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Dust mite induces leukocyte exosomes with proinflammatory effects toward airway epithelial cells



To the Editor:

House dust mite (HDM), a major indoor perennial aeroallergen, is a prevalent cause of atopic sensitization worldwide. Exposure to mite allergens is in fact a common cause and trigger for asthma, a disease that affects 300 million persons worldwide.¹ Crosstalk between multiple cell types is known to maintain chronic inflammation in patients with allergic airway disease, although the exact mechanisms are complex and still uncertain.² To date, intercellular communication between leukocytes and airway epithelial cells has been largely attributed to cytokines and chemokines. However, the inflammatory milieu also contains other soluble mediators that are yet unappreciated and potentially pathogenic in airway inflammation. Growing evidence suggests

that exosomes, nano-sized membrane-bound vesicles, are contributors to cell-to-cell signaling and important conveyors of inflammation in patients with airway disease.

Initial studies have implicated exosomes in the regulation of immune responses and inflammation in patients with allergic disease.³⁻⁵ Exosomes containing nucleic acid and protein cargo are released by virtually all cell types and act as a shuttle to transfer material to recipient cells, where they exert functional and regulatory effects. In our study we aimed to determine whether dust mite extract (DME) regulates production of exosomes from human mononuclear cells and whether they have immunoregulatory effects on airway epithelial cells.

Across disciplines, exosome release appears to be cell dependent and regulated by both internal conditions and exogenous factors, although the external stimuli required for exosome secretion are still largely unknown.⁶ Additionally, the majority of studies on exosome release have been completed with cancer cell lines. By using HDM extract as a relevant inflammatory model, our study fills this gap by examining the effects of DME exposure on exosome secretion in human PBMCs from patients with dust mite allergy.

We analyzed exosomes derived from PBMCs of donors with dust mite allergy (for subjects' characteristics, see Table E1 in this article's Online Repository at www.jacionline.org) cultured with DME or PBS vehicle control for 48 hours. Exosomes were isolated from media by using a well-established, volume-crowding, polymer precipitation method reported in the Methods section in this article's Online Repository at www.jacionline.org and then analyzed by using electron microscopy and nanoparticle tracking analysis. Exosomes from both unstimulated and DME-stimulated leukocytes had similar characteristics, including size (108.68 ± 3.00 and 112.88 ± 1.93 nm, respectively) and morphology (see Fig E1 in this article's Online Repository at www.jacionline.org).

PBMCs constitutively produced exosomes without stimulation. PBS vehicle-treated cells produced an average of 512 ± 51 million particles/mL in a 48-hour period. Exposure to DME increased PBMC exosome levels to 1.081 ± 0.886 billion particles/mL, nearly doubling exosome release in the same

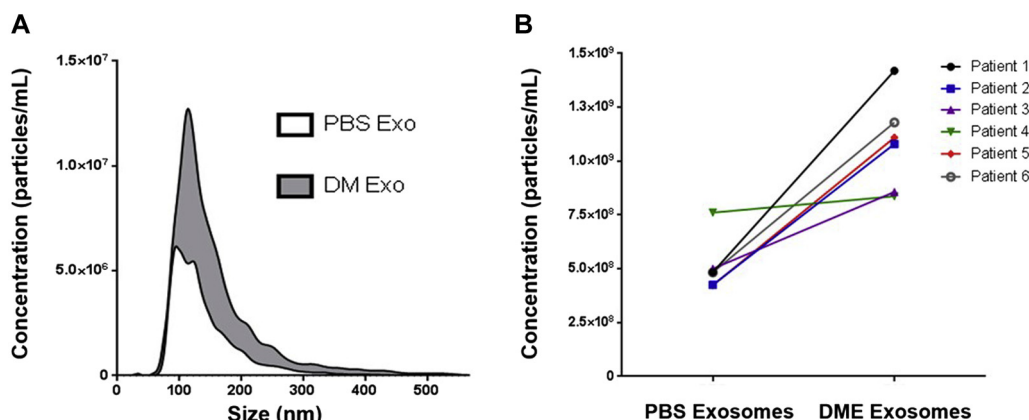


FIG 1. DME stimulates PBMC production of exosomes. PBMCs from donors with dust mite allergy ($n = 6$) were stimulated *in vitro* with $50 \mu\text{g}$ of DME or PBS vehicle for 48 hours. Exosomes were isolated from cultured media and measured by using nanoparticle tracking analysis. **A**, Histogram of mean particle concentration for each size. **B**, Plot of total particle concentration from unstimulated versus stimulated patient-matched cells. Dust mite allergen significantly increases leukocyte production of exosomes, $P = .0313$ determined by the Wilcoxon signed pairs rank test. *DM Exo*, DME exosome; *PBS Exo*, PBS-exosome.

