Proinflammatory polarization of monocytes by particulate air pollutants is mediated by induction of trained immunity in pediatric asthma

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Abstract

The impact of exposure to air pollutants, such as fine particulate matter (PM), on the immune system and its consequences on pediatric asthma are not well understood. We investigated whether the ambient levels of fine PM with aerodynamic diameter [?] 2.5 microns (PM $_{2.5}$) are associated with alterations in circulating monocytes in children with or without asthma. Increased exposure to ambient PM $_{2.5}$ was linked to specific monocyte subtypes, particularly in children with asthma. Mechanistically, we hypothesized that innate trained immunity is evoked by a primary exposure to fine PM and accounts for an enhanced inflammatory response after secondary stimulation *in vitro*. We determined that the trained immunity was induced in circulating monocytes by fine particulate pollutants, and it was characterized by upregulation of proinflammatory mediators, such as TNF, IL-6, and IL-8, upon stimulation with house dust mite or LPS. This phenotype was epigenetically controlled by enhanced H3K27ac marks in circulating monocytes. The specific alterations of monocytes after ambient pollution exposure suggest a possible prognostic immune signature for pediatric asthma, and pollution-induced trained immunity may provide a potential therapeutic target for asthmatic children living in areas with increased air pollution.

INTRODUCTION

Air pollution is a global concern with detrimental effects on health and a significant socioeconomic burden worldwide. Approximately 4.2 million people die every year due to exposure to a specific air pollutant, particulate matter with aerodynamic diameters [?] 2.5 micrometer ($PM_{2.5}$) (*1*). Elevation of $PM_{2.5}$is a risk factor associated with prevalence of inflammatory diseases such as asthma (*2, 3*). However, the mechanisms underlying $PM_{2.5}$ effects on asthma prevalence are unclear.

Asthma is the most common chronic disease among children, typically associated with inflammation of the airways and massive structural changes collectively called airway remodeling (*4*). Despite various endotypes of asthma, eosinophilic-rich type 2 immunity is a characteristic feature of the disease in nearly half of the patients. Type 2-low asthma is associated with neutrophilic inflammation and refractoriness to corticosteroids (*5*). However, the role of monocytes in asthma has remained to be fully understood. It has

been previously reported that monocytes accumulate in the airways of children with fatal asthma attacks (*6*). In addition, our previous studies showed that circulating monocytes are enriched in adolescents highly exposed to $PM_{2.5}$ (*7*). Deep phenotyping of circulating monocytes in healthy versus asthmatic children exposed to various levels of $PM_{2.5}$ may uncover a specific endotype of the disease.

Monocytes encompass a heterogeneous population that can be divided into distinct subsets based on expression of immune markers under inflammatory conditions (*8*). We postulated that immune signatures reflecting phenotypic and functional changes of circulating monocytes differ in healthy versus asthmatic children exposed to low versus high levels of $PM_{2.5}$. These immune signatures may provide novel biomarkers to better stratify disease progression and outcomes, particularly in children. Furthermore, they may enable development of treatments for patients living in highly polluted areas, which is an unmet clinical need.

Monocytes undergo innate training, a cellular process by which they are educated to respond differently to secondary stimulation compared to the initial encounter (*9*). This process is tightly regulated by epigenetic reprogramming. Diverse triggers of epigenetic reprogramming for trained immunity include β-Glucan (*10*), viral infections (*11*), catecholamines (*12*), and Bacillus Calmette–Guérin (BCG) vaccine (*13*). It is not known whether particulate pollutants are able to train innate immune cells to elicit a specific hyper-inflammatory response that could eventually predispose individuals to inflammation via epigenetic mechanisms such as histone modifications (*10, 14*). Acetylation of histone 3 at lysine 27 residue (H3K27ac) is a canonical epigenetic mark that distinguishes active enhancers from inactive/poised enhancer elements and contributes to underlying induction of trained immunity in monocytes (*10, 15, 16*).

In this study, we compared circulating monocytes from healthy versus asthmatic children exposed to varying levels of ambient $PM_{2.5}$ to determine differences in expression of monocyte-specific markers as detected by mass cytometry time-of-flight (CyTOF). In addition, we postulated that fine particulate pollutants provoke trained immunity characterized by elevation of pro-inflammatory mediators in circulating monocytes after stimulation with house dust mite serving as an asthma-relevant aeroallergen *in vitro* . Using chromatin immunoprecipitation sequencing (ChIP Seq), we further aimed to understand whether pollutant-induced trained immunity in monocytes is mediated via regulation of histone modifications, particularly H3K27ac.

RESULTS

Frequency of classical versus non-classical circulating monocytes is inversely altered in children highly exposed to $PM_{2.5}$

A subset of fifty children (mean age of 8.02±0.60 years) from a previously characterized air pollution cohort (*17*) were selected based on exposure to the lowest (7.47±1.45 μg/m^3, N=25) versus highest (21.33±4.61 μg/m^3, N=25) ambient levels of $PM_{2.5}$ (P <0.001) (Fig. 1A). Subjects were excluded if they had taken oral immune suppressants within 5 days of the blood draw, had a history of allergen immunotherapy within 1 year of the clinical visit, had a chronic disease other than asthma, or had an acute infection. We utilized CyTOF analysis of a 35-marker panel focused on identifying circulating monocyte changes (Supplementary Table 1). Cell populations other than monocytes were manually excluded (Supplementary Fig. 1 and Fig. 1B). Major monocyte subsets from children exposed to low versus high levels of $PM_{2.5}$ were computationally visualized (Fig. 1C). Environmental exposure to high levels of $PM_{2.5}$ was associated with significantly increased frequency of total (Fig. 1D, P =0.008) and classical ($CD14^+/CD16^-$; Fig. 1E,P =0.004) but not intermediate ($CD14^+/CD16^+$; Fig. 1F,P =0.071) monocytes. Conversely, the frequency of non-classical monocytes ($CD14^{lo/-}/CD16^+$) was significantly diminished in those highly exposed to $PM_{2.5}$ (Fig. 1G, P =0.015). Elevation of classical and reduction of non-classical circulating monocytes in children highly exposed to $PM_{2.5}$ was further confirmed by comparison of absolute number (Supplementary Fig. 2). Monocyte subsets did not differ between healthy and asthmatic children exposed to high levels of $PM_{2.5}$. Therefore, these results suggest that the level of total and major monocyte subsets, classical and non-classical, could reflect the impact of exposure to the fine particulate pollutants. Importantly, monocytes have been found to be more heterogeneous than the major subsets indicate (*18*), leading us to take an unsupervised approach to identify additional subsets for our investigation of the impact of exposure to $PM_{2.5}$ on circulating monocytes

from healthy versus asthmatic children.

Distinct monocyte clusters are associated with exposure to $PM_{2.5}$ *levels in healthy versus asthmatic children*

Using an unsupervised FlowSOM approach (*19*), we identified 8 monocyte clusters that were projected onto a two-dimensional Uniform Manifold Approximation and Projection (UMAP) plot (Fig. 2A-B). Also, we determined the proportion of each monocyte cluster per subject and found considerable interpersonal variation (Supplementary Fig. 3). UMAPs in Supplementary Fig. 4 represent the expression levels of individual markers within the predicted monocyte clusters.

We next addressed whether specific monocyte clusters display an association with ambient exposure to $PM_{2.5}$. In accordance with our initial gating approach (Fig. 1), the frequencies of classical monocytes from clusters 1, 2, and 3 were significantly increased in subjects highly exposed to $PM_{2.5}$. In these subjects, the frequency of cluster 1 [FcεR1a^{-} CCR5^{-}CD163^{dim} CD123^{dim}CLEC4D^{+} CD11b^{+}CD14^{+}], the major monocyte population, as well as cluster 3 [FcεR1a^{hi} CCR5^{+}CD163^{+} CD123^{+}CLEC4D^{-} CD11b^{dim}CD14^{+}] were particularly enhanced in children diagnosed with asthma compared to the healthy control group. The frequency of these monocytes was not associated with the disease status in subjects exposed to low levels of $PM_{2.5}$ (Fig. 2C).

Similarly, frequency of cluster 4 [CD16^{+}CD11c^{hi} CD11b^{dim}CD64^{dim} CD14$^{dim/-}$], i.e., intermediate monocytes, was associated with asthma in subjects highly exposed to $PM_{2.5}$. In contrast, no significant association was observed in those exposed to low levels of $PM_{2.5}$ regardless of disease status (Fig. 2C). Among non-classical monocytes, the frequency of cluster 5 [CD16^{+} CX3CR1^{dim}CD11b^{-} CD14^{-}] was negatively associated with $PM_{2.5}$ exposure. However, decreased frequency of these monocytes in asthmatic individuals was not significant compared to a healthy control group with exposure to high levels of $PM_{2.5}$. Interestingly, a higher frequency of cluster 5 was associated with asthma diagnosis in subjects exposed to low levels of $PM_{2.5}$. Other clusters did not show a significant difference between individuals exposed to low versus high $PM_{2.5}$ or to those with or without asthma (Fig. 2C).

