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Transcriptomic Profiles of Well-Differentiated Airway Epithelial Cells in Response to Environmental Triggers of Asthma Exacerbation

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To the Editor:

Environmental exposures, including pollutants, allergens, and microbes may trigger asthma exacerbations.[1] Inhaled exposures interact with airway epithelial cells, which then activate the innate immune system resulting in the release of DAMPs (danger-associated molecular patterns) and cytokines via pattern recognition receptor (PRRs), including TLRs. The activated immune system further orchestrates inflammation and Th2 immune responses in the airway.[2,3] Exposure combinations may potentiate exacerbations. In patients with asthma, eosinophilic lung inflammation in response to house dust mite (HDM) allergen may be further amplified by concomitant bacterial lipopolysaccharide (LPS) exposure.[4] Transcriptomic responses to environmental stimuli (both individually and in combination), may uncover gene targets relevant for exposures, with implications for asthma pathogenesis and gene by environment interaction studies.

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We performed transcriptomic analyses of well-differentiated primary human bronchial epithelial cells (HBECs) from 3 donors with fatal asthma and 3 without asthma (**Supplemental Table 1**). We stimulated HBECs with HDM, LPS, or their combination. To identify differential expression (stimulus vs. vehicle), we performed RNA sequencing (RNA-seq) (**Supplemental Methods**) and used DESseq2 (R statistical software) for our statistical analyses.

In all subjects, the combination of HDM and LPS exhibited the greatest number of differentially expressed (DE) genes (adjusted p <0.05) (2,103 genes), followed by LPS (854 genes) and then HDM (46 genes) (**Figure 1A, 1K**). The majority (63%) of DE genes with the combined exposure were not observed by either HDM or LPS alone (**Figure 1A)** . For HDM alone, the top-ranked DE genes included NOD-like receptor pathway (*IL-8*), ribosomal (*RPS5, RPL37*), and histone genes (*HIST1H4E* , *HIST1H4C)*(**Supplemental Table 2)** . For LPS alone, the top-ranked DE genes included IL-17 signaling (*IL17C)* , NFKB (*BCL2A1*), nitric oxide synthesis (*NOS2*), and NOD-like receptor signaling genes (*CCL2*) (**Supplemental Table 3).** For the combination of HDM and LPS, the top-ranked DE genes overlapped with the results for each stimulus and then extended to ubiquitin oxidoreductases and mitochondrial encoded cytochromes pathways. (**Supplemental Table 4** , **Figure 1F-1J**)

Next, to identify networks, we mapped DE genes onto protein-protein interactions using the String Interactome feature in Network Analyst (www.Networkanalyst.ca) (**Figure 1B-1I**). Compared to the HDM-specific network (**Figure 1B**), the LPS-specific network (**Figure 1C**) contained a greater number of nodes and included hubs for *NFKB1, SRC, RAC1* , and *CTNNB1* . By far, the most complicated network was for the combined exposure (**Figure 1D-1E**).

When stimulated, HBECs from donors with asthma (vs. non-asthma) demonstrated a much larger number of DE genes, and much denser, more highly connected networks with additional gene hubs **(Figure 2A-2D)** . For asthmatic HBECs (vs. non-asthmatic HBECs), HDM produced 315 DE genes (vs. 59), LPS 277 genes (vs. 119), and the combination of HDM + LPS 1,709 genes (vs. 335). The HDM-specific network in asthmatic cells showed immune regulatory genes such as *TNFAIP3, NFKBIA* , and*FAT1* , none which were nodes in the HDM-specific network for non-asthmatic cells (**Fig 2A**). In the LPS-specific networks, both asthmatic and non-asthmatic cells had nodes for *NFKB1, NFKB1A* and *TNFAIP3* represented; however, the hub *JAK2* is only observed for asthmatic cells (**Fig 2B**).

We performed two validation studies. First, qPCR confirmed differential expression of select top genes (**Supplemental Figure 1**). Second, we performed another set of exposure experiments in HBECs from additional donors with non-fatal asthma and without asthma and performed qPCR analysis, which also produced similar results (**Supplemental Figure 2**). These validations confirm generalizability of our results across asthma phenotypes (i.e. HBECs from fatal asthma and non-fatal asthma responded similarly).