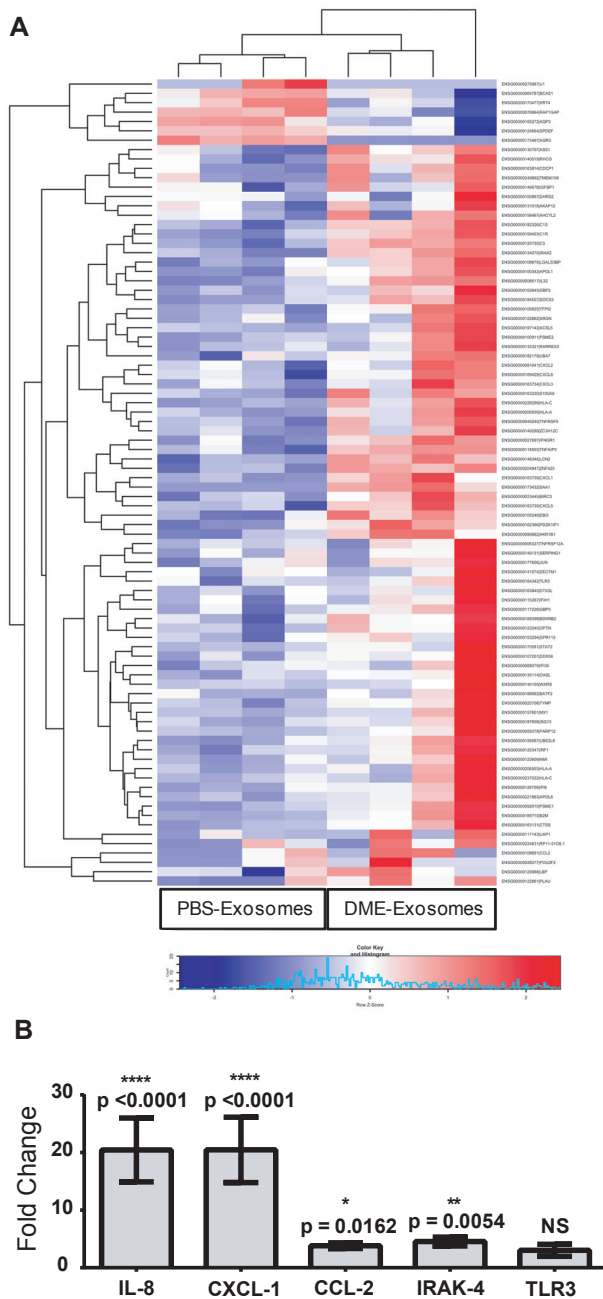


FIG 2. Exosomes from DME-stimulated cells alter transcriptome of airway epithelial cells. **A**, Heat map showing differential expression of transcripts in airway epithelial cells treated with either exosomes from PBS-treated PBMCs (*PBS-Exosomes*) or exosomes from DME-stimulated PBMCs (*DME-Exosomes*). **B**, Validation of RNA sequencing candidates by using real-time quantitative PCR. Data are represented as means $\pm$ SEMs ($n = 12$ donors with dust mite allergy).

amount of time ($P = .0313$, Fig 1). Others have previously shown in an HDM mouse model of allergic asthma that dust mite exposure *in vivo* leads to increased exosome production by the murine airway.⁷ However, the source of these exosomes, airway epithelial cells versus leukocytes, was not clear, and this process has not been well studied in human subjects. Our results indicate that PBMCs are a source of DME-induced exosomes in human subjects with allergic rhinitis.

