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Effect of Inhaled Endotoxin on Regional Particle Deposition in Mild Asthmatics

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To the Editor

Atopic asthmatic patients are reported to be more sensitive to the effects of environmental endotoxin (LPS) than healthy volunteers (HVs) ¹. However, we recently found that the airway inflammatory response to inhaled LPS challenge is blunted in allergic asthmatics (AAs) compared with that seen in healthy volunteers ². While the percentage of neutrophils in induced sputum was increased in both HVs and AAs 6 hours after inhaled LPS challenge, the absolute numbers of leukocytes and PMNs recruited to the airways were significantly lower in AA patients compared with those seen in HVs. Neither group had any change in spirometric function associated with LPS challenge, but it is widely recognized that spirometry is not a very sensitive measure for detecting subtle changes in airway physiology. We recently showed an increase in bronchial airway deposition and increased “patchiness” of particle deposition post house dust mite inhalation challenge in AAs despite

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the fact that spirometric function had returned to pre-challenge values³. Consequently in the group of HVs (5M/13F) and AAs (5M/8F) from the Hernandez et al study² we also assessed the effect of inhaled LPS challenge (20,000 EU Clinical Center Reference Endotoxin (CCRE)) on regional deposition of inhaled particles post challenge as a sensitive index of mild, heterogeneous changes in airway caliber that may not be evident from spirometry^{3,4,5}. Informed written consent was obtained from all subjects prior to their participation in the study that was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill. The study was listed on clinicaltrials.gov (NCT00839124).

We compared regional particle deposition on a baseline, non-challenge study day to that 4 hours post LPS challenge day by gamma scintigraphy (figure 1B and C) following controlled inhalation (tidal flow and breathing rate at 500ml/sec and 30/min) of ^{99m}Tc - sulfur colloid aerosol (mass median aerodynamic diameter of 5µm, GSD = 2.0). Regional particle deposition in the right lung was then determined by central-to-peripheral (C/P) ratio of activity (primary outcome) and the skew of the histogram distribution (counts/pixel vs. #pixels) for the deposition images⁶ (figure 1B and C). A xenon (¹³³Xe) equilibrium lung scan (figure 1A) was recorded for each subject on their baseline visit to allow the creation of suitable regions of interest (ROIs) and normalization of the C/P ratio of particle deposition to account for differences in lung volume between C and P³, e.g. a C/P of 1.0 reflects equal deposition in each region. Increases in C/P to values greater than unity reflect an increase in central vs. peripheral deposition primarily as a result of increased bronchial deposition. We also assessed regional deposition heterogeneity as the skew of the histogram distribution (counts/pixel vs. #pixels)⁶ within the whole right lung ROI, increasing with increased patchiness of deposition and frequency of “hot spots” in the lung, and independent of the specific region within the lung (e.g. C vs. P). Frequency distribution histograms were constructed from the right lung deposition images, i.e. number of pixels with a given count value (expressed as a fraction of total pixels) vs. count values, and the third moment about the mean or skew of the histogram was calculated⁶. Heterogeneity of deposition increases with increasing skew (i.e. more pixels with high counts/pixel). The Wilcoxon matched-pairs signed ranks test was used for pre vs. post LPS and baseline vs. post LPS challenge comparisons. The study was powered on primary inflammation endpoints associated with Hernandez et al² but the n for allergic asthmatics was also similar to that for which we observed a change in regional deposition associated with allergen challenge³.

There were no differences in pre vs. post LPS challenge lung function (FEV1) for either HVs or AAs for any time measured up to 6 hours post challenge. There was no significant difference in baseline C/P or skew between HVs and AAs. The AAs showed an increase in bronchial airway deposition post-endotoxin challenge compared to baseline (increase in both C/P and skew compared to baseline $p < 0.05$, table 1). Figure 1B and C shows an example of a deposition image for baseline vs. post LPS challenge in an AA subject. On the other hand, there was no baseline vs. challenge difference in regional deposition in the HVs (table 1).

We found that asthmatics showed a change in regional deposition associated with LPS challenge not seen in the healthy non-asthmatic subjects. We hypothesize that this may be due to increased secretion of mucus in the airways post challenge in asthmatics, leading to enhancement of deposition in these airways⁷. LPS has been shown to stimulate production and expression of MUC5AC in animal and in-vitro models^{8,9}. This observation that LPS modifies deposition of inhaled particulates in the airways also supports the hypothesis that low-level airway inflammation in asthmatics may result in increased particle deposition and therefore, increased total and local airway doses of subsequent inhaled allergen, consistent with our finding that 2-hour exposure to low-level LPS enhanced the immediate response to inhaled allergen¹⁰. The increased airway deposition (C/P and skew) with LPS challenge in

the AA was also similar to what we observed following dust mite challenge in a similar cohort of AA³.

In summary, these data show that a mild exposure to endotoxin produces an acute increase in airway deposition of inhaled particles in asthmatics likely associated with endotoxin-induced accumulated mucus in the airways. Our observation suggests that innate immune response of asthmatics to environmental agents such as LPS may modify deposition of bioactive particulate matter, enhancing response to such agents.

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List of Abbreviations

LPS	lipopolysaccharide
HV	healthy volunteer
AA	allergic asthmatic
M	male
F	female
CCRE	Clinical Center Reference Endotoxin
C/P	Central to Peripheral
FEV1	Forced expiratory volume in 1 second
MUC	mucin

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Figure 1.

Lung images for a representative AA subject. Activity in the right lung for images B and C are normalized to same total counts.

A (far left). Xenon equilibrium scan for one patient showing whole lung and central ROIs. Peripheral region is that area lying between the central and whole lung ROI.

B (middle). Deposition image on baseline, non-challenge study day, $C/P = 1.64$, skew = 1.70.

C (far right). Deposition image on LPS challenge study day, $C/P = 1.90$, skew = 3.32.

Table 1

Median (interquartile range) of regional deposition indices for each cohort on baseline and LPS challenge study days.

	C/P	Skew
HV		
Baseline	1.73 (0.58)	1.80 (0.74)
Post LPS Challenge	1.78 (0.52)	1.91 (0.61)
Post LPS - Baseline	0.08 (0.46)	0.04 (0.45)
AA		
Baseline	1.64 (0.28)	1.67 (0.49)
Post LPS Challenge	1.90 (0.50) [*]	1.78 (0.89) [#]
Post LPS - Baseline	0.18 (0.31)	0.37 (0.25)

^{*} P < 0.05 and

[#] P < 0.005 by Wilcoxon signed-rank test for baseline vs. LPS challenge.