found in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Phenotypic analysis of cell-surface receptor and TLR expression by flow cytometry

Dendritic cell cultures were washed and resuspended in fluorescence-activated cell sorting (FACS) staining buffer (PBS supplemented with 2.5% vol/vol FBS, 20 mmol/L HEPES buffer pH 7.4 and 0.02% wt/vol sodium azide) and incubated for 5 minutes in 0.5% vol/vol normal human AB serum in PBS to block Fc γ receptors, then stained with an isotype-matched control antibody or test mAb for 45 minutes in the dark at 4°C. A

FACSCaliber flow cytometer (Becton Dickinson, San Diego, Calif) was used for data analysis using Cellquest software (Becton Dickinson). Data were collected as geometric mean fluorescence intensity and percent fluorescent positive cells.

Functional assay of the endocytosis of low-molecular-weight fluorescein isothiocyanate-conjugated dextran by DCs

This assay was conducted as described.¹⁵ DCs were incubated at 37°C and pulsed with fluorescein isothiocyanate (FITC)-dextran (molecular weight 40,000) at set time points to assess endocytosis.¹⁵ Samples were washed with ice-cold FACS buffer and resuspended in 250 μ L of FACS buffer before analysis on a FACSCaliber flow cytometer.

Assay of cytokine secretion by cultures of resting and stimulated DCs

Details of this procedure can be found in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Coculture of allogeneic $CD4^+$ T cells and PM-exposed DCs

Details of this procedure can be found in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Use of wild-type and MyD88 null bone marrow-derived DCs to test for APM bioactivity

These experiments are described in detail in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Biometry and statistical measurements

Data are expressed as means $\pm$ 1 SD or 1 SEM as described. For experiments with $CD34^+$ PBPC-derived DCs, a minimum of $n = 8$ independent experiments were completed as described in the text. Comparisons made between data sets were tested for significance using the Student t test and post hoc correction according to the method of Bonferroni. Statistical significance was set an α value of at least $p < 0.05$ and as indicated in the text.

RESULTS**Modulation of costimulatory molecule expression by APM-stimulated DCs**

We compared different concentrations of PM with LPS at 100 ng/mL as a positive control and carbon black particles (CBs) as a negative control. The expression of CD40 by DCs was enhanced after stimulation with LPS, compared with resting DC ($P < .05$; Fig 1, A). The expression of CD40 was also augmented by APM-stimulated DCs as compared with resting DCs ($P < .05$). The expression of CD80 (Fig 1, B) and CD86 (Fig 1, C) was enhanced on DCs that had been stimulated with either APM or LPS compared with resting DCs (Fig 1, B and C, respectively; $P < .01$). Enhanced expression of CD40, CD80, and CD86 by APM-stimulated DCs is consistent with conventional maturation of activated DC. The expression of these markers were not