Training of circulating monocytes with particulate matter pollutant induces a robust inflammatory response

We hypothesized that alteration of monocytes associated with exposure to $PM_{2.5}$ is mediated via induction of trained immunity. To test our hypothesis, we enriched circulating monocytes from N=10 donors and then stimulated them for 24h with a well-characterized particulate pollutant, standard reference material (SRM1648a). Next, we rested the monocytes for 6 days and then stimulated them with lipopolysaccharide (LPS) or house dust mite (HDM) aeroallergen for 24h (*20*). Finally, the levels of pro-inflammatory mediators were measured in supernatants (Fig. 3A). Training of monocytes with SRM1648a induced a significant upregulation of TNF, IL-6, and IL-8 after LPS or HDM stimulation (Fig. 3B).

We next asked whether prior exposure to ambient $PM_{2.5}$contributes to trained immunity in monocytes. To address this question, we analyzed the responses, based on exposure to extremely low (N=5) versus extremely high levels of $PM_{2.5}$ (N=5). Monocytes from individuals highly exposed to ambient $PM_{2.5}$displayed increased TNF, IL-6, and IL-8 levels compared to monocytes from individuals exposed to low $PM_{2.5}$ levels after stimulation with LPS or HDM (Fig. 3C). Additionally, monocytes were trained with β-glucan as a positive control and demonstrated a robust induction of trained immunity (N=5 per group, data not shown). Next, we demonstrated that the trained immunity phenotype induced by SRM1648a is retained if the same pollutant is used as the secondary stimulant. Notably, the level of IL-6 did not significantly change upon pollutant re-exposure, suggestive of a specific pattern of trained immunity elicited by a second encounter with pollutants (Fig. 3D).

We further validated pollution-mediated trained immunity by measuring secreted mediators in supernatants from monocyte cultures 7 days after induction of trained immunity using a Luminex assay (N=5 subjects with lowest versus 5 subjects with highest exposure to $PM_{2.5}$). Training of circulating monocytes by the SRM1648a promoted a hyper-inflammatory response upon stimulation with LPS or HDM compared to the control untrained group; specifically, we observed significant upregulation of pro-inflammatory cytokines TNF, IL-1β, IL-6, GM-CSF, and G-CSF (Fig. 4A). In addition, SRM1648a provoked a significant increase in

the levels of chemokines including, IL-8, MIP-1α, MIP-1β, GRO, and IP-10 after LPS or HDM stimulation (Fig. 4B). Chemokine responses were relatively specific, as MCP-1 and MDC levels did not significantly change after training of monocytes with SRM1648a compared to the control group (Fig. 4C). Secretion of MCP-3 was significantly elevated upon pollutant training regardless of the type of stimulation, LPS or HDM (Fig. 4C). Finally, the levels of EGF and FGF-2, were significantly elevated by either LPS or HDM stimulation after pollutant training (Fig. 4C), whereas VEGF level did not change compared to the control group (data not shown). Multivariate profiling of mediators secreted from monocytes upon pollution-induced training further demonstrated the relationships between analytes during re-stimulation (Supplementary Fig. 5). The differences between the analyte levels for LPS and HDM suggest distinct hyper-inflammatory profiles dependent on the type of stimulant. Altogether, we found that a pollution model analogous to $PM_{2.5}$ exposure robustly induces trained immunity in monocytes.

Particulate pollutant-induced trained immunity is mediated by H3K27ac in circulating monocytes

Next, we asked whether pollution-induced trained immunity is specifically mediated by H3K27ac modifications. Epigenomic ChIP Seq profiling of pollutant (SRM-1648a) versus media trained circulating monocytes, freshly purified from a healthy donor, revealed H3K27 acetylation patterns, depending on the training type, throughout the genomic regions that include promoter-transcription start sites (TSS) (Fig. 5A-B). In promoters, operationally defined as regions within 3 kb of a TSS, we identified H3K27ac marks that specifically associate with pollutant exposure, 6 days post training (Fig. 5C). In line with the inflammatory signature (Fig. 3-4), we detected an enhanced H3K27ac in the promoter-TSS of the genes encoding inflammatory mediators such as IL-1β, IL-6, IL-8, and MIP-1α upon pollutant training, arguing that this training drives a pro-inflammatory transcriptional program in monocytes (Fig. 5D). Training with pollutant enhanced H3K27ac marks in the promoter-TSS of *IRF* 8 and *AHR* , which encode essential transcription factors, Interferon Regulatory Factor 8 and Aryl Hydrocarbon Receptor. Furthermore, the strongest H3K27ac signal among transcription factors was detected upstream of *TCF3* , encoding transcription factor 3. Finally, pollution-induced training of monocytes increased H3K27ac in promoter-TSS of *SETD1A* encoding SET Domain Containing 1A, a dedicated H3K4 methyltransferase (Fig. 5D). To understand the biological pathways affected during pollutant-induced trained immunity, we performed a gene ontology (GO) and KEGG pathway analysis using clusterProfiler R package (*21*). As shown in Fig. 5E, H3K27ac was highly enriched in the promoter-TSS of genes involved in inflammatory responses such as chemokine and TNF signaling, consistent with increased secretion of inflammatory mediators such as IL-8, and TNF. Interestingly, other inflammatory pathways relevant to asthma immunopathogenesis such as MAPK signaling, TCR activation, Fcγ receptor-mediated phagocytosis, autophagy, NOD-like receptor signaling were found among the pathways with highest H3K27ac mark specifically activated by particulate pollutants in circulating monocytes. The comprehensive list of genes, peaks, and pathways specific to pollutant-induced training have been summarized in Supplementary Table 2-4, respectively. In addition, untrained monocytes, baseline control group, possess a specific H3K27ac pattern of specific genes and peaks as well as biological pathways, *e.g.* tight junctions, cell adhesion, and insulin secretion as shown in Fig. 5E and Supplementary Table 5-7. Finally, monocytes underwent training with β-glucan, served as positive control, for 6 days (Table 8). Collectively, pollution training leaves H3K27ac scars in specific genome regions that are not affected in the control untrained monocytes.

DISCUSSION

In this study, we demonstrated associations between high ambient exposure to $PM_{2.5}$ and the frequency of specific circulating monocyte subtypes. These associations were enhanced for certain monocyte clusters in asthmatic children who are chronically exposed to high levels of $PM_{2.5}$. Thus, the frequency of monocyte subtypes as determined by deep phenotyping could be considered a novel measure of disease status in highly polluted areas. To model a global real-life air pollution issue, we focused on the total level of $PM_{2.5}$ as averaged from 4 monitoring stations located in the same area in Fresno, CA. Of key importance, no significant associations between circulating monocyte subsets and the levels of ambient pollutants other than $PM_{2.5}$, including PM_{10}, CO, and NO, were observed. For example, see data for PM_{10} in Supplementary Fig. 6.

Our results thus suggest a novel monocyte association specific to $PM_{2.5}$.

Association between episodic exposure to $PM_{2.5}$ and elevation of total circulating monocytes, but not specific subsets, has been previously reported (*22*). Classical monocytes are the most abundant circulating monocytes. Thus, it is not surprising to observe elevation in the level of total monocytes when the classical subset is increased. Also, previous studies report that classical monocytes typically play a pro-inflammatory role, while non-classical monocytes often act as anti-inflammatory cells and they are activated, for instance, in viral infections (*23, 24*). Based on our results, associations between exposure to high levels of $PM_{2.5}$and increased frequency of classical monocytes may favor a pro-inflammatory condition. This notion is further supported by a significant reduction in frequency and number of anti-inflammatory non-classical monocytes in children highly exposed to $PM_{2.5}$. Changes in major monocyte subsets did not show a specific impact on asthma onset in our pediatric population.

