

Jay M. Portnoy, MD
 Children's Mercy Hospitals & Clinics
 Kansas City, Missouri
 jportnoy@cmh.edu

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Ancient organisms and modern lifestyles

Human response to endotoxin has a long history in the immunology community. Nevertheless, the role of this biological polymer in the genesis and exacerbation of asthma remains unsettled. There are undoubtedly many discoveries related to endotoxin exposure and the human immune system yet to be made; a recent article by Sheehan and colleagues in *Annals*¹ make incremental but important contributions because it begins to place children's endotoxin exposure into the context of overall environmental exposure and asthmatic disease. Although we typically think of endotoxin exposure in agricultural and industrial environments, it is a substance that each one of us comes in contact with daily; at home, in daycare, in schoolrooms, and in the workplace.

Evolution of our understanding of endotoxin can be traced to *Limulus polyphemus*, an ancient organism that defended itself against the harmful effects of bacteria before mammals evolved. Its defensive mechanism centered on detecting the presence of endotoxin or lipopolysaccharide (LPS), substances common to many bacteria. Endotoxin entered the immunology sphere when animal experiments of the early 1960s indicated a single massive but sublethal dose given to young animals modified their endotoxin response as adults.² These experiments came to fruition with the awarding of a portion of a 2011 Nobel Prize to Bruce Beutler³ for demonstrating that toll-like receptor 4 is the LPS receptor. Endotoxin or its common constituent, LPS, are polymers on a similar theme but with varying components. Endotoxins are typically the size of small proteins (approximately 10 kDa) with polymeric aggregates sometimes approaching 100 times larger. Because they are identified more by their origin (bacteria) and recognition by *L. polyphemus* amoebocytes and human interleukin 14/toll-like receptor than for any structural homology, they are hard to quantify. Endotoxin is found nearly everywhere. Temperatures greater than 250°F for 30 minutes are required to destroy 99.9% of endotoxin even on clean glassware. An excellent history of the use of *Limulus* in the detection of LPS can be found on the Marine Biological Laboratory internet site.⁴

Paracelsus is credited with saying, "All things are poison, and nothing is without poison; only the dose permits something not to be poisonous." Endotoxin seems to fit this saying, because long-standing experiments with endotoxin have not fully determined whether it is a boon or a bane. Additionally, it is not merely the dose but also the route of exposure (intravenous or inhaled) that determines its significance. Important human-re-

lated effects of varying dose ranges and routes of exposure of endotoxin include: intravenous injection of nanogram amounts⁵ causes fever, and inhalation of large amounts produces asthma symptoms.

When inhaled by individuals with allergic asthma, endotoxin can exacerbate airway obstruction and inflammation⁶; and allergic individuals have heightened response to inhaled endotoxin.⁷ Experimental airway exposure to LPS in humans with asthma and mice modulates allergen response.⁸ However, mere exposure to endotoxin-containing dust does not produce airway disease. Factors including the genetic background of the exposed organism as well as the timing of the exposure appear to determine whether endotoxin has a positive or negative influence. Environmental endotoxin exposure does not occur alone but is accompanied by protein allergens, beta glucans, airborne fungi, and bacteria as well as communicable diseases, these days mostly of a viral origin.

With all of the contradictory information concerning endotoxin, and in light of the hygiene hypothesis, the reader likely wonders whether more endotoxin exposure might be better. Is exposure to an "endotoxin soup" good for the patient with asthma when viewed from a larger perspective? Endotoxin exposure is certainly higher in crowded environments such as Mumbai and Mexico City; however, these places have not been historically noted for high rates of asthma. The absence, in the present study, of a seasonal variation in endotoxin is interesting in light of the common belief that asthma attacks occur more frequently when school starts in the fall as outdoor aeroallergens peak and viral exposure increases. Perhaps inner-city children are better in school as opposed to being in their homes with multiple asthma triggers (smoke, allergens, cleaning agents, and so forth). Maybe the endotoxin helps them, or maybe it does not make any difference.

The study methodology is primarily designed to understand the sources and exposure of school endotoxin. The possibilities are that school endotoxin levels are derived from home endotoxin carried by the children, or there is some endogenous source of endotoxin production associated with the school itself. Perhaps crowding or the population density of the classrooms is a factor. The same factors are probably not operating in all schools, because 2 of the 12 schools have lower exposures, and 2 of the schools have essentially the same exposures as homes. Investigators did not offer any explanations why schools 10 and 11 had less endotoxin. The correlation between school cat and dog allergen levels and increasing dust endotoxin levels is interesting, because we presume cat and dog allergen is brought from

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home by many students. One may easily assume that the higher bacterial levels associated with materials shed or excreted by these animals would be major contributors to endotoxin levels. Perhaps, as the author's state, low cat and dog ownership in the population with asthma resulted in lower levels in those homes. Also, the absence of cockroach allergens may be viewed as a factor limiting the universal application of the findings.

Poor uniformity in endotoxin measurements between laboratories makes comparisons with other studies difficult. The Limulus assay is used mostly in the pharmaceutical industry to ensure non-pyrogenicity for injected preparations. Attempts to standardize endotoxin assays between laboratories have not been successful. Only results produced by the same laboratory should be compared directly. The authors recognize that their median endotoxin concentrations (approximately 13 EU/mg dust in schools and 7 EU/mg in homes) are lower than other studies (18–80 EU/mg 95% CI for 2,552 homes⁹), but levels were generally lower in bedrooms. However, the reader is left to wonder whether the difference of 6 EU/mg dust makes a real difference in exposure when natural levels can be 1,000 times higher. The answer to this speculation remains unknown, but it is necessary to perform comparative studies such as the one by? to attempt to make sense of the complicated endotoxin exposure puzzle.

Charles S. Barnes, PhD
Chitra Dinakar, MD
Section of Asthma
Allergy and Immunology
Children's Mercy Hospital
Kansas City, Missouri
barnescs@umkc.edu

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