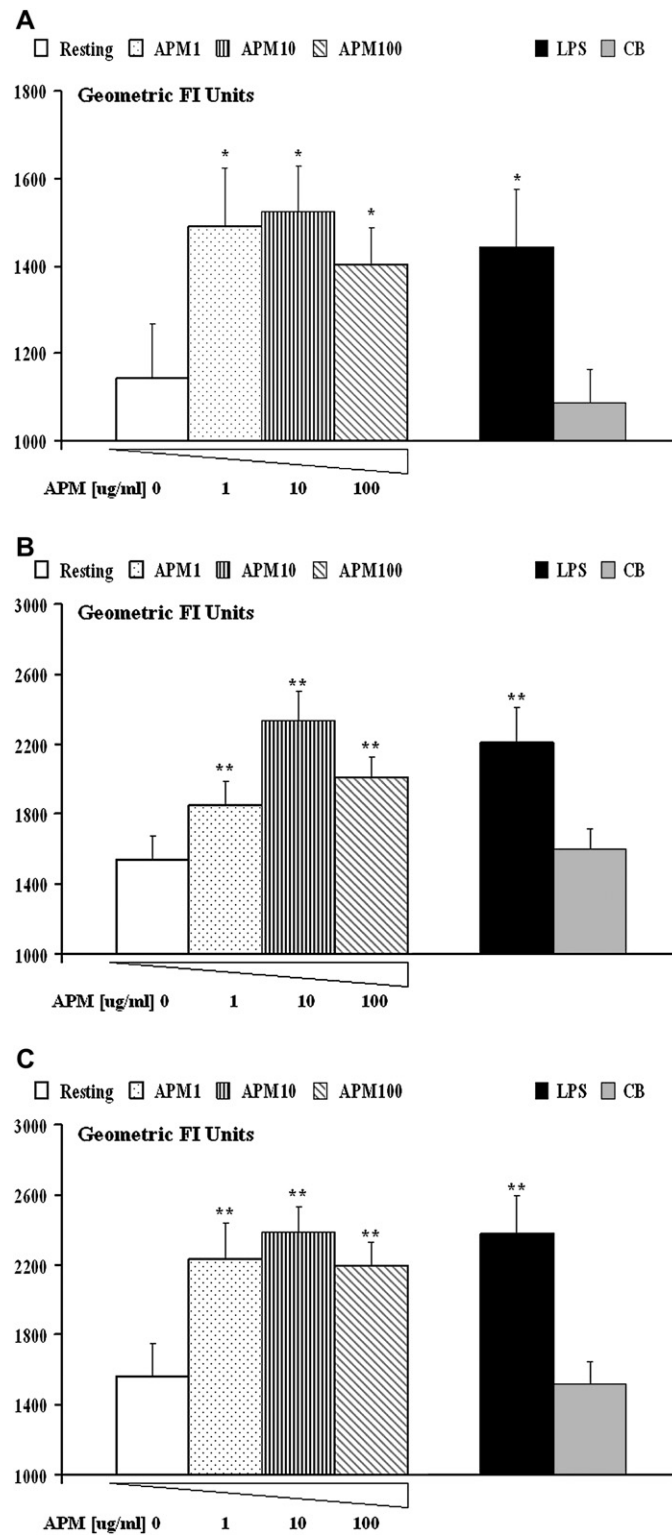


FIG 1. Flow-cytometric quantitation of the costimulatory molecules CD40 (A), CD80 (B), and CD86 (C) on the cell surface of resting and stimulated CD34⁺ PBPC-derived DCs. DCs were stimulated with APM (100 μ g/mL), LPS (100 ng/mL), or CBs (100 μ g/mL) for 48 hours as indicated. Data are recorded as geometric mean fluorescence intensity (FI) units $\pm$ SDs. The levels of significance shown are * P < .05 and ** P < .01 compared with resting DCs.

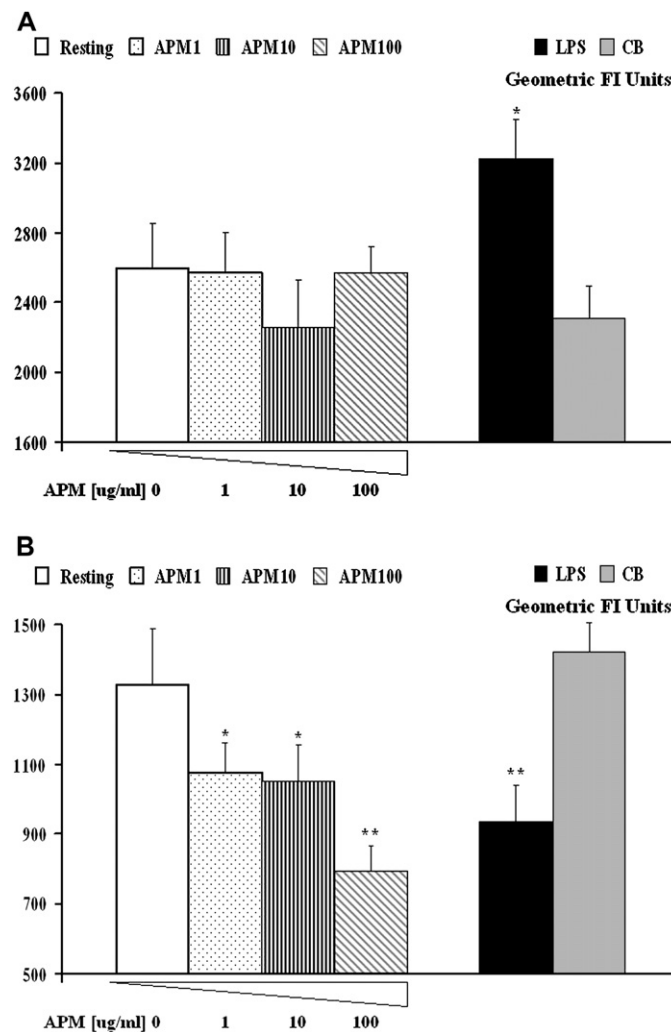


FIG 2. Flow-cytometric quantitation of the cell-surface expression of MHC class II/HLA-DR (**A**) and CD1a (**B**) for resting and stimulated CD34⁺ PBPC-derived DCs as in Fig 1. Data are recorded as geometric mean fluorescence intensity (FI) units $\pm$ SDs. The levels of significance shown are * $P < .05$ and ** $P < .01$ compared with resting DCs.

affected by incubation with CBs, indicating a specific effect of the constituents of APM.

