

Letter to the Editor

Effect of inhaled endotoxin on regional particle deposition in patients with mild asthma

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 asthmatic (AA) patients compared with that seen in HVs.² Although the percentage of neutrophils in induced sputum was increased in both HVs and AA patients 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 after house dust mite inhalation challenge in AA patients despite the fact that spirometric function had returned to prechallenge values.³ Consequently, in the group of HVs (5 male and 13 female subjects) and AA patients (5 male and 8 female subjects) from the study by Hernandez et al.,² we also assessed the effect of inhaled LPS challenge (20,000 EU Clinical Center Reference Endotoxin) on regional deposition of inhaled particles after challenge as a sensitive index of mild heterogeneous changes in airway caliber that might not be evident from spirometry.³⁻⁵

Informed written consent was obtained from all subjects before their participation in the study, which 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, nonchallenge study day with that 4 hours after the LPS challenge day by using gamma scintigraphy (Fig 1, B and C) after controlled inhalation (tidal flow and breathing rate of 500 mL/s and 30 breaths/min) of ^{99m}Tc-sulfur colloid aerosol (mass median aerodynamic diameter, 5 μ m; geometric SD, 2.0). Regional particle deposition in the right lung was then determined based on the

central/peripheral (C/P) ratio of activity (primary outcome) and the skew of the histogram distribution (counts per pixel vs number of pixels) for the deposition images (Fig 1, B and C).⁶ A xenon (¹³³Xe) equilibrium lung scan (Fig 1, A) was recorded for each subject on the baseline visit to allow the creation of suitable regions of interest and normalization of the C/P ratio of particle deposition to account for differences in lung volume between central and peripheral activity,³ such as a C/P ratio of 1.0, which reflects equal deposition in each region. Increases in the C/P ratio to values greater than unity reflect an increase in central versus peripheral deposition, primarily as a result of increased bronchial deposition. We also assessed regional deposition heterogeneity as the skew of the histogram distribution (counts per pixel vs number of pixels)⁶ within the whole right lung region of interest, increasing with increased patchiness of deposition and frequency of “hot spots” in the lung and independently of the specific region within the lung (eg, central vs peripheral). Frequency distribution histograms were constructed from the right lung deposition images (ie, 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 calculated.⁶ Heterogeneity of deposition increases with increasing skew (ie, more pixels with high counts per pixel). The Wilcoxon matched-pairs signed-rank test was used for pre-LPS versus post-LPS and baseline versus post-LPS challenge comparisons. The study was powered on primary inflammation end points associated with the study by Hernandez et al.,² but the *n* for AA patients was also similar to that for which we observed a change in regional deposition associated with allergen challenge.³

There were no differences in pre-LPS versus post-LPS challenge lung function (FEV₁) for either HVs or AA patients for any time measured up to 6 hours after challenge. There was no significant difference in baseline C/P ratio or skew between HVs and AA patients. The AA patients showed an increase in bronchial airway deposition after endotoxin challenge compared with baseline values (increase in both C/P ratio and skew compared with baseline, *P* < .05; Table I). Fig 1, B and C, shows an example of a deposition image for baseline versus post-LPS challenge in an AA patients. On the other hand, there was no baseline versus challenge difference in regional deposition in the HVs (Table I).

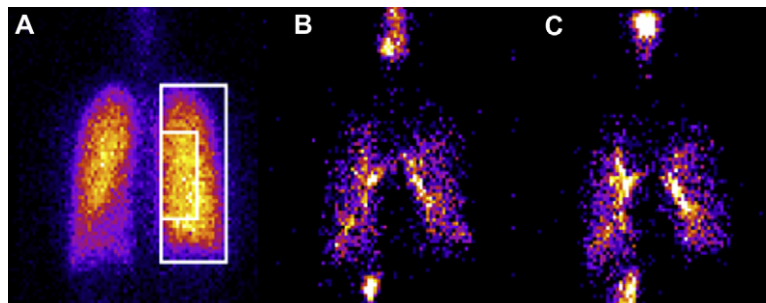


FIG 1. Lung images for a representative AA patient. Activity in the right lung for Fig 1, B and C, is normalized to the same total counts. **A**, Xenon equilibrium scan for 1 patient showing whole-lung and central regions of interest. The peripheral region is the area lying between the central and whole-lung regions of interest. **B**, Deposition image on baseline, nonchallenge study day (C/P, 1.64; skew, 1.70). **C**, Deposition image on LPS challenge study day (C/P, 1.90; skew, 3.32).

TABLE I. Regional deposition indices for each cohort on baseline and LPS challenge study days

	C/P ratio	Skew
HV's		
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 patients		
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)

Values are presented as medians (interquartile ranges).

* $P < .05$ and † $P < .005$, Wilcoxon signed-rank test for baseline versus LPS challenge.

We found that asthmatic patients showed a change in regional deposition associated with LPS challenge not seen in the healthy nonasthmatic subjects. We hypothesize that this might be due to increased secretion of mucus in the airways after challenge in asthmatic patients, leading to enhancement of deposition in these airways.⁷ LPS has been shown to stimulate the 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 asthmatic patients might result in increased particle deposition and therefore increased total and local airway doses of subsequent inhaled allergen, which is consistent with our finding that 2-hour exposure to low-level LPS enhanced the immediate response to inhaled allergen.¹⁰ The increased airway deposition (C/P ratio and skew) with LPS challenge in the AA patients was also similar to what we observed after dust mite challenge in a similar cohort of AA patients.³

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

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