Our computational unsupervised clustering method revealed additional heterogeneity, in addition to the canonical classical, intermediate and non-classical monocyte subsets. Among 8 distinct monocyte subtypes, 2 classical clusters (1 and 3) were found to not only be associated with $PM_{2.5}$ levels, but also with asthma diagnosis in children. Maurer *et al* . have previously reported that the expression of FcεRI, the high affinity IgE receptor and a key marker of monocyte cluster 3, is enhanced on circulating monocytes from allergic patients compared to those of non-allergic individuals (*25*). In addition, treatment of allergic asthmatic patients with glucocorticoids lowers IL-4-mediated upregulation of FcεRI on circulating monocytes, suggesting an anti-type 2 mechanism of action (*26*). Interestingly, similar to dendritic cells, aggregation of this receptor on human monocytes has been shown to activate NF-κB signaling (*27*). Finally, relevant to our findings, $PM_{2.5}$induces FcεRI-mediated signaling in bone marrow-derived mast cells, leading to degranulation and cytokine production (*28*). Effects of $PM_{2.5}$ on FcεRI expression on monocytes had not been examined previously; our findings demonstrate the correlation between exposure to high levels of $PM_{2.5}$ with significantly increased frequency of monocytes expressing FcεRI (cluster 3).

Increased frequency of cluster 4 [$CD16^{+}CD11c^{hi}$ $CD11b^{dim}CD64^{dim}$ $CD14^{dim/-}$], a subtype within intermediate monocytes, was associated with asthma status in children chronically exposed to elevated levels of $PM_{2.5}$. This warrants further investigation because, to our knowledge, there is no study investigating the potential contribution of these cells in pollution-mediated pathogenesis of pediatric asthma. It would be tempting to speculate that clusters 1, 2, and 4 might undergo trained immunity upon exposure to high levels of $PM_{2.5}$ through metabolic and epigenetic reprogramming. Finally, among non-classical subtypes, cluster 5 [$CD16^{hi}$ $CD11b^{-}CCR2^{-}$ $CCR5^{-}CX3CR1^{+}$ monocytes] was associated with low levels of $PM_{2.5}$ exposure, particularly in asthmatic children. CX3CR1 is suppressed in circulating non-classical monocytes from severe asthmatic patients (*29*). Signaling by this chemokine receptor preserves lung function in fungal-induced allergic airway inflammation, which is mediated via a novel regulatory mechanism (*30*). Our results suggest an anti-inflammatory role of this specific subtype, reversely linked to ambient $PM_{2.5}$ level. Collectively, our CyTOF data provides a detailed analytical approach that yielded novel monocyte clusters which, if confirmed as biomarkers, may allow improved management of asthma in children living in areas with elevated levels of $PM_{2.5}$.

Analysis of inflammatory mediators after induction of trained immunity by particulate pollutants indicates that the pattern is a relatively specific innate immunity response. The levels of certain mediators, such as MCP-1 and MDC, did not significantly change upon pollution-induced trained immunity. Interestingly, the type of secondary stimulation, HDM versus LPS, was a determining factor in the case of EGF secretion after primary training with the particulate pollutant. To our knowledge, the impact of trained immunity has not been tested after re-exposure to the primary stimulant. In our model, a trained immunity phenotype was sustained upon pollutant re-exposure for IL-8 and TNF, but not IL-6. These results imply that the nature of secondary stimulus affects the final functional outcome.

A major strength of our study was that we specifically linked innate training of monocytes with a fine particulate pollutant to the activation of certain inflammatory pathways. We report not only increased secretion of pro-inflammatory mediators, but also differential H3K27 acetylation peaks in proximity to the

transcription start sites of the genes encoding these mediators. Our evidence supports the dynamic role of H3K27ac, a mark of active transcription, in trained immunity at both promoter and enhancer sites (*10*) and its differential landscape in subjects highly exposed to $PM_{2.5}$, particularly for genes involved in immune cell activation (*14*). Besides cytokines and chemokines, pollutant-induced H3K27ac marks at the transcription factors *IRF* 8 and *AHR* reflect the impact of air pollution on monocyte development and xenobiotic detoxification via a previously unknown trained immunity mechanism (*31, 32*). In line with H3K27ac mark at *AHR* , the pathway analysis revealed that xenobiotic metabolism is also highly enriched, suggesting a novel immuno-detoxification pathway, as previously reported in the liver (*33*) and kidney (*34*). Furthermore, identification of the strongest H3K27Ac signal among transcription factors upstream of*TCF3* was unexpected as this transcription factor is known as a master regulator in B cell fate (*35*). The H3K27ac mark at promoter-TSS region of *SETD1A* , a dedicated H3K4 methyltransferase, suggests an active methylation axis under H3K27 acetylation (*36*). Identification of MAPK signaling pathway with a high H3K27ac mark indicates pollutant-induced training could be mediated by metabolic reprogramming in monocytes (*37*).

Lastly, our analysis of biological pathways on ChIP Seq data highlighted the inflammatory pathways relevant to asthma such as chemokines, TNF, and MAPK signaling, as well as autophagy (*38*) and axon guidance (*39, 40*) which were epigenetically activated upon pollution training in monocytes. In addition, other pathways that contribute to pathogenesis of cardiovascular, infectious, metabolic diseases, and cancers were found to be activated upon induction of innate trained immunity by air pollution in monocytes. Although these pathways are beyond the focus of current study, these results suggest the importance of pollution-induced training of monocytes as a potential risk factor underlying several diseases. Finally, similar to β-glucan-trained control group, changes in cell culture media from pollutant-trained monocytes support a plausible metabolic reprograming towards glycolysis to be measured the lactate levels in the future.

Our study had some limitations and challenges to be considered for interpretation of results and development of future directions. First, $PM_{2.5}$ contains several organic and inorganic constituents, making it difficult to dissect which compound(s) specifically induce phenotypic and functional changes in circulating monocytes. Variations in chemical composition and the source of ambient $PM_{2.5}$ should be considered when designing sample collection strategies and monitoring environmental pollution levels across different populations or across monitoring seasons for the same population. Another limitation is that our study was cross-sectional. Continuous exposure to high levels of $PM_{2.5}$ over time might be associated with disease progression or exacerbation. Therefore, monitoring of monocytes in blood samples longitudinally collected from the same subjects could determine the pattern of alterations in monocyte subtypes as a potential biomarker of asthma status. Also, sample collection was prioritized based on pollution exposure levels and the asthma diagnosis was a secondary co-morbidity. Therefore, including several samples from children diagnosed with asthma exposed to low versus high levels of $PM_{2.5}$ and their corresponding healthy controls was not possible. In our trained immunity *in vitro* studies, we selected the subjects with lowest versus highest levels of exposure to $PM_{2.5}$ regardless of asthma status due to small sample size. Our hypothesis to understand the epigenetic mechanism underlying trained immunity by pollutants was centered on H3K27ac. However, various histone marks might be modified, and these could be comprehensively deciphered using the mass cytometry technology, EpiTOF (*37, 41*). Additionally, treatment of monocytes with specific pharmacologic inhibitors against H3K27ac in future studies would be important to corroborate our proposed mechanism.

Uncovering the full epigenetic mechanism is a high priority for future studies. Targeting epigenetic alterations of monocytes induced by pollution exposure may provide novel therapeutic strategies for individuals living in areas with increased ambient levels of particulate pollutants.

METHODS

Detailed experimental procedure has been provided in the Supplement.

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Author Contributions: H.M. designed and performed experiments, analyzed the data and prepared the manuscript. M.P. managed the pollution cohort samples and contributed to design the experiments and to prepare the manuscript. D.D., X.Z., and E.S. assisted in performing CyTOF experiments and data acquisition. S.A. and Z.H., and X.Z. contributed to CyTOF data analysis. A.K.W., M.S., and M.P.S. contributed to ChIP Seq experiments. A.F. and A.K. performed ChIP-Seq data analysis. A.K. also contributed to statistical analysis througout the manuscript. G.R.A.M. and P.S. assisted in Luminex assay. P.S. and E.D.M. contributed to the conceptualization of pollution-induced trained immunity and provided expertise in relevant experiments. K.C.N. supervised the study. All authors reviewed and edited the final manuscript.

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List of supplementary materials

Supplementary Fig. 1. Gating strategy to identify circulating monocytes out of total PBMC based on surface expression of inflammatory markers (N=25 per group). Related to Fig. 1 and 2.

Supplementary Fig. 2. Comparison of circulating monocyte subsets absolute numbers between children exposed to low versus high levels of $PM_{2.5}$. Related to Fig. 1.

Supplementary Fig. 3. Variation in frequency of monocyte clusters per subject. Related to Fig. 1 and 2.

Supplementary Fig. 4. Expression of individual monocyte markers in each cluster per cell on UMAP. Related to Fig. 2.

Supplementary Fig. 5. Multivariate analysis of Luminex data using Early Bird Cytoanalytics. Related to Fig. 4.

Supplementary Fig. 6. Association between circulating monocytes and ambient PM_{10} levels in children. Related to Fig. 1.

Supplementary Table 1) Panel of 35 markers labeled with heavy metal isotopes to characterize circulating monocytes in subjects exposed to $PM_{2.5}$ using mass cytometry CyTOF.