Modulation of cell-surface receptors important in antigen presentation by APM-stimulated DCs

Both major histocompatibility complex (MHC) class II (HLA-DR) and CD1a are important receptors involved in the presentation of antigen to T cells. Although CD1a presents lipid antigen to CD4⁺ or CD8⁺ T cells, HLA-DR is important in presentation of endogenously-processed linear peptides to CD4⁺ T cells. The expression of HLA-DR by DCs was enhanced after stimulation with LPS compared with resting DCs ($P < .05$; Fig 2, A). However, at none of the doses tested did APM alter MHC class II expression (Fig 2, A). By contrast, the expression of CD1a by LPS-stimulated DCs was diminished compared with resting DCs (Fig 2, B; $P < .01$). CD1a

expression was also suppressed in a dose-dependent manner when DCs were stimulated with APM (Fig 2, B; $P < .01$ for APM at 100 $\mu\text{g/ml}$ and $P < .05$ for APM at 10 or 1 $\mu\text{g/ml}$). The downregulation in CD1a expression by DCs is consistent with a normal activation of DCs. CBs were biologically inert in these experiments.

TLR2 and TLR4 are markedly downregulated by APM-stimulated DCs

Toll-like receptors are important in directing the recognition of microbial signals by DCs. Thus we asked the question how the expression of these receptors may be modulated by APM compared with classic TLR signals such as those provided by LPS. The expression of TLR2 by DCs was markedly downregulated after stimulation with LPS ($P < .001$; Fig 3). DCs that were exposed to APM also exhibited a dramatic suppression of TLR2 ($P < .001$). Even at the lowest concentration of APM

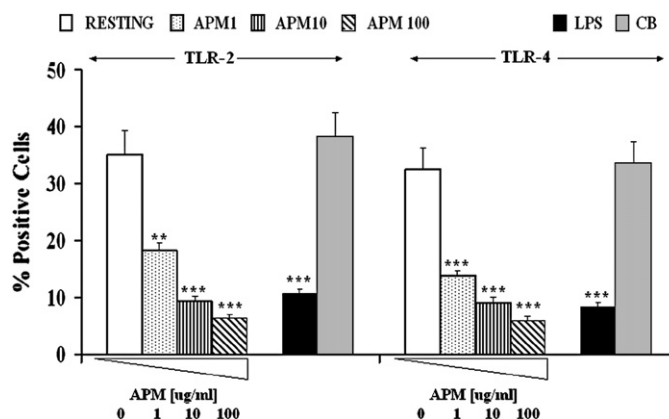


FIG 3. Flow-cytometric quantitation of the cell-surface expression of TLR2 and TLR4 for resting and stimulated DCs as in Fig 1. Data are recorded as geometric mean fluorescence intensity (FI) units $\pm$ SDs. The levels of significance shown are ** $P < .01$ or *** $P < .001$ compared with resting DCs.

tested (1 $\mu\text{g/mL}$), we observed marked suppression in TLR2 expression ($P < .01$). The expression of TLR4 (Fig 3) followed the same pattern of response to LPS and APM as was observed for TLR2.

Alteration in expression of the macrophage mannose receptor CD206 and endocytic function of DCs

One of the most important functions of immature DCs is their ability to take up extracellular particulate antigen by endocytosis. This function tends to be lost in favor of antigen presentation by DCs undergoing maturation. Consistent with this phenomenon, we observed that under resting conditions, immature DCs expressed CD206, the receptor that plays a role in extracellular antigen uptake (Fig 4, A). Concordant with DC maturation, LPS-stimulated DCs displayed suppressed expression of CD206 ($P < .05$). Moreover, stimulation of DCs with the highest concentration of APM induced a more pronounced decrease in the expression of CD206 (Fig 4, A; $P < .01$), an effect that was also observed when DCs were stimulated with lower concentrations of APM (Fig 4, A; $P < .05$).