Although our pilot study shares novel and preliminary results, we have not identified the exact mechanisms for enhanced exosome release. Because HDM extract contains a complex mix of proinflammatory molecules, it is possible that effects could be driven by 1 or more of these immunogenic components. Given the relatively low yield of exosomes isolated from cell-culture experiments, we were unable to fully characterize the effects of different components of HDM extract. It is possible that allergen-dependent T-cell receptor signaling is a stimulus for exosome release in T cells. Alternatively, recent work demonstrated that pattern recognition receptor activation leads to exosome release, and DME might increase exosome secretion through innate immune signaling.⁸ Future work will need to investigate this mechanism, untangle specific triggers for exosome secretion, and also establish the relative contributions of each cell population in PBMCs.

We hypothesized that dust mite-induced exosome production from PBMCs promotes an inflammatory response in airway epithelial cells, representing a novel method of communication between these cells. We performed proof-of-concept *in vitro* coculture assays by transferring exosomes from unstimulated (*PBS-Exosomes*) or DME-stimulated (*DME-Exosomes*) PBMCs to the apical surfaces of A549 airway epithelial cells (as described in the [Methods](#) section in this article's Online Repository) and measured the transcriptomic effects using RNA sequencing. Compared with *PBS-exosomes*, *DME-exosomes* significantly altered the transcriptome of 86 genes in airway epithelial cells, upregulating 81 and downregulating 5 targets (Fig 2, A). Specifically, *DME-exosomes* upregulated expression of proinflammatory genes, including chemokines, cytokines, and members of innate immune signaling pathways in airway epithelial cells that included genes that were part of both early and late asthmatic responses to allergen (see [Table E2](#) for functional classification of genes and [Table E3](#) for a complete list of genes in this article's Online Repository at www.jacionline.org). We validated a panel of targets by using real-time quantitative PCR (Fig 2, B). The innate immune signaling pathway members Toll-like receptor 3 and IL-1 receptor-associated kinase-like 2 were both induced by *DME-Exosomes*, supporting a role for exosomal signaling in early events of allergic airway disease. Furthermore, *DME-exosomes* significantly upregulated chemoattractants for neutrophils and monocytic cells, CXCL8, CXCL1, and CCL2, which amplify and sustain the inflammatory response in the airway. Consistent with *in vivo* studies of murine HDM models,⁷ our findings support a model in which exosome induction of airway chemokines might contribute to leukocyte recruitment, which can perpetuate inflammation. Further work will need to be conducted to fully elucidate the physiologic and pathogenic role of exosomes in this context, particularly *in vivo*.

In summary, we found that PBMCs from patients with HDM allergy produced not only greater numbers of exosomes after stimulation with DME but also particles with unique functional properties. Our data suggest that exosomes are a novel cell-to-cell communication mechanism to promote airway inflammation. Modulation of exosome secretion or function might have therapeutic implications. Because exosomes are emerging messengers of the immune system, characterization of their cargo and elucidation of their mechanisms of action on inflammatory pathways require additional investigation.

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Kristin A. Lambert, BS^a
Man-Hsun Hsu, BS^a
Zoe C. Laryea, BS^a
Yuanjun Zhao, MD, PhD^b
Alanna N. Roff, MS^c
Jeffrey M. Sundstrom, MD, PhD^b
Faoud T. Ishmael, MD, PhD^{a,d}

From ^athe Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, and ^bthe Department of Ophthalmology, Penn State Milton S. Hershey Medical Center, Hershey, Pa; ^cthe Department of Biostatistics and Informatics, University of Colorado Denver, Anschutz Medical Campus, Aurora, Colo; and ^dthe Department of Allergy and Sleep Medicine, Mount Nittany Medical Group, State College, Pa. E-mail: faoud.ishmael@mountnittany.org.

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The FOXP3Δ2 isoform supports Treg cell development and protects against severe IPEX syndrome



To the Editor:

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare syndrome of immune dysregulation characterized by severe autoimmunity that is often fatal in early life. IPEX syndrome is caused by mutations in the forkhead box protein 3 (*FOXP3*) gene on chromosome Xp11.23.¹ *FOXP3* is a key transcription factor for the development and function of regulatory T (Treg) cells, which are essential for immune tolerance.¹

