

REVIEW

Modulating the early allergic response with endotoxin

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'It would seem that hay fever has, of late years, been considerably on the increase...The persons who are most subjected to the action of the pollen belong to a class which furnishes the fewest cases of the disorder, namely, the farming class.' Blackley CH. Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay fever or Hay asthma), 1873 [1].

In Charles Blackley's seminal publication of his research revealing the role of pollens in hay fever, there is a lesser known observation, in a chapter entitled, 'On the greater prevalence of hay fever and on the increase of its predisposing and exciting causes', of the paradox that those with the greatest exposure to the pollens — farmers — rarely manifest the symptoms of hay fever [1]. Nearly 125 years later, it seems that some researchers have picked up where Blackley left off, in search of explanations for this paradox on the farms. Numerous atopy prevalence studies in modern farming communities (including several papers published recently in *Clinical and Experimental Allergy*) have consistently revealed primarily less allergic rhinitis and allergen sensitization, and some have reported less asthma, in children raised in farming communities [2–6].

Thus, the farming community environment and lifestyle seem to have a protective influence on atopic disease. While the possible explanations are many, Von Mutius and colleagues [7], have further explored the potential of environmental endotoxin to mediate this effect, by Th1-type induction. Endotoxin (a cell wall component of gram-negative bacteria) is a potent inducer of the cytokines supportive of Th1-type development: IL-12 and IFN γ [8,9]. What other evidence currently supports this hypothesis?

Can environmental endotoxin induce Th1-type immune development and abrogate allergen sensitization?

In a study we recently published in *The Lancet* [10], we investigated a potential atopy-protective effect of chronic endotoxin exposure in infants (9–24-month-old) with at least three provider-documented episodes of wheezing. Infants found to be allergen-sensitized (by prick skin testing) had significantly lower concentrations of endotoxin in their house-dust samples (Fig. 1). Importantly, in a random subset of subjects, house-dust endotoxin levels correlated with the proportions of Th1-type cells (i.e. IFN γ -producing CD4⁺ and CD8⁺ cells) in their peripheral blood (Fig. 2), providing an immune correlate that strengthens the hypothesis of Th1-type induction by chronic endotoxin exposure. Thus, this study raises the possibility of Th1-type induction in early life, separate from serious infection, as a means to down-modulate Th2-type immune development, and prevent atopy and possibly asthma.

In search of a common thread

A lower prevalence of atopy and asthma has also been demonstrated in children raised in places other than farms, primarily in children raised in rural areas of developing countries. For example, compelling studies in Africa have exploited exercise-induced bronchospasm (EIB) as an objective measure of asthma, and in comparing the prevalence of EIB in Africans living in their rural native homelands and homesteads, compared with those living in a nearby metropolitan area or in relative affluence, the rural natives are nearly EIB-free [reviewed in 11]. The logic of Th1-type induction in early life being atopy-protective has seemed most fitting for life in rural areas of developing countries, where public hygiene measures are substantively lacking, and thus serious chronic infections in childhood are expected to be the norm. Indeed, in studying disease prevalence in these rural areas of developing countries, the possibility of survivor bias is a concern, meaning that those who die from serious infections may be the ones who

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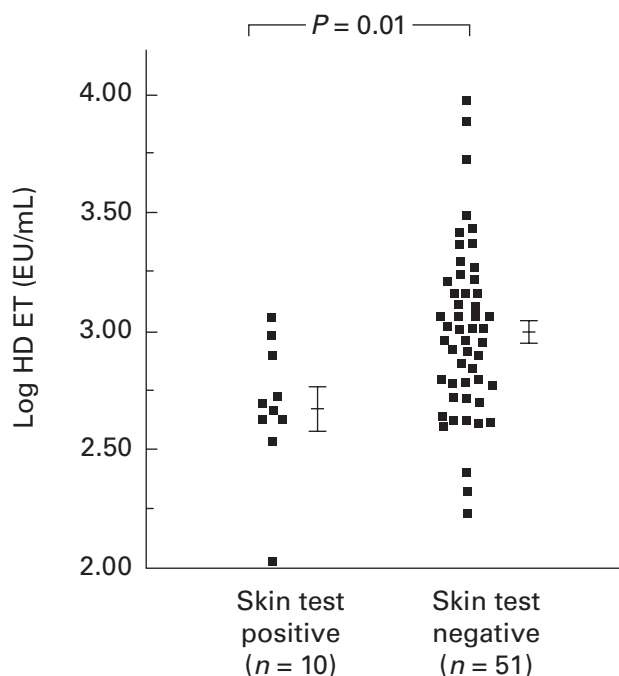


Fig. 1. House dust endotoxin concentrations (HD ET) in allergy skin test positive and skin test negative infants with repeated wheezing episodes. Sixty-one infants (9–24-month-old) with at least three provider-documented wheezing episodes were allergy skin-prick tested to common inhalant and food allergens. Endotoxin levels were measured in house-dust extracts using a Limulus amoebocyte assay-based method standardized by the United States Food and Drug Administration. Log transformation of HD ET values normalized the distribution of the data, allowing for parametric statistical analysis (*t*-test). Reprinted with permission from [10].

would have gone on to have atopy and asthma. This is exemplified in the study in Guinea-Bissau, West Africa of measles infection associated with less risk of atopy (by skin testing) [12]. However, 25% of the children of Guinea Bissau died from this measles epidemic. One might glean from this example that studies that can identify Th1-type induction exposures, separate from potentially fatal infections, may serve to clarify a possible atopy-protective effect.