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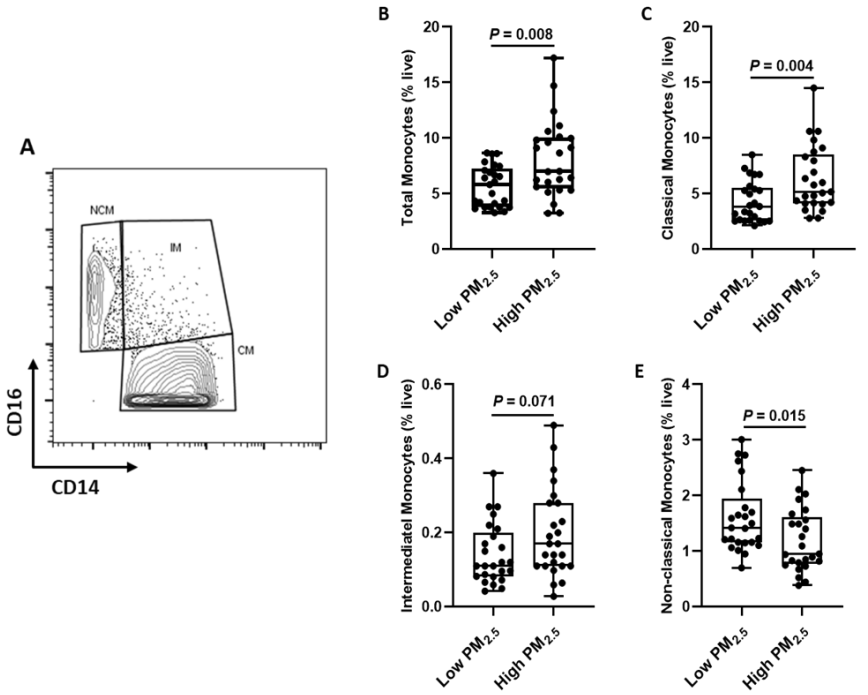
Figure 1

Age (Months)	Sex (M/F)	BMI percentile	Average $PM_{2.5}$ one-week preceding sampling (mg/m^3)	Asthma status (Physician diagnosis)	Secondhand smoke exposure	Ethnicity	Previous oral steroid treatment	Other atopies
96.34 ±7.20	M: 25 F: 25	73±24.6	Low: 7.47±1.45 High: 21.33±4.61	Yes: 20 (10 low vs 10 high $PM_{2.5}$) No: 30 (15 low vs 15 high $PM_{2.5}$)	Yes: 9 No: 41	Hispanic: 36 African- American: 9 White: 3 Others: 2	Yes: 7 No: 13 (asthmatic subjects)	Yes: 13 (5 eczema + 4 hay fever + 4 medication) No: 37



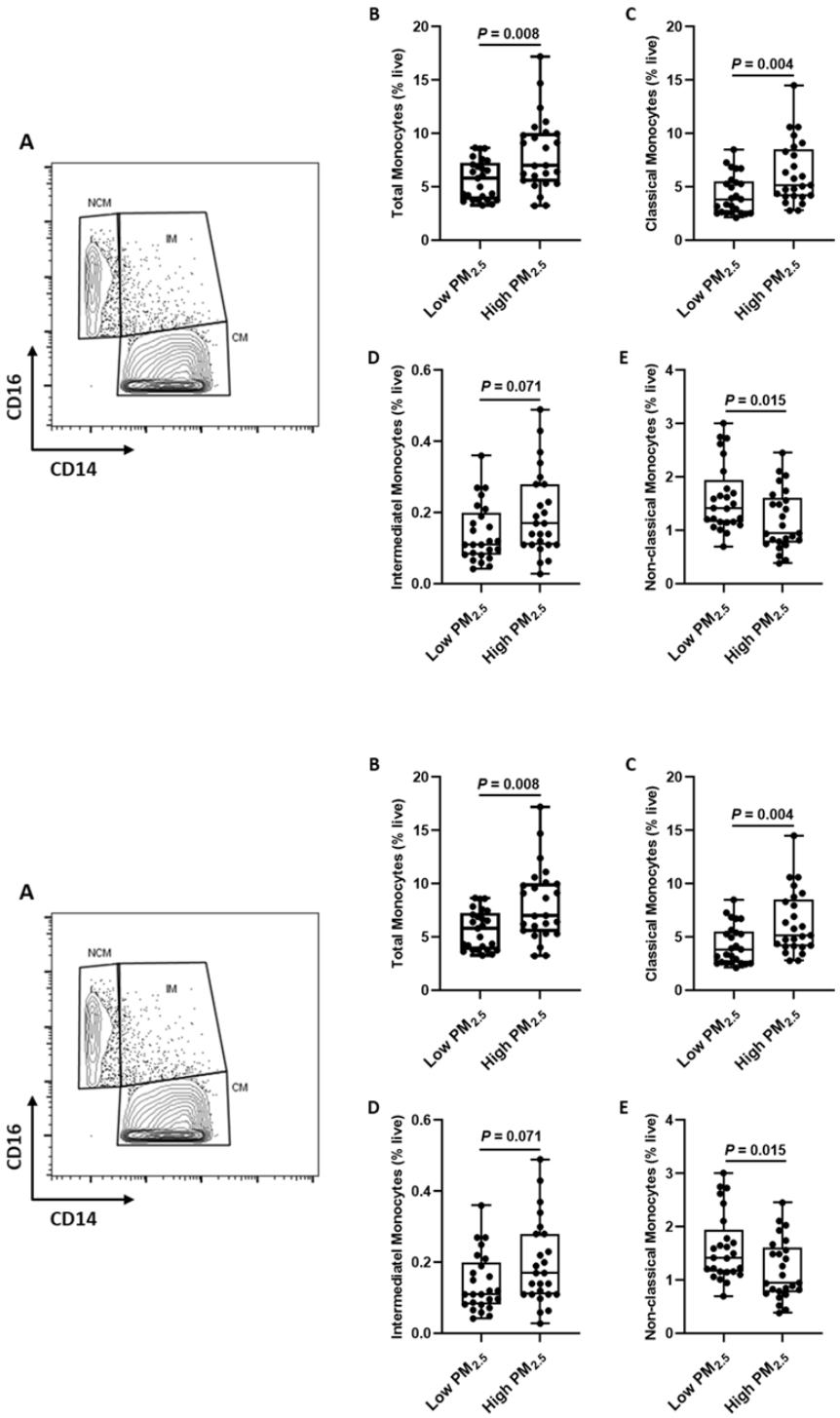


Fig. 1. *Monocyte subsets are associated with ambient exposure to $PM_{2.5}$ levels.* Demographics and clinical characteristics of individuals enrolled in the study. Subjects were divided into two groups according to

ambient levels of $PM_{2.5}$ (A). Major monocyte markers CD14 and CD16 were utilized to characterize classical, intermediate, and non-classical monocytes after gating out other inflammatory cell types (B). Diffusion maps were generated using cytofkit package to visualize the relationship between major monocyte subsets (C). The frequency of total (D), classical (E), intermediate (F), and non-classical (G) monocytes were compared between children exposed to low versus high levels of $PM_{2.5}$. The statistical significance was computed using a non-parametric Wilcoxon sum test. CM: Classical Monocyte; IM: Intermediate Monocyte; NCM: Non-classical Monocyte