FOXP3 protein is comprised of 4 functional domains with distinct roles in gene regulation: a carboxyl terminal forkhead domain important for nuclear localization and DNA binding and 3 protein/protein interaction domains, including a central zinc finger, a leucine zipper, and a proline-rich transcriptional repressor domain (see Fig E1 in this article's Online Repository at www.jacionline.org).¹ Alternative splicing of human *FOXP3* results in expression of 4 naturally occurring isoforms: a full-length protein containing all domains (*FOXP3fl*) and shortened isoforms as a result of exclusion of either exon 2 (*FOXP3Δ2*), exon 7 (*FOXP3Δ7*), or both exons 2 and 7 (*FOXP3Δ2Δ7*) from the *FOXP3* transcripts.²⁻⁴ Differential expression of *FOXP3* isoforms has been found in patients with diseases, such as Crohn disease, although the regulation and function of distinct isoforms are incompletely understood.⁵ This is compounded by the fact that mice naturally express only the full-length protein, thus limiting the utility of mouse models to elucidate the functional significance of distinct *FOXP3* isoforms in human subjects.²⁻⁴ The most abundant isoforms of human *FOXP3* are *FOXP3fl* and *FOXP3Δ2*.^{1,4} Recently, we have found that *FOXP3Δ2* is preferentially induced on Treg cell activation and that high *FOXP3Δ2* use is associated with atherosclerotic plaque stability.⁴ However, to date, it has been impossible to study the *in vivo* function of either *FOXP3* isoform. Exceedingly rare reports of IPEX syndrome caused by mutations predicted to cause loss of exons 2 or 7, which have assessed isoform expression, have not provided specific clinical data or detailed Treg cell analysis.^{1,5-7}

Here we report the molecular phenotype of 2 patients with an autoimmune phenotype and a carrier female subject from the same family, with a novel *FOXP3* frameshift mutation (c.305delT; p.Phe102fs), which results in loss of *FOXP3fl* and *FOXP3Δ7* expression. The proband (P1) is a 25-year-old man who had type 1 diabetes (insulin-dependent diabetes mellitus [IDDM]) at 6 months of age and was strongly positive for glutamic acid decarboxylase autoantibodies. In adolescence he had inflammatory bowel disease and primary sclerosing cholangitis, both of which have gone into long-term remission with minimal intervention. He possessed additional autoantibodies, including anti-PR3, anti-gliadin IgA/IgG, as well as ASCA, and had normal lymphocyte subsets and levels of serum immunoglobulins (see Table E1 in this article's Online Repository at www.jacionline.org). Since late adolescence, he has had recurrent staphylococcal boils, sometimes requiring drainage. He has one sister who is well and not a carrier.

P2 is the 72-year-old maternal grandfather of P1, who was in good health until 40 years of age when he had psoriatic arthritis. He has type 2 diabetes and chronic obstructive pulmonary disease, having smoked heavily until 5 years prior. There is no other family history of note (see Fig E2 in this article's Online Repository at www.jacionline.org).

Panel next-generation sequencing testing (Agilent custom capture v3 Illumina/HiSeq; Illumina, San Diego, Calif) in P1 at age 20 years identified a novel hemizygous frameshift mutation in exon 2 of *FOXP3* (NM_014009.3:c.305delT), which is suggestive of IPEX syndrome (see Methods in this article's Online Repository at www.jacionline.org). *FOXP3* exon 2 encodes amino acids 71 to 105 of the 431-amino-acid protein, and c.305delT is predicted to result in protein termination at amino acid 102 (p.Phe102fs). Familial segregation studies using Sanger sequencing confirmed the patient's mother (C1), aged 47 years and healthy, to be a carrier of this variant. c.305delT was also

Online Repository Document

Dust mite induces leukocyte exosomes with proinflammatory effects towards airway epithelial cells

Kristin A. Lambert¹, BS, Man-Hsun Hsu¹, BS, Zoe C. Laryea¹, BS, Yuanjun Zhao, MD, PhD²,
Alanna N. Roff, MS, 3 Jeffrey M. Sundstrom, MD, PhD², Faoud T. Ishmael MD, PhD^{1,4}

METHODS

Patient Selection

This study was approved by the Penn State College of Medicine Institutional Review Board and all subjects provided written informed consent. Dust-mite (DM) allergic patients were enrolled according to clinical criteria, history and symptoms. DM allergic patients (n=17) had either positive skin test (≥ 4 mm wheal) or positive serology (serum ImmunoCap test to *Dermatophagoides farinae*). All DM-allergic individuals in our cohort reported symptoms consistent with allergic rhinitis and sensitivities to dust. About half of this group also reported a history of asthma (n=9).