To determine the importance of depressed levels of CD206 seen on DCs stimulated with either LPS or APM, we modeled the functional ability of DCs to take up extracellular antigen using FITC-dextran (Fig 4, B). As would be expected of immature DCs, resting DCs exhibited a prolific and time-dependent uptake of FITC-dextran (Fig 4, B). By contrast, LPS-matured DCs exhibited suppressed uptake of FITC-dextran. Consistent with the observations made for LPS, stimulation of DCs with APM at 100, 10, or 1 $\mu\text{g/mL}$ provoked a near abolition in the ability of DCs take up FITC-dextran (Fig 4, B), which is characteristic of maturing DCs.

Inflammatory cytokine production by DCs is modulated by Baltimore APM

Cytokine secretion by activated DCs contributes to inflammation and influences T_H cell polarization. Thus,

we next investigated the effects of PM on DC cytokine production (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Induction of inflammatory cytokine production in an allogeneic DC/CD4⁺ T-cell costimulation assay

The hallmark of activated DCs is their ability to activate T cells. Therefore, we next studied how exposure to APM altered the ability of DCs to induce T-cell cytokine production and proliferation using DC:T-cell cocultures. Details of these experiment can be found in the Online Repository (see this article's Fig E3, A and B, at www.jacionline.org).

APM modulates the ability of DCs to stimulate allogeneic CD4⁺ T-cell proliferation appropriately

Consistent with the immature state of unstimulated DCs, we saw only modest stimulation of CD4⁺ T-cell proliferation. Details can be found in the Online Repository (see this article's Fig E3, C, at www.jacionline.org).

Ambient particulate matter promotes a T_H2 -like cytokine response by stimulated naive CD45RA⁺CD4⁺ T cells

To explore further the ability of APM-exposed DCs to influence the T_H -type cytokine production, we titrated APM as well as the equivalent amounts of LPS found in APM against DCs and cocultured them with highly purified naive CD45RA⁺CD4⁺ T cells. We observed a dose-dependent increase in IFN- γ secretion in response to APM (Fig 5, A). We also noted that 50 $\mu\text{g/mL}$ LPS was sufficient to stimulate the production of IFN- γ , an amount of LPS found in 100 $\mu\text{g/mL}$ APM (see this article's Fig E2, A, in the Online Repository at www.jacionline.org). We observed similar responses for IL-5 (Fig 5, B) and IL-13 (Fig 5, C). However, when naive CD4

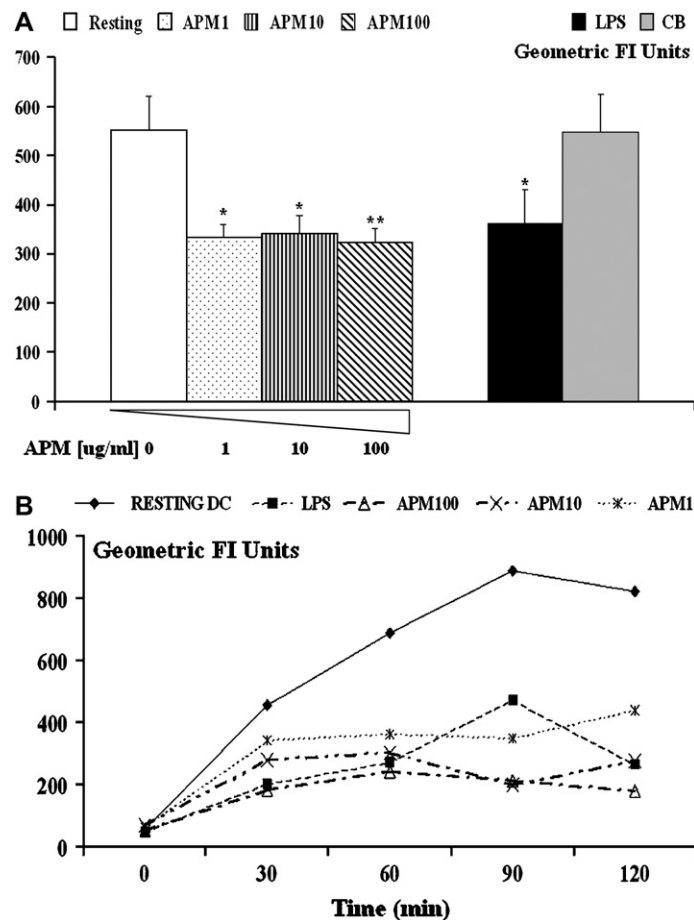


FIG 4. Flow-cytometric quantitation of DC surface expression of CD206 (**A**) and time-dependent uptake of FITC-conjugated dextran (**B**). Data are recorded as geometric mean fluorescence intensity (FI) units $\pm$ SDs. The levels of significance shown are * $P < .05$ and ** $P < .01$ compared with resting DCs. The functional uptake of FITC-dextran by CD34⁺ PBPC-derived DCs was also measured (**B**). Data are expressed as geometric mean FI units.