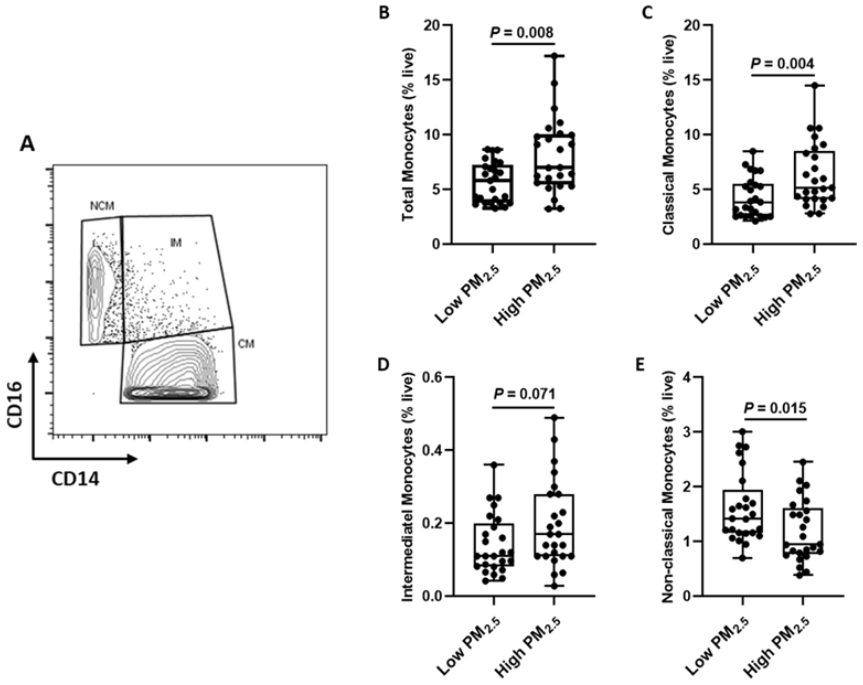
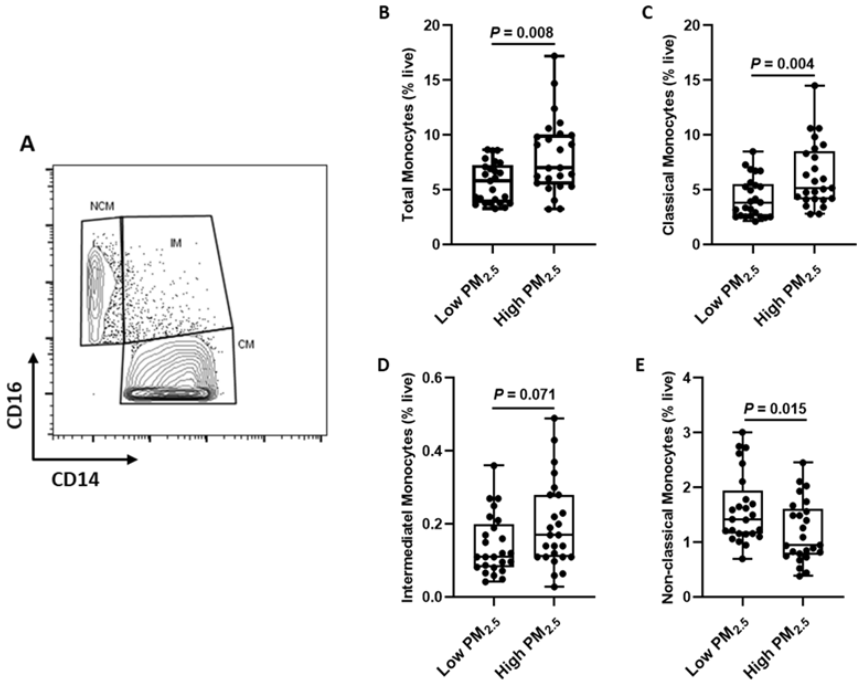
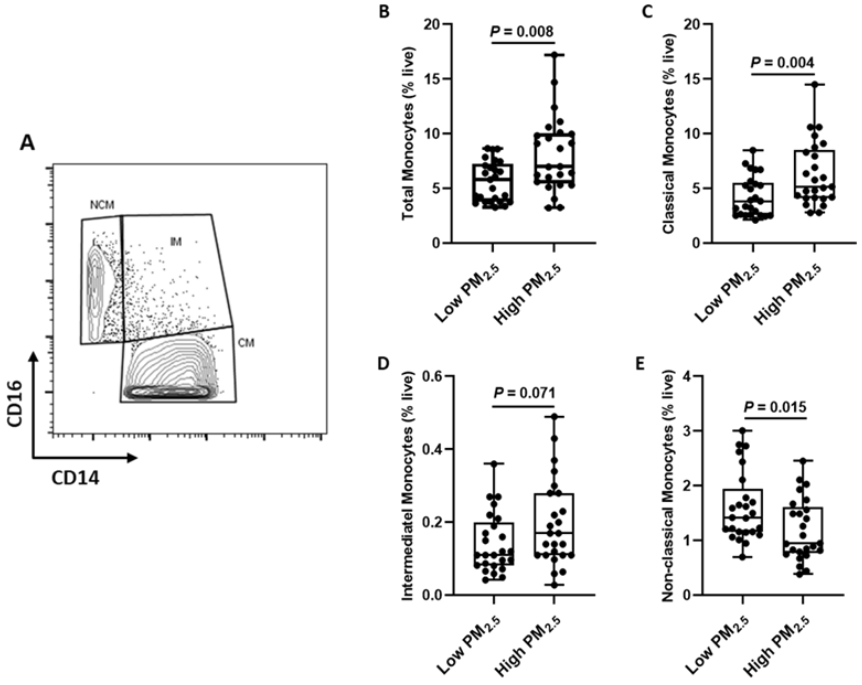
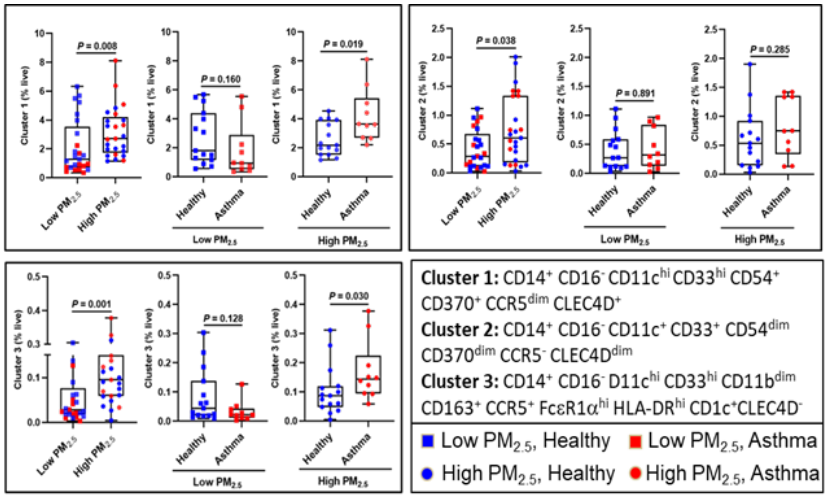
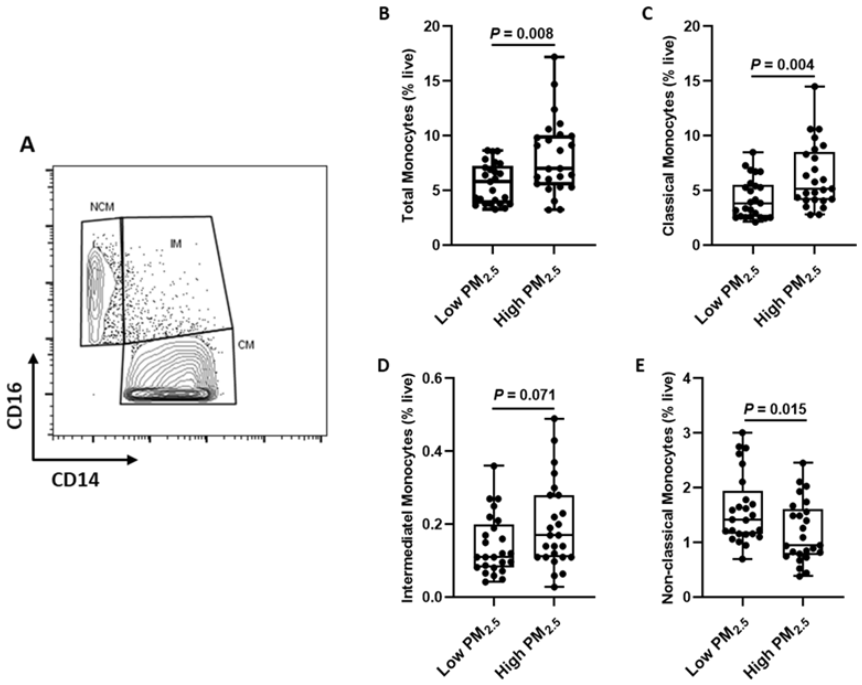