PBMC isolation and treatment

40 mL of whole blood was collected from subjects by venipuncture, layered on a 15 mL Ficoll Paque (GE Healthcare) gradient and centrifuged at 630 x g for 30 minutes to separate peripheral blood mononuclear cells (PBMCs). Isolated cells were resuspended in AIM-V (Life Technologies) medium at 1 million cells/mL. Where indicated, PBMCs were stimulated with 50 μ g/mL of *Dermatophagoides farinae* house dust mite extract (GREER labs) or PBS vehicle and incubated at 37° C for 48 hours (1, 2). The house dust mite extract used in our study is a staple of published mouse models and *in vitro* human studies, where proinflammatory effects of the extract are compared to PBS diluent controls (3-10).

Exosome isolation

Exosomes were isolated via polymer precipitation, a method of exosome purification suited for high yield separation of particles while preserving their biological function (11,12). Exosome purification with Thermo Total Exosome Isolation Reagent was previously shown to be an effective purification method for harvesting exosomes from asthmatic patient-derived materials (13) and was subsequently selected for our study to collect human exosomes with intact activity for our functional assays. According to manufacturer's protocols, cells and media were collected at time of harvest and centrifuged at 2,000 x g for 30 minutes at room temperature to remove cells and debris. Cell-free supernatant was transferred to a new tube and total exosome isolation reagent (Thermo Fisher Scientific), Sample was incubated with the exosome isolation reagent for 24 hours at 4°C and then centrifuged at 10,000 x g for 1 hour at 4°C. Supernatant was aspirated and the exosome pellet was resuspended in 100 μ L of PBS.

Transmission electron microscopy

Exosomes were visualized using transmission electron microscopy (TEM). Exosomes were resuspended in PBS and 10 μ L of this solution was mounted on a formvar coated copper grid for 20 minutes. Mounted samples were then fixed with 4% paraformaldehyde for 20 minutes, washed three times with PBS, and crosslinked with 1% glutaraldehyde for 5 minutes. Exosome samples were then negatively stained with 1% uranyl acetate for 20 seconds and observed on a JEOL 1400 TEM.

Nanoparticle tracking analysis (NTA)

Exosome quantity or concentration and particle size were measured using a NanoSight NS300 (Malvern) as previously published (14). Equipment was calibrated using 100 nm polystyrene latex beads (Malvern). Exosomes were diluted 50 fold with particle-free water before injection

into the NanoSight. Five independent, 60-second videos were captured of each exosomes sample at room temperature and detection threshold 5, using the sCMOS camera scatter mode.

Airway epithelial cell co-culture with exosomes

Human A549 airway epithelial cells (ATCC) were cultured in F12-K supplemented with 10% FBS and maintained in a 37°C incubator with 5% CO₂. For co-culture experiments, A549 cells were plated in fresh media without FBS, a known source of exosomes. A549s were treated for 24 hours with apical addition of 10 µg of exosomes derived from PBS vehicle treated cells and DM treated cells. Biorad protein assay was used according to manufacturer's instructions to quantitate exosome concentration.

RNA Sequencing and analysis

The cDNA libraries were prepared using the NEXTflex™ Illumina Rapid Directional RNA-Seq Library Prep Kit (BioO Scientific) as per the manufacturer's instructions. Briefly, polyA RNA was purified from 200 ng of total RNA using oligo (dT) beads. The extracted mRNA fraction was subjected to fragmentation, reverse transcription, end repair, 3'– end adenylation, and adaptor ligation, followed by PCR amplification and SPRI bead purification (Beckman Coulter). The unique index sequences were incorporated in the adaptors for multiplexed high-throughput sequencing. The final product was assessed for its size distribution and concentration using BioAnalyzer High Sensitivity DNA Kit (Agilent Technologies). Pooled libraries were diluted to 2 nM in EB buffer (Qiagen) and then denatured using the Illumina protocol. The denatured libraries were diluted to 10 pM by pre-chilled hybridization buffer and loaded onto a TruSeq SR v3 flow cells on an Illumina HiSeq 2500 for 50 cycles using a single-read recipe (TrueSeq SBS Kit v3) and run for 50 cycles using a single-read recipe according to the manufacturer's instructions.