T cells were cocultured with high-dose (5000 pg/mL) LPS-stimulated DCs, the secretion of IFN- γ exceeded the levels of IL-13 and IL-5 production compared with the manner by which low contaminating doses of LPS found in the APM suspensions stimulated production of these cytokines.

We analyzed the ratio of cytokines produced by naive CD4⁺ T-cell and DC cocultures using the low dose of 50 pg/mL LPS per 100 $\mu\text{g/mL}$ APM, the coordinate dose of APM (100 $\mu\text{g/mL}$), and contrasted these with high-dose LPS (5000 pg/mL) effects (Fig 5, D). These analyses suggested a T_H2-like bias in the cytokine responses of APM-exposed DC/naive CD4⁺ T-cell cocultures. Because IFN- γ was still produced in these allogeneic cocultures, we refer to this as a T_H2-like bias and not strictly consistent with T_H2-polarization per se of the responding naive CD4⁺ T cells.

We also explored the dose-dependent ability of APM-exposed DCs to stimulate naive CD4⁺ T-cell proliferation (Fig 6, A) compared with DCs stimulated with LPS at the equivalent levels found in titrated APM suspensions (Fig 6, B). DCs stimulated naive CD4⁺ T-cell proliferation in an APM dose-dependent manner (Fig 6, A). In these

assays, APM did not inhibit the ability of DCs to stimulate naive T-cell proliferation. We also found that LPS at doses found in the APM suspensions did not alter T-cell proliferation (Fig 6, B). Therefore, the levels of LPS contamination found in APM were not responsible for CD4⁺ T-cell responses after their interactions with APM-exposed DCs. Indeed, a concentration of at least 500 pg/mL was required to stimulate naive CD4⁺ T cells adequately. However, this does not exclude the possibility of other TLR-like ligands from engaging and augmenting DC function.

DISCUSSION

How the innate immune system responds to inhaled environmental pollutants including APM is not well understood. The potential links between APM exposure and allergic immunity also require further study. Here we show that APM exposure induces a unique phenotype of DC activation characterized by enhanced costimulatory molecule expression and induction of a mixed T_H1/