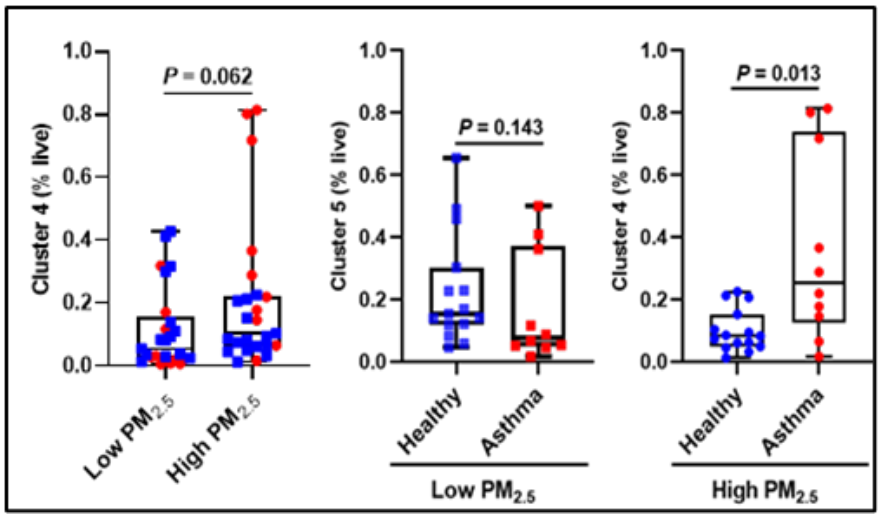
Figure 2

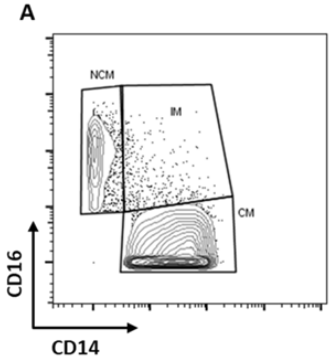


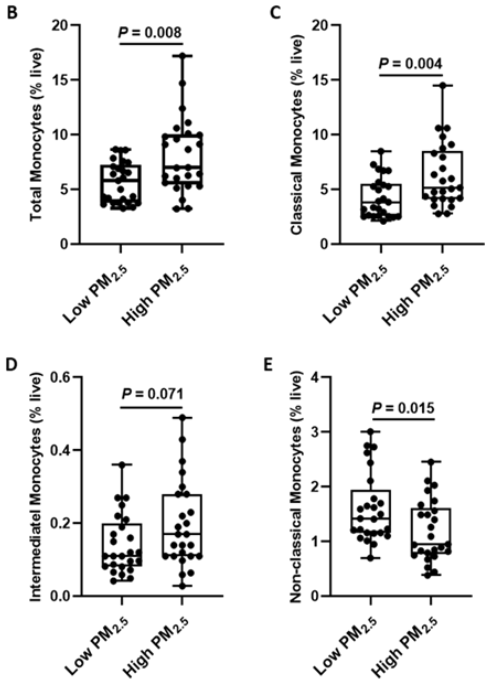












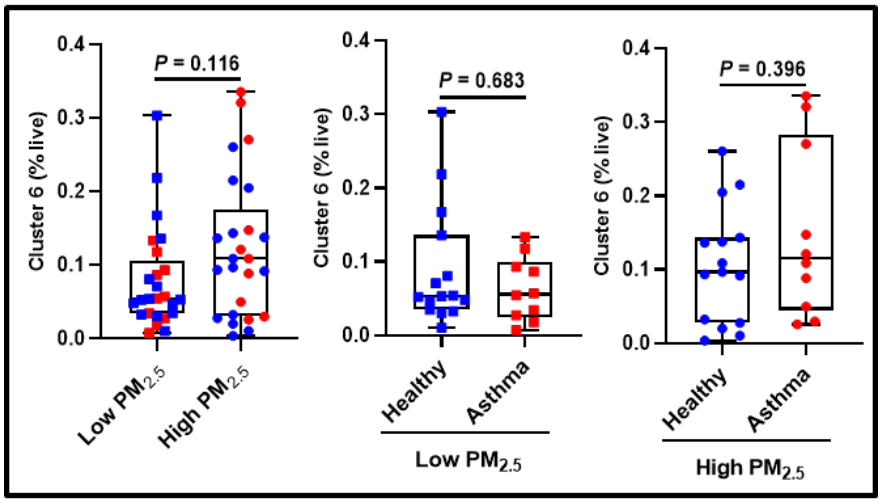
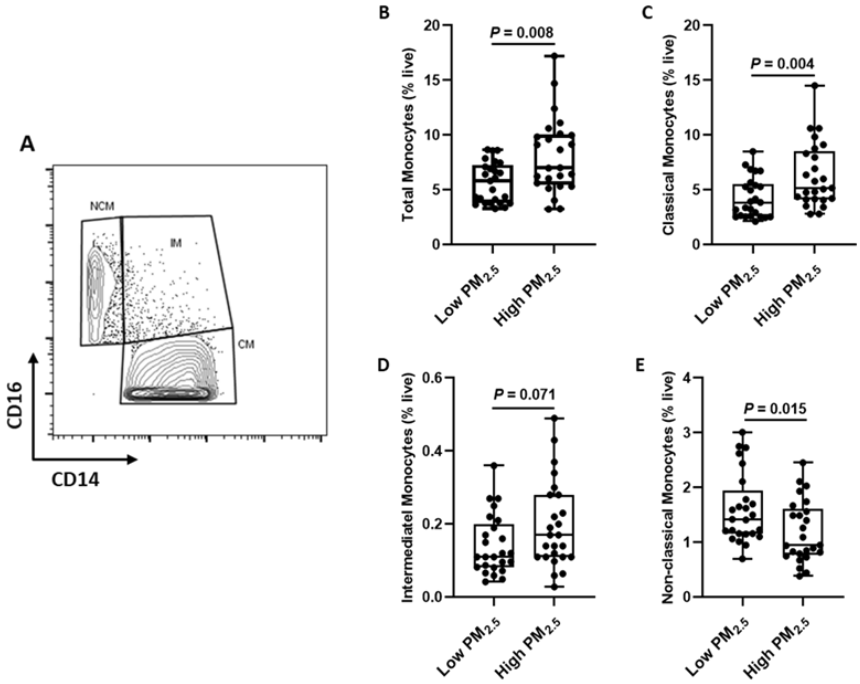
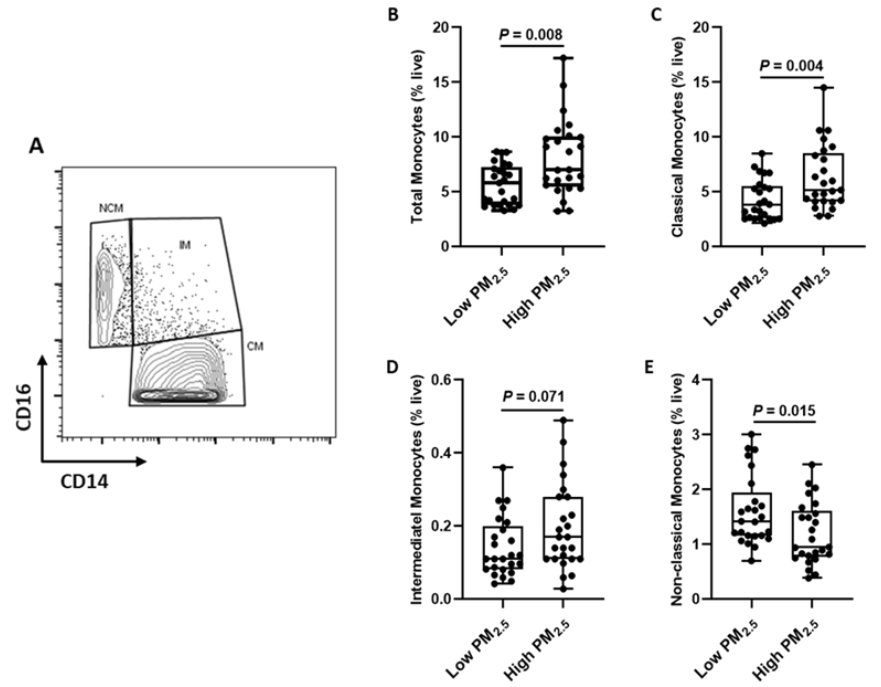
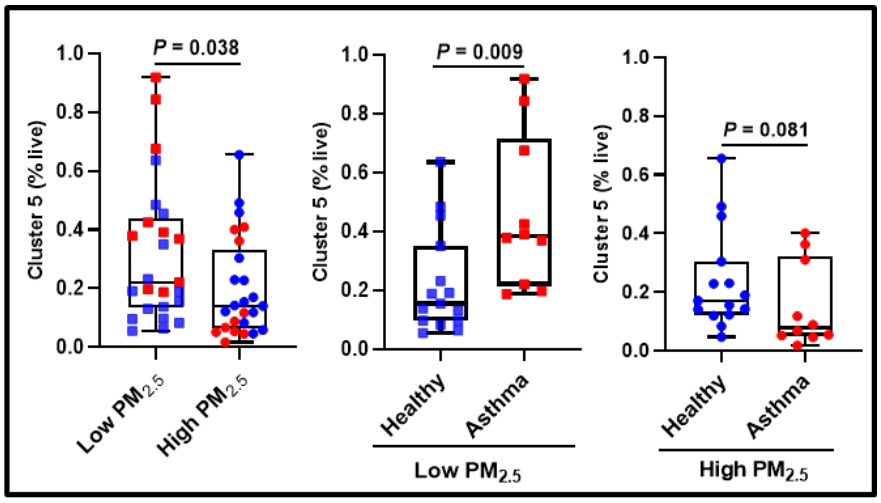