Life on the farm, with endotoxin

Thus, the farm provides fertile ground for more than crops and livestock. From a public hygiene standpoint, modern farms are presumably much closer to how most people live in modernized metropolitan areas. If so, then farming communities may provide an environment to further study atopy- and asthma-protective mechanisms that do not seem inherently harmful.

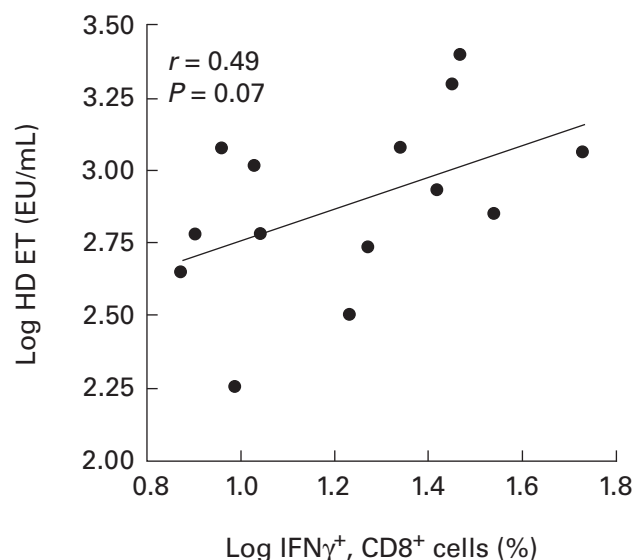
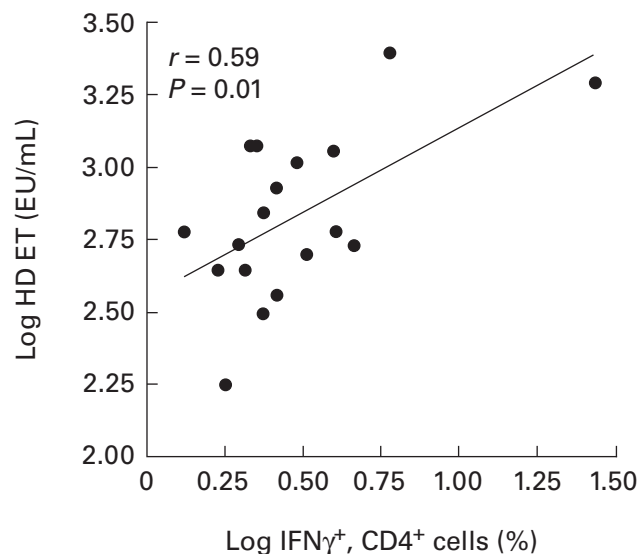


Fig. 2. Correlations of house dust endotoxin (HD ET) concentrations with proportions of IFN γ -producing CD4 + and CD8 + T lymphocytes from infant peripheral blood samples. Proportions of T-lymphocytes primed to produce IFN γ were measured in peripheral blood samples by cell-surface and intracellular cytokine staining after a brief 4-h stimulation, with flow cytometry. Log transformation of HD ET values and cell percentages normalized the distributions of the data, allowing for parametric statistical analysis (pairwise correlation, *r* = Pearson coefficient). Reprinted with permission from [10].

One common theme in some of the recent farm studies has strengthened the association of atopy protection with livestock exposure, either by regular animal contact reported for the child [4–6], or part-time vs full-time

farming by the parent [3]. If endotoxin exposure, as a potential Th1-type inducer, is increased as a result of close contact with livestock, then could endotoxin be making an atopy-protective impression on the farms? The paper by Von Mutius and colleagues in the September edition of *Clinical and Experimental Allergy* [7] is the first to document higher levels of house-dust endotoxin in farm homes vs others. Further details of this investigation are interesting: (i) dust from different places in homes were sampled, revealing much higher levels of dust endotoxin in bedding than from kitchen floors. It is intriguing to speculate that, like indoor allergen exposure, we attribute greatest controllable indoor exposure to what is in the bedroom, and particularly in the bed, where the most indoor time is spent each day; (ii) the air sampling is also a valuable entry, taking advantage of much that is already known about airborne dust endotoxin exposure and asthma or obstructive lung disease in occupational settings [reviewed in 13]. Indeed, personal endotoxin monitoring has become the standard in occupational asthma assessments, and when applied to this question of environmental endotoxin and atopy protection, air sampling (ideally personal) may help to clarify if endotoxin may be inducing a protective effect directly in the airways, or if house-dust endotoxin is more a marker of endotoxin exposure through other means (e.g. GI tract).