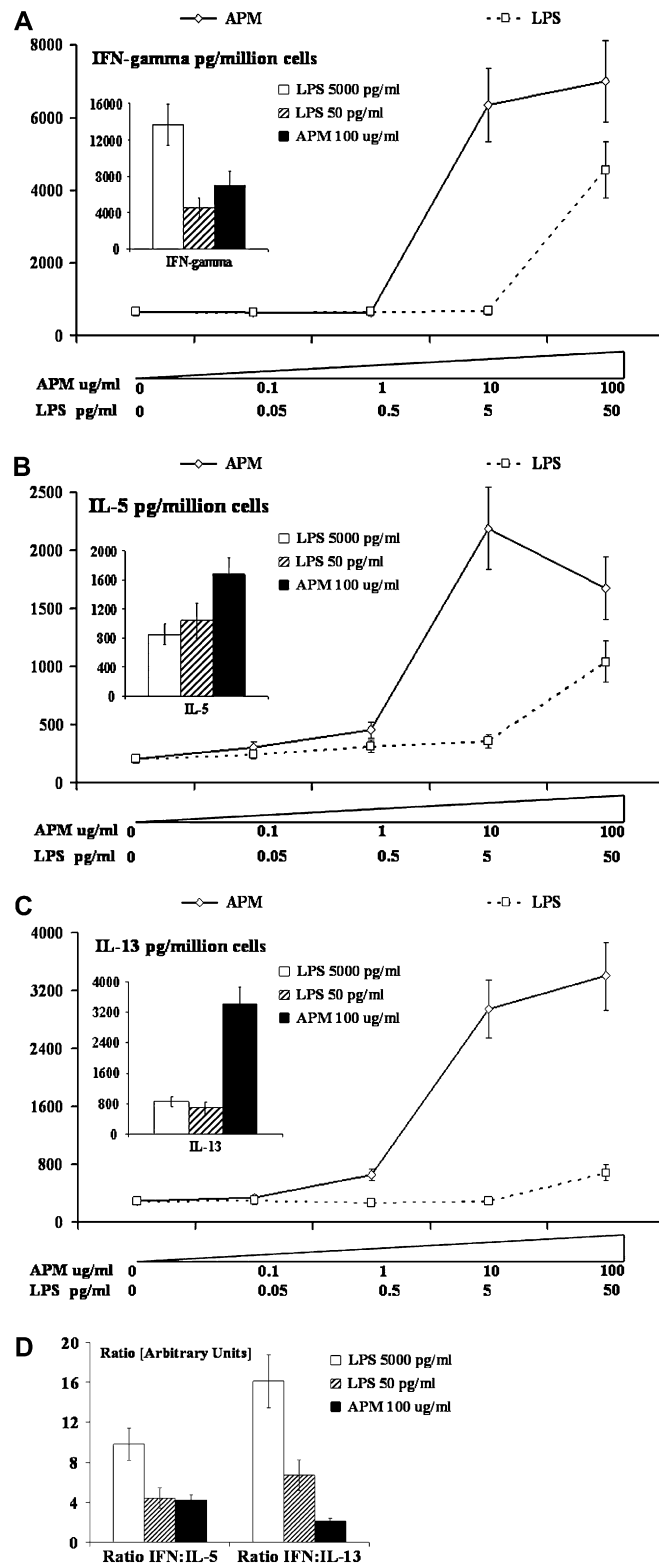


FIG 5. Dose-dependent stimulation of cytokines by naive alloreactive CD4⁺ T cells stimulated by DCs. T-cell-mediated secretion of IFN- γ (A), IL-5 (B), and IL-13 (C) is shown. In addition, we have shown (inset bar charts) cytokine production after prestimulation of DCs with high-dose LPS (5000 pg/mL) compared with APM 100 μ g/mL and the corresponding concentration of LPS at 50 pg/mL found in that dose of APM for comparison. Data are presented as picograms secreted product per million naive CD4⁺ T cells $\pm$ 1 SD of the mean. The ratio-metric analysis of IFN- γ to IL-5 and IFN- γ to IL-13 produced by these cocultures is also shown (D).