De-multiplexed and adapter-trimmed sequencing reads were generated using Illumina CASAVA pipeline (released version 1.8). The sequencing reads were subjected to quality filtering used FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit) to keep only reads that have at least 80% of bases with a quality score of 20 or more (conducted by fastq_quality_filter function) and reads left with > 10 bases after being trimmed with reads with a quality score of < 20 (conducted by fastq_quality_trimmer function). Filtered reads were mapped to the human reference genome (GRCh38) using TopHat (version 2.0.9) supplied by Ensembl annotation file; GRCh38.78.gtf. After normalization was performed via the median of the geometric means of fragment counts across all libraries, differential expression was determined using Cuffdiff tool which is available in Cufflinks version 2.2.1. All genes passing FDR criteria of 0.05 were considered differentially expressed genes. Heatmaps were generated using the gplots R package version 3.0.1 (<https://CRAN.R-project.org/package=gplots>).

Analysis of inflammatory gene expression

Inflammatory gene expression was quantified in exosome treated cells using real-time quantitative PCR using methodology previously published by our group (15, 16). Exosome-treated airway cells were harvested and RNA extracted from cell pellets using TRIzol, followed by column isolation of total RNA using the Direct-zol RNA miniprep kit (Zymo). RNA was quantified by UV absorbance at 260 nm and reverse transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) as described by the manufacturer. Quantitative real-time PCR (qRT-PCR) was performed using 1:5 diluted cDNA as

template, 2x iTaq Universal SYBR Green Supermix (Bio-Rad), and gene-specific primers. qRT-PCR reactions were run on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad) using the following program: 2 minute hot start at 95°C, 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C, and 2 minutes at 10°C. Gene expression fold changes were calculated using the $\Delta\Delta C_t$ method with either GAPDH as the normalization control where appropriate.

Supplemental Table 1. Dust-Mite Allergic Patient Demographics (n=17)

Age (mean years $\pm$ SEM)	39.88 $\pm$ 3.74
Sex (Ratio Male : Female)	7 : 10
Race/ Ethnicity (Ratio White: Non White)	11 : 6
Allergic Rhinitis (Ratio AR: No AR)	17 : 0
Asthma (Ratio Asthma : No Asthma)	9 : 8

Supplemental Figure 1. Characterization of exosomes shed from PBMCs of dust-mite allergic human donors. Transmission electron microscopy visualization of exosome morphology for particles derived from (A) vehicle-treated human PBMCs and (B) dust-mite stimulated PBMCs. Representative images are shown. (C) Mode particle size analyzed by nanoparticle tracking analysis (NTA) for exosomes derived from both vehicle treated and dust mite stimulated PBMCs (n =6 dust mite allergic donors). Data represented as mean $\pm$ SEM of mode size particle measured in five independent, 10-second video captures of each exosome sample from each individual donor (n=30 videos for each condition). Statistical differences were determined by Wilcoxon signed pairs rank test. $p < 0.05 = *$.

Supplemental Table 2. Gene Ontology enrichment analysis of genes differentially expressed in airway epithelial cells treated with exosomes derived from DME-stimulated PBMCs.

Gene Ontology (GO) Terms	Genes	Adjusted P Value
Immune response	CXCL1, IFIH1, CCL2, CXCL5, C3, CXCL3, CXCL2, TLR3, IL32, C1R, C1S, IFI35, B2M, POU2F2, LBP, EBI3, SECTM1, HLA-A, HLA-C, SERPING1, CTSS	3.61E-13
Defense response	CXCL1, IRAK2, IFIH1, CCL2, NMI, C3, CXCL3, S100A9, CXCL2, TLR3, IL32, SERPING1, HLA-C, C1R, BDKRB2, C1S, DDX58, LGALS3BP, APOL1, SAA1	1.64E-09
Inflammatory response	IRAK2, CXCL1, CCL2, NMI, C3, CXCL3, CXCL2, S100A9, TLR3, SERPING1, C1R, BDKRB2, C1S, SAA1, LBP	3.72E-07
Response to wounding	CXCL1, IRAK2, CCL2, NMI, C3, CXCL3, CXCL2, S100A9, TLR3, SERPING1, C1R, BDKRB2, C1S, SAA1, LBP, IGFBP1, TFPI2, PLAU	3.4E-07
Extracellular Space	CXCL1, SECTM1, CCL2, CXCL5, C3, CXCL3, CXCL2, IL32, SERPING1, LGALS3BP, APOL1, ISG15, SAA1, AKR1B1, IGFBP1, LBP, EBI3, SRGN	9.84E-07
Extracellular Region	CXCL1, CCL2, CXCL5, C3, CXCL3, CXCL2, CDCP1, IL32, C1R, C1S, B2M, LGALS3BP, ISG15, SAA1, LBP, TFPI2, EBI3, SRGN, SECTM1, SERPING1	8.60E-07
Response to Virus	DDX58, LCN2, IFIH1, ISG15, TLR3, MX1, IFNGR1, IFI35, STAT2	1.74E-05
Extracellular Region Part	CXCL1, SECTM1, CCL2, CXCL5, C3, CXCL3, CXCL2, IL32, SERPING1, LGALS3BP, APOL1, ISG15, SAA1, AKR1B1, IGFBP1, LBP, TFPI2, EBI3	8.06E-06
Innate Immune Response	DDX58, IFIH1, APOL1, C3, TLR3, C1R, SERPING1, C1S, LBP	8.95E-05
Chemotaxis	CXCL1, TYMP, CCL2, CXCL5, SAA1, CXCL3, CXCL2, S100A9, PLAU	2.35E-04