***Fig. 2C** continued*







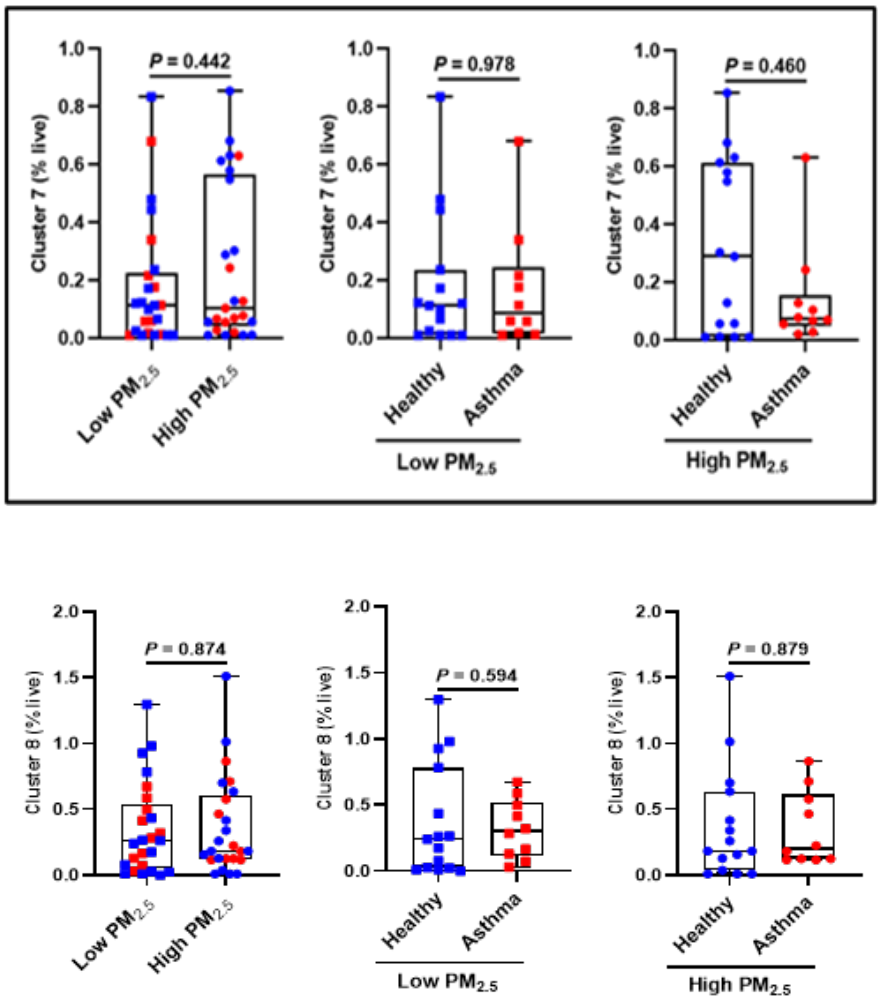


Fig. 2. *Specific monocyte clusters are associated with ambient exposure to $PM_{2.5}$ levels in healthy versus asthmatic subjects.* Computational analysis was performed on mass cytometry data from PBMCs from 6-8 years old subjects exposed to low versus high $PM_{2.5}$ (N=25 including 15 healthy and 10 asthmatic subjects per group). Eight unsupervised distinct clusters were identified using FlowSOM based on expression of phenotypic and functional monocyte markers. Heatmap representing expression intensity of each marker and the frequency of monocyte clusters (A). Data visualization using Uniform Manifold Approximation and Projection (UMAP) for dimension reduction demonstrated the neighborhood pattern of monocyte clusters. The colors in the UMAP correspond to the colors of the cluster IDs, as indicated in the heatmap. (B). Non-parametric statistical analysis identified monocyte signatures significantly associated with exposure to $PM_{2.5}$ in children diagnosed with or without asthma (C). *P* values, computed using non-parametric Wilcoxon-rank sum test, indicate significance of difference in frequency of circulating monocyte clusters from children exposed to low and high levels of $PM_{2.5}$.

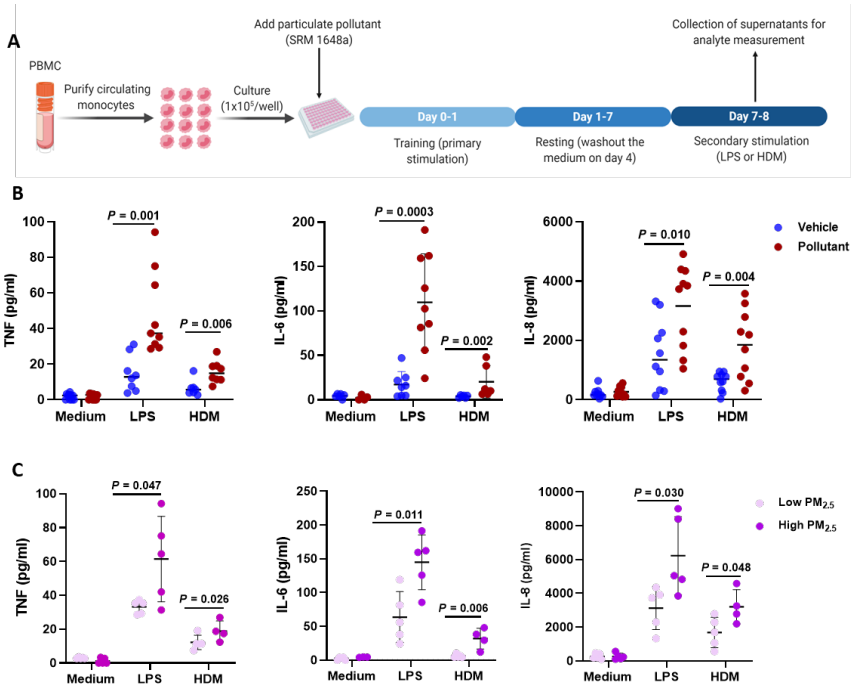


Figure 3

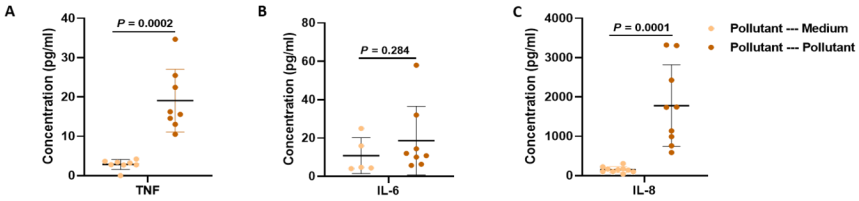


Fig. 3. *In vitro exposure to a particulate pollutant induces trained immunity in human circulating monocytes.*Schematic representation of a standard protocol for induction of trained immunity in monocytes (A). The levels of TNF, IL-6, and IL-8 were compared in supernatants collected from monocytes under training with SRM 1648a pollutant (red), or vehicle (blue) after stimulation with medium, LPS, or HDM. Training of monocytes with SRM1648a pollutant induced a robust increase in LPS or HDM-mediated elevation of TNF, IL-6, and IL-8 levels. (N=10 subjects including 7 healthy and 3 asthmatic children per group) (B). The role of environmental exposure to $PM_{2.5}$ on induction of trained immunity was studied by comparing these mediators in subjects exposed to low (pink) versus high (purple) levels of ambient $PM_{2.5}$ (N=5 subjects including 4 healthy and 1 asthmatic children per group) (C). The impact of re-exposure to pollutant on trained immunity was determined by measuring the levels of TNF, IL-6, and IL-8 in supernatants (D). The graphs represent a single set of measurements from one of 2 independent cytokine experiments. Statistical significance was determined using non-parametric Wilcoxon sum test.