Our findings provide evidence that the combined exposure to environmental triggers of asthma exacerbation increases the number of DE genes and the complexity of transcriptomic responses in HBECs as compared to individual stimuli. Moreover, cells from donors with asthma are more sensitive to environmental asthma triggers, eliciting a greater number of DE genes. While our data should be confirmed in a larger cohort, they serve as a solid basis for future studies of the effect of multiple exposures (i.e. the exposome) on molecular signaling in the bronchial epithelium during asthma exacerbations.

References

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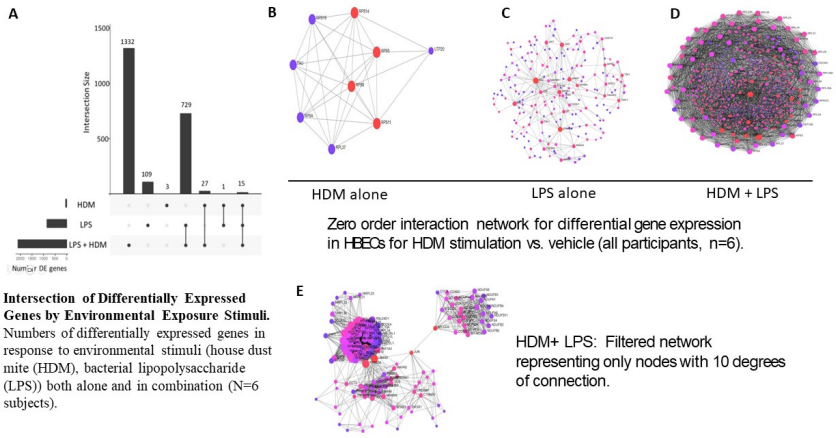
Conclusion Paragraph For Letter:

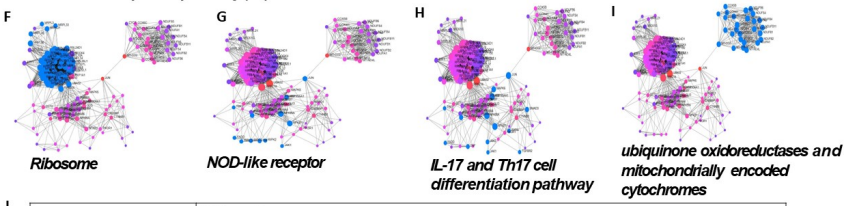
In this study, we tested two environmental exposure stimuli, bacterial lipopolysaccharide (LPS) and house dust mite (HDM), in an in vitro model of primary human bronchial epithelial cells (HBECs) from 6 individuals, to identify differentially expressed genes using RNAseq analysis in response to these exposures both individually and in combination. Our findings illustrate that exposure mixtures increase the strength and complexity of transcriptomic responses in HBECs as compared to individual stimuli alone, and that asthmatic's cells are more sensitive to these stimuli, eliciting a greater number of differentially expressed genes vs. controls. Our results provide a basis for future studies in this area, such as the expansion of this in vitro model to test multiple exposure combinations (i.e. the exposome) and their influence on molecular signaling in the bronchial epithelium.

Figure 1. Differential gene expression in HBECs



Intersection of Differentially Expressed Genes by Environmental Exposure Stimuli. Numbers of differentially expressed genes in response to environmental stimuli (house dust mite (HDM), bacterial lipopolysaccharide (LPS)) both alone and in combination (N=6 subjects).

HDM+ LPS: Filtered network representing only nodes with 10 degrees of connection. ***Highlighted in blue indicates each specific pathway (F-I)***