Supplemental Table 3 Differentially expressed genes in airway epithelial cells treated with exosomes derived from DME-stimulated PBMCs. 86 genes were identified as differentially expressed between airway epithelial cells treated PBS-Exosomes and DME-Exosomes.

Gene	Log Fold Change	P.Value	adj.P.Val
TNFRSF12A	1.03724	5.00E-05	0.0119906
IL32	4.17733	5.00E-05	0.0119906
BIRC3	1.35413	5.00E-05	0.0119906
TYMP	2.1495	5.00E-05	0.0119906
IFNGR1	0.852058	5.00E-05	0.0119906
TNFRSF9	2.9411	5.00E-05	0.0119906
PARP12	1.16993	5.00E-05	0.0119906
IFI35	1.86886	5.00E-05	0.0119906
RAP1GAP	-1.22825	5.00E-05	0.0119906
CXCL2	1.83717	5.00E-05	0.0119906
PSME1	1.37761	5.00E-05	0.0119906
APOL1	2.02223	5.00E-05	0.0119906
PSME2	1.42574	5.00E-05	0.0119906
EBI3	2.62695	5.00E-05	0.0119906
TFPI2	0.917521	5.00E-05	0.0119906
DDX58	1.16716	5.00E-05	0.0119906
CCL2	1.50166	5.00E-05	0.0119906
UAP1	1.4316	5.00E-05	0.0119906
GBP3	2.07635	5.00E-05	0.0119906
TNFAIP3	2.17196	5.00E-05	0.0119906
NMI	1.26209	5.00E-05	0.0119906
SPDEF	-0.932155	5.00E-05	0.0119906
IRF1	2.28907	5.00E-05	0.0119906
C3	2.0694	5.00E-05	0.0119906
IFI6	2.22733	5.00E-05	0.0119906
ASS1	1.55014	5.00E-05	0.0119906
AKAP12	0.96953	5.00E-05	0.0119906
RARRES3	3.73642	5.00E-05	0.0119906
IRAK2	1.58672	5.00E-05	0.0119906
OASL	3.52114	5.00E-05	0.0119906
WARS	2.31326	5.00E-05	0.0119906
RHCG	2.4216	5.00E-05	0.0119906
IGFBP1	1.43179	5.00E-05	0.0119906
LCN2	2.94251	5.00E-05	0.0119906
SERPING1	2.27958	5.00E-05	0.0119906