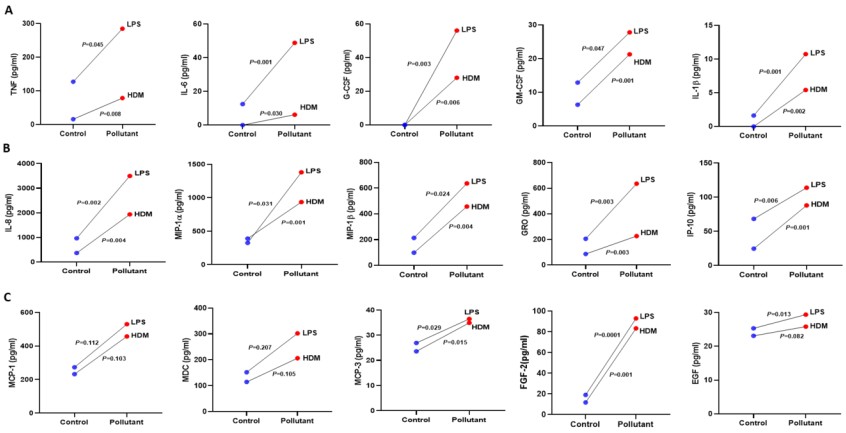


Figure 4

Fig. 4. *Pollution-induced trained immunity is characterized by enhanced secretion of inflammatory mediators in monocytes.* Impact of *in vitro* overnight exposure to the particulate pollutant SRM1648a in monocytes was assessed 6 days later by measuring the levels of pro-inflammatory cytokines including TNF, IL-6, G-CSF, GM-CSF, and IL-1β upon stimulation with LPS or HDM (A). The levels of chemokines, IL-8, MIP-1α, MIP-1β, GRO, and IP-10 were also measured in monocytes after training with SRM1648a pollutant and then LPS or HDM stimulation (B). Training with the SRM1648a pollutant did not induce MCP-1 and MDC secretion; whereas MCP-3 level was increased in monocytes stimulated with HDM but not LPS (C). Release of FGF-2 and EGF was higher in supernatants from monocytes trained with the SRM1648a pollutant compared to untrained control group after LPS or HDM stimulation (C). The data were obtained from 2 independent Luminex experiments in which statistical significance was determined using non-parametric Wilcoxon sum test. Blue dots represent the average values of Luminex data form monocyte culture supernatants obtained from 5 donors without training with SRM1648a pollutant (control group). Red dots represent the average values of Luminex data form monocyte culture supernatants obtained from the same 5 donors as of control group but after training with SRM1648a. EGF: Epidermal Growth Factor; FGF 2: Fibroblast Growth Factor 2; G-CSF: Granulocyte Colony Stimulating Factor; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; GRO-α: Growth-Related Oncogene Alpha; IP10: Interferon gamma-induced Protein 10; MCP: Monocyte-Chemotactic Protein; MDC: Macrophage-Derived Chemokine; MIP: Macrophage Inflammatory Protein.

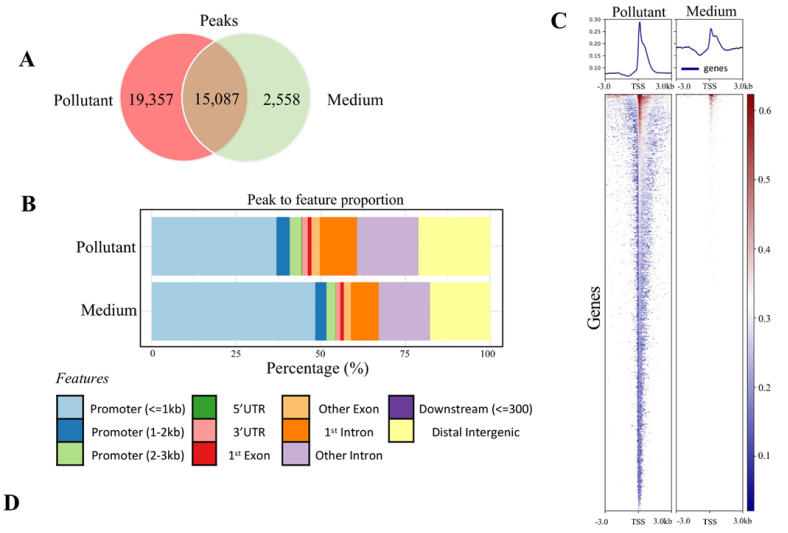
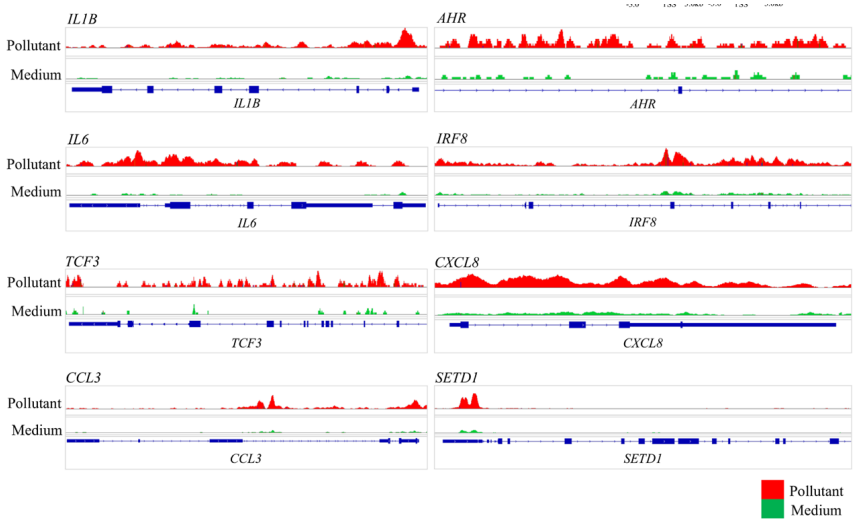


Figure 5



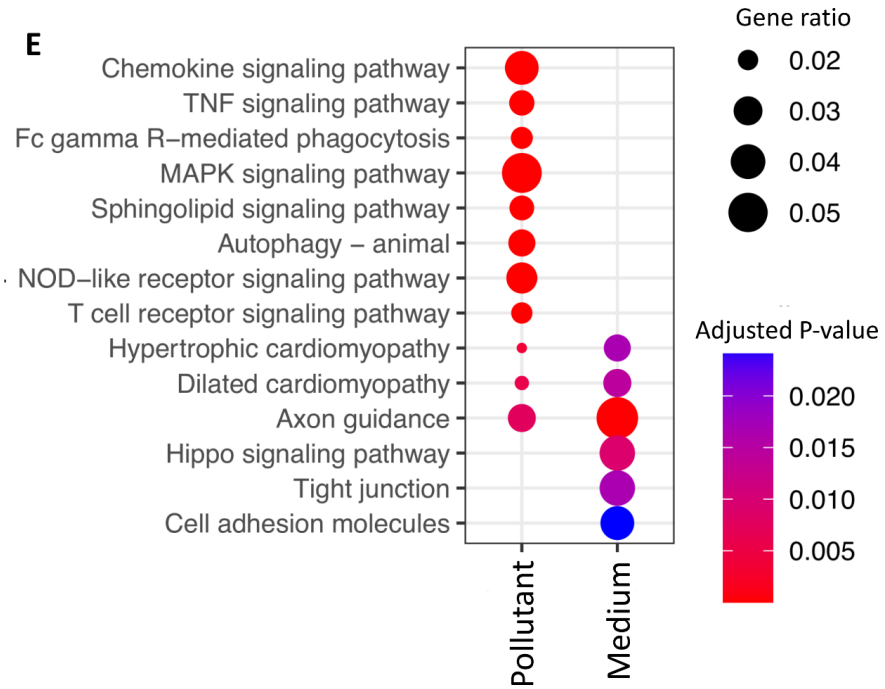


Fig. 5 continued

Fig. 5. *Pollution-induced trained immunity is epigenetically mediated by enhancing H3K27ac in circulating monocytes.*Human circulating monocytes freshly enriched from a healthy individual were trained with the vehicle (negative control) or SRM1648a pollutant for 24h followed by resting for 6 days. ChIP Seq was utilized to study H3K27ac. Intersect plot consensus peaks call between pollutant versus vehicle-trained samples were determined. The specific, overlapping, and total number of the peaks per training condition were summarized in a Venn diagram (A). Peaks annotation stacked barplot was generated by calculating the proportion of peaks assigned to genomic features by HOMER (*45*) (B). Pileup heatmap of the H3K27ac mark specific to pollutant in comparison with medium at enhancers and TSSs. Rows are genomic regions from -3 to +3 kb around the center of the peaks; the signal intensity was determined in windows of 300 bp. (C). Genome browser screen shots of H3K27ac ChIP Seq landscape at *IΛ1β, IΛ6, ΞΛ8, ΛΛ3, AHP, IPΦ8, TΦ3* , and *SETD1A* loci as representative inflammatory, transcription factor, and epigenetic signatures associated with pollution training. Super-enhancer regions of each gene are highlighted with dashed line boxes. (D). The KEGG enrichment analysis of the closest genes assigned to the pollutant-specific peaks was performed using MSigDB Hallmark 2020 (E).

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`Table S2.csv` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S3.csv` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S4.csv` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S5.csv` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S6.xlsx` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S7.csv` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma

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`Table S8.xlsx` available at https://authorea.com/users/327588/articles/594359-proinflammatory-polarization-of-monocytes-by-particulate-air-pollutants-is-mediated-by-induction-of-trained-immunity-in-pediatric-asthma