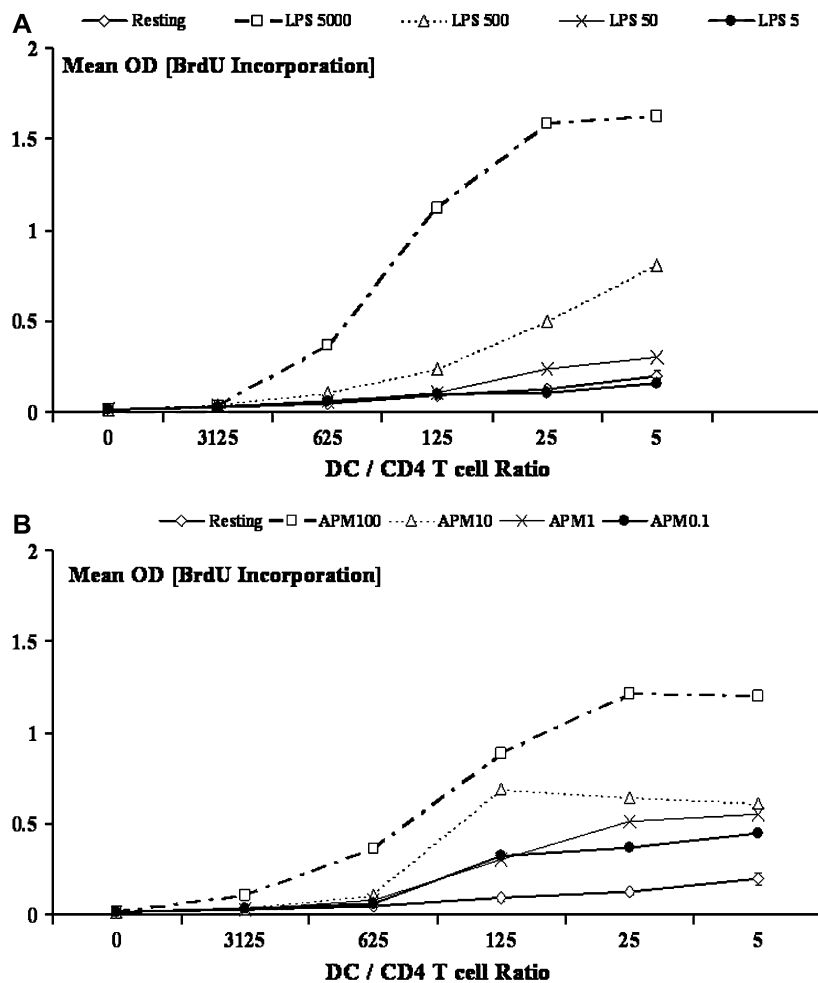


FIG 6. APM and DC-stimulated proliferation of naive CD4⁺ alloreactive T cells. The dose-dependent effects of LPS-stimulated DCs (A) as well as the dose-dependent effects of APM-stimulated DCs on the proliferation of naive CD4⁺ T cells (B) is shown. LPS is titrated against DCs at doses (50 and 5 pg/mL) that were found in the appropriate concentrations of APM used to stimulate DCs, as well as 10-fold and 100-fold higher concentrations of LPS (500 and 5000 pg/mL LPS, respectively) for comparison. Data are recorded as mean OD from a bromodeoxyuridine (BrdU) DNA incorporation cell proliferation ELISA.

T_H2-like profile in coculture with naive T cells. We used well established protocols that are thought to yield immature DCs representative of those at mucosal sites *in vivo*.^{11,14,24} We acknowledge that we have not studied respiratory tract DCs per se, but note that uncertainties about the precise phenotype of these cells and their very low abundance would preclude the kind of detailed analysis reported herein. Because respiratory tract DCs are derived from circulating precursors,^{17,24} we believe our experiments provide a reasonable approximation of DC-PM interactions *in vivo*. Current thinking is that DCs are richly interdigitated throughout the respiratory epithelium, and may directly sample the bronchial lumen.²⁴ Furthermore, PM-exposed epithelia secrete the DC chemoattractant macrophage inflammatory protein 3 α .²⁴ Therefore, it seems likely that DCs will be among the first cells to interact with and respond to inhaled PM.

We have shown that APM adversely affects important parameters of DC activation. The enhanced expression of

the costimulatory molecules CD40, CD80, and CD86 and suppressed expression of CD206 and CD1a by APM-exposed DCs were consistent with a classic pattern of maturation of DCs. However, the unaltered expression of MHC class II by APM-exposed DCs was inconsistent with a mature DC phenotype. These data suggest that stimulation of DCs with APM is functionally distinct from that provided by the TLR4 ligand LPS, and that APM is not a classic “danger” signal. The physiologic consequences of this are uncertain, and it remains to be determined whether APM exposure affects the processing or display of peptides by DCs.

A consistent and unexpected observation from our experiments was the marked decreases in TLR2 and TLR4 expression after exposure of PBPC-derived DCs to APM. Both TLR2 and TLR4 serve critical functions in linking innate and adaptive immunity, and because they recognize microbial products, they initiate immune responses to a broad array of microbial challenges.²⁵⁻²⁷ Thus, the lack of

expression of TLR2 and TLR4 on human monocyte-derived and PBPC-derived DCs after stimulation with APM has several potential consequences. For example, the idea that exposure to LPS early in life redirects the developing immune system away from a T_H2 bias at birth is a key provision of the hygiene hypothesis.²⁸ Our data suggest that APM exposure during childhood may be particularly deleterious because the protective effects of LPS would be attenuated. This revision of the hygiene hypothesis would need to be addressed in separate experiments.

Others have suggested the idea that PM may be immunosuppressive from studies in animal models.²⁹⁻³¹ For examples, exposure of already infected mice to inhaled environmental particulate matter (PM_{10}) worsened both local infection and altered local pulmonary and systemic immunity.³¹ Because exacerbations of asthma or chronic obstructive pulmonary disease are usually driven by airway infections, our data suggest that 1 link between PM exposure and exacerbations of these conditions could be caused by a relative immune suppression.

Endotoxin is ubiquitous in the environment and has the potential to “contaminate” APM.³² However, our data do not support the concept that the effects of APM on DC activation were mediated by contaminating endotoxin. We showed that although APM dose-dependently altered the surface expression of important molecules and the production of immunomodulatory cytokines, the equivalent doses of LPS found in APM had no effect. Additional evidence came from our studies of bone marrow-derived DCs obtained from mice lacking the MyD88 adaptor protein that is crucial for TLR signaling. We tested the dose-dependent effects of APM and LPS on DC activation and found that CD80 and MHC class II were actually upregulated in MyD88 knockout DCs after stimulation with APM. This suggests that an MyD88-independent pathway controlled surface expression of these molecules in response to APM.