ZC3H12C	1.03926	5.00E-05	0.0119906
GPR115	1.28642	5.00E-05	0.0119906
UBE2L6	1.92346	5.00E-05	0.0119906
MX1	3.02393	5.00E-05	0.0119906
AHCYL2	1.21292	5.00E-05	0.0119906
C1R	2.51851	5.00E-05	0.0119906
PDZK1IP1	3.61703	5.00E-05	0.0119906
GBP2	2.49296	5.00E-05	0.0119906
CTSS	1.64796	5.00E-05	0.0119906
S100A9	2.28319	5.00E-05	0.0119906
CXCL3	1.48445	5.00E-05	0.0119906
CXCL5	1.33686	5.00E-05	0.0119906
CXCL1	2.84704	5.00E-05	0.0119906
CDCP1	1.998	5.00E-05	0.0119906
DTX3L	1.43933	5.00E-05	0.0119906
TLR3	2.67613	5.00E-05	0.0119906
AQP3	-0.99335	5.00E-05	0.0119906
B2M	2.0271	5.00E-05	0.0119906
BATF2	2.43526	5.00E-05	0.0119906
CXCL8	2.13536	5.00E-05	0.0119906
KRT4	-2.20514	5.00E-05	0.0119906
STAT2	1.03057	5.00E-05	0.0119906
SAA1	10	5.00E-05	0.0119906
AGR3	0.1	5.00E-05	0.0119906
UBA7	2.64837	5.00E-05	0.0119906
C1S	1.84475	5.00E-05	0.0119906
SOCS3	1.0272	5.00E-05	0.0119906
ISG15	2.39524	5.00E-05	0.0119906
ACSL5	3.08932	5.00E-05	0.0119906
HLA-A	1.40229	5.00E-05	0.0119906
APOL6	1.99358	5.00E-05	0.0119906
HLA-C	2.19805	5.00E-05	0.0119906
HLA-C	1.33527	5.00E-05	0.0119906
TMEM158	1.85806	5.00E-05	0.0119906
DHRS2	1.27699	0.0001	0.0229819
SRGN	0.727174	0.0001	0.0229819
SECTM1	2.14818	0.0001	0.0229819
BCAS1	-1.236	0.00015	0.0335412
JUN	0.981854	0.00015	0.0335412
POU2F2	2.12248	0.0002	0.0424282

AKR1B1	0.782061	0.0002	0.0424282
LBP	1.05345	0.0002	0.0424282
ZNF425	1.91119	0.0002	0.0424282
LGALS3BP	1.18219	0.00025	0.0481017
IFIH1	2.37468	0.00025	0.0481017
PLAU	0.803382	0.00025	0.0481017
OPTN	1.11182	0.00025	0.0481017
BDKRB2	0.808588	0.00025	0.0481017
HLA-A	1.15507	0.00025	0.0481017
RP11-51O6.1	0.763222	0.00025	0.0481017
U1	0.1	0.00025	0.0481017

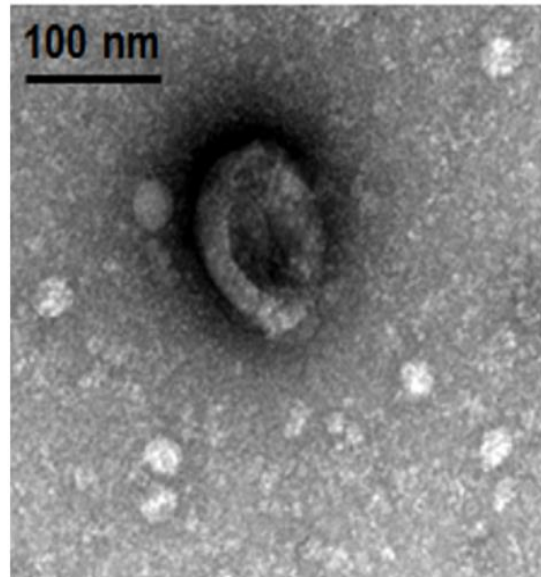
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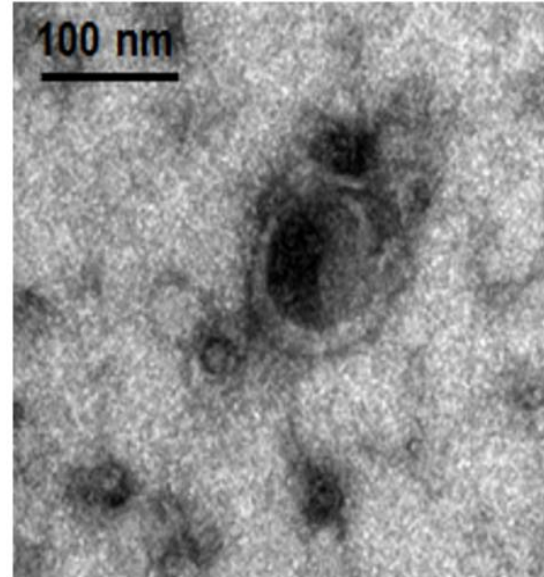
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