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	Pathway Genes
Ribosome	*RPS27A, UBA52, RPS3, RPL23A, RPS18, RPL11, RPL9, RPL23, RPL35, RPS8, RPS16, RPS11, RPL4, RPS2, RPL5, RPS25, RPL27, RPS5, RPS23, RPS3A, RPS6, RPS29, RPS14, RPL31, RPL6, RPL30, RPS20, FAU, RPS13, RPL19*
NOD-like receptor	*GABARAPL2, MAPK13, RIPK2, CCL2, NFKB1, NOD2, CXCL8, STAT2, HSP90AA1, IFNAR2, TXN, TANK, BCL2, IL6, FADD, BIRC3, BIRC2, JAK1, NFKB1, JUN, TBK1, NFKB1A*
IL-17 signaling and Th17cell differentiation	*MAPK13, CCL2, NFKB1, MAPK6, IL1B, CXCL8, FOSL1, MMP1, HSP90AA1, IL6, LCN2, IKBKE, TNFAIP3, JAK1, JAK2, FADD, TGFBR2, TNF, JUN, MAPK6, TBK1, SMAD3, NFKB1A, NFKBIE, IFNGR2, TGFBR2, GATA3, RXRA*
ubiquinone oxidoreductases and mitochondrially encoded cytochromes	*COX5B, COX4I1, NDUFS4, NDUFB6, CYCS, NDUFB6, NDUFS4, NDUFB11, NDUFB2, NDUFB1, NDUFB4, NDUFA2, NDUFB9, NDUFA1, UQCRH, NDUFB1, NDUFA4, MT-CO1, MT-CYB, MT-ATP6, MT-ND1, MT-ND4L, MT-ND5, MT-CO2, MT-ND4, MT-ND2, MT-ATP8, GNAI3, COX7A2, NDUFA1, NDUFS5, UQCRQ, NDUFB6, PARK7, NDUFA6, NDUFB2, COX7C, COX6C, APAF1, COX4I1*

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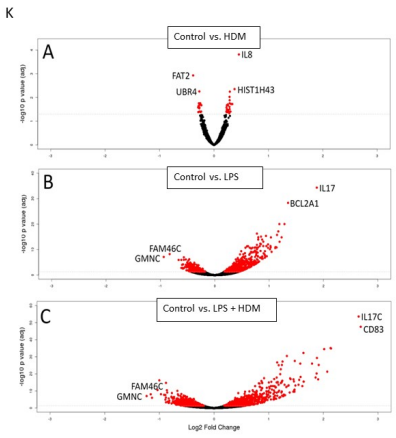
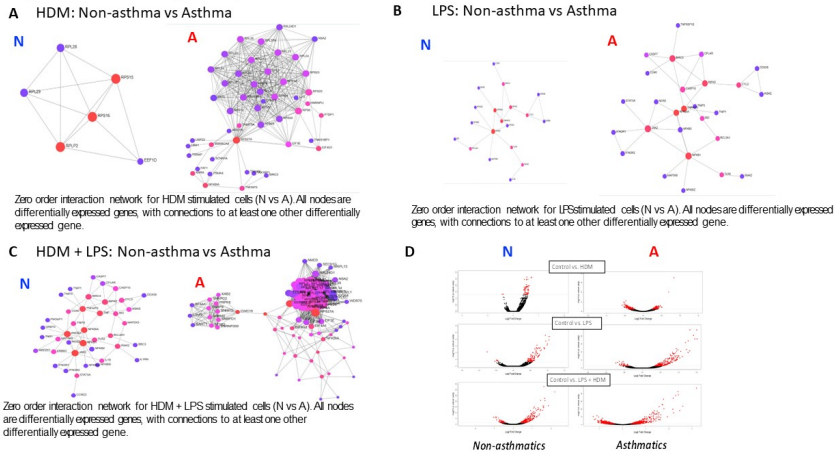
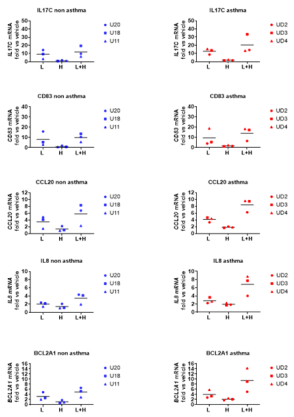


Figure 2. Differential gene expression in Asthma vs. Non-Asthma



Supplemental Figure 1. Validation of RNA-seq results by qPCR using RNA from 3 donors without asthma (blue) and 3 donors with fatal asthma (red). L=LPS H=HDM stimulation, L + H = LPS + HDM combination



Supplemental Figure 2. Additional HBEC experiments by qPCR using RNA from 3 donors without asthma (blue) and 3 donors with non-fatal asthma (red). L=LPS H=HDM stimulation, L + H = LPS + HDM combination