Moreover, only the secretion of IL-12p70 and IL-6 was dampened in MyD88 knockout DCs. By contrast, the secretion of TNF remained largely intact in MyD88 knockout DCs, implicating either MyD88-independent production of TNF in response to APM stimulation or non-TLR pattern-recognition receptor dependence of TNF production. Stimulation of MyD88 or wild-type DCs with endotoxin at the levels present in APM suspensions had little effect on cytokine secretion, and a dose of at least 5000 pg/mL purified LPS was required.

Previous studies of the effects of settled house dust or ambient PM^{33,34} have implicated the involvement of TLRs in causing DC activation and lung inflammation, especially TLR2 and TLR4. It seems likely that both TLR and non-TLR dependent danger signals can be presented by APM, and our data support both MyD88-dependent and independent pathways of DC activation. However, complicating this argument is our observation that both TLR2 and TLR4 were downregulated after APM exposure. The mechanism for regulating TLR signaling is poorly understood but may involve ubiquitylation and proteolytic degradation.³⁵ This is an effective strategy for

limiting the scope of a hyperinflammatory immune response to danger and subsequent tissue damage or cell death. This process is thought to involve recruitment of TRIAD3 to the cytoplasmic Toll/interleukin-1R (TIR) domain of a number of TLRs, including TLR1, TLR3, TLR4, TLR5, and TLR9, but not TLR2.³⁵

Myeloid DCs are not by default pro- T_H1 or pro- T_H2 ; rather, it is the nature of the stimulus received by the DCs that defines their ability to support T-cell differentiation programs.^{36,37} However, it is thought that DCs secreting high levels of IL-12 will favor a T_H1 differentiation program by activating signal transducer and activator of transcription 4, which initiates IFN- γ transcription leading to transcription factor T-box expressed in T cells (T-bet) induction and stabilization of the T_H1 phenotype. By contrast, DCs that exhibited suppressed IL-12 production will more likely favor a T_H2 program of differentiation. We found that stimulation of DCs with APM provoked a pattern of cytokine secretion that was distinct from that seen with LPS. Although APM augmented IL-12p40 secretion compared with resting DCs, the levels of IL-12p40 produced were dampened compared with stimulation of DCs with LPS.

Moreover, APM dose-dependently enhanced production of IL-18 by DCs, whereas LPS-stimulated DCs exhibited unaltered production of this cytokine. IL-18 is a unique cytokine that may promote either T_H1 or T_H2 immune responses. In association with IL-12, IL-18 promotes T_H1 -mediated immunity and enhanced production of IFN- γ . However, IL-18 is also capable of assisting a T_H2 -type immune response and allergic inflammation. Enhanced production of IL-18 has been reported in individuals with allergic disorders.^{38,39} We have shown that APM-exposed DCs exhibited suppressed IL-12p40 production and enhanced production of IL-18, conditions favorable for T_H2 responses and allergic inflammation. Further, in cocultures of naive T cells and APM-exposed DCs, we saw decreased production of IFN- γ and elevated secretion of IL-13 and IL-5. These are characteristic of a largely mixed T_H1/T_H2 -type immune response.

We also observed that the levels of IL-6 secreted by APM-stimulated DCs were lower than those levels seen after stimulating DCs with LPS, an effect that was also seen after interactions of APM-exposed DCs with all-or-eactive $CD4^+$ T cells. IL-6 is a multifunctional cytokine that may assist T-cell activation by mechanisms that rely in part on the suppressive function of T-regulatory cells against their effector T-cell counterparts.⁴⁰ Suppressed IL-6 production seen in APM-exposed DCs when either cultured alone or in DC/T-cell cocultures may contribute to quenching of $CD4^+$ T-cell proliferation (see this article's Fig E3, C, in the Online Repository at www.jacionline.org).

We conclude that APM-exposed DCs exhibit a non-classic mode of immunological activation that is functionally distinct from DCs activated by a classic stimulus such as LPS. Moreover, DCs activated by APM direct a complex pattern of cytokine responses both by the DCs themselves and the T cells with which they react. Our data

suggests that a complex T_H1/T_H2 -like pattern of T-cell activation is induced by APM-exposed DCs, particularly when one appreciates the cytokine profile generated from naive $CD4^+$ T cells cocultured with APM-exposed DCs. Future studies of the molecular and cellular mechanisms of action of APM at the level of DC activation and T-cell interactions are warranted.

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