Indeed, amongst the rural poor, intimate living with animals is nearly a given. Therefore, endotoxin may be a common thread of the environments and lifestyles on the farms and in rural areas of developing countries, sharing an intimate relationship of humans with their livestock. However, if endotoxin has such an atopy-protective effect, it may be more difficult to observe in rural areas with poorer public hygiene and presumably a greater Th1-type influence due to more serious and chronic infections. It is on the farms that we might envision a better chance of observing a potential atopy-protective effect owing to endotoxin, separate from a relatively high volume of serious infections. Similarly, one might wonder if this may also be true for pet-owners living in modern urban communities, considering several recent reports of less atopy among children raised with pets (e.g. cats, dogs) [14–16].

Other noteworthy differences in the studies between rural areas of developing countries and European farming communities (perhaps meriting deeper investigation) include: (i) the European farm studies are questionnaire-based for atopic disease diagnoses [3–6], whereas in rural studies, and especially Africa studies, diagnoses of asthma and atopy are mostly based on EIB and/or physical exam [reviewed in 11]; and (ii) the European farm studies report primarily an effect on airways atopic disease, not eczema, and a relatively weaker effect on asthma — does this

suggest a Th1-type protective mechanism primarily in the airways, owing to local airways exposure? It seems plausible that the highest deposition of dust-bound endotoxin would be in the nasopharynx, where its local atopy-protective action may therefore be the strongest. In animal models utilizing Th1-type induction to prevent the development of the asthmatic phenotype, comparing modes of BCG administration reveals greater efficacy when BCG is administered in the nasopharynx vs either subcutaneously or intraperitoneally [17]. Alternatively, increased environmental endotoxin may be a marker of bacterial colonization (e.g. GI tract and skin colonization, of humans and their associated animals) by primarily endotoxin-containing bacteria, or by bacteria with higher endotoxin content [18].

Endotoxin — teasing out the good from the bad

In today's scientific arena, endotoxin is not alone as a microbial derivative being explored for its Th1-type inductive capacity, without necessitating serious infection. For example, mycobacterial vaccinations and bacterial CpG-motif immunostimulatory DNA are currently being pursued in human studies as a means of guiding immune development away from atopy, sparing infection [19–22]. Whether these current attempts at Th1-type interventions are early enough in disease development to be optimally effective will become clearer with time.

There are also concerns. For example, endotoxin augments asthma and allergies [23–25]. Indeed, allergen exposure in sensitized asthmatics increased endotoxin receptor/enhancer binding protein levels (i.e. lipopolysaccharide-binding protein, soluble CD14) in the airways considerably, providing a potential mechanism for increased airways sensitivity to endotoxin [26]. In this regard, a new paradigm for the role of specific environmental exposures in atopy and asthma is emerging: can an exposure like endotoxin augment most aspects of allergy and asthma in patients with established atopic disease, and yet provide a protective influence on immune development prior to disease onset? The potential double-edged sword of endotoxin in atopy and asthma is well-modelled in a recently published study in a rat-induced asthma model, demonstrating that a single dose of inhaled endotoxin (lipopolysaccharide, LPS) exposure, either before or within the first 4 days of sensitization to ovalbumin, abrogated the asthma phenotype; however, from day 6 and later, nebulized LPS augmented many manifestations of the asthma phenotype, from airways hyperresponsiveness to inflammation [27].

Equally exciting then, is recently published work that identified a polymorphism in the endotoxin receptor, CD14, promoter region and its association with varying

levels of soluble CD14, IgE, and allergen sensitization [28]. Taken together, these studies suggest that immune responsiveness to environmental endotoxin may provide a valuable opportunity to study gene–environment interactions underlying atopy/asthma development, where relevant gene targets have been elucidated, and both the immune response and the environmental exposure to endotoxin can be quantified.

This wealth of recent research, cited above, suggests that the important next steps in understanding the role of endotoxin exposure in atopy and asthma prevention are assuredly not lost amongst those well positioned to carry out rigorous research in this area. We are in need of well-controlled disease outcomes studies that strengthen the connection between higher levels of environmental endotoxin with less atopy and asthma. Ideally, the addition of other disease outcomes potentially influenced by such Th1-type immunomodulation, such as severe responses to infections and autoimmune diseases, will attempt to discern if Th1-type induction in early life is not just effective, but also safe. If endotoxin or similar avenues of Th1-type induction appear promising, then other important questions to optimize atopy-preventive intervention include: (i) dosing that is effective and safe; (ii) mode of exposure or administration; (iii) when in disease development (e.g. how early in childhood); and (iv) who will likely acquire a protective influence from environmental endotoxin exposure (e.g. enviro-genetics). It is exciting to speculate that, should these and/or other atopy and asthma prevention research identify effective prevention strategies, we may see a reversal in the global rise in allergy and asthma prevalence, in time for the 150th year anniversary of Blackley's publication noting the rising prevalence of hay fever.

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