

Hygiene hypothesis and endotoxin: what is the evidence?

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Purpose of review

The hygiene hypothesis has gained strong support over the past few years. Exposure to microbial products in early life could be an underlying factor in this hypothesis, but the mechanisms that lead from a less clean and more crowded environment to a lower prevalence of asthma and allergies are not known. Among the variety of potential microbial molecules that may confer protection against the development of asthma and allergies, endotoxin, a component of Gram-negative bacteria, has incited lively as well as controversial discussions. This review focuses on recent studies on endotoxin and its role in the context of the hygiene hypothesis.

Recent findings

Results from cross-sectional surveys, prospective cohorts, and experimental studies *in vitro* and in rodents suggest that exposure to house dust endotoxin in early life protects from atopic sensitization and IgE-mediated diseases, but is a risk factor for wheezing in infancy.

Summary

Numerous studies have supported the hygiene hypothesis, but whether endotoxin by itself confers the protection or whether it acts as a marker for another environmental exposure is still unclear. The challenge for the future will be to identify those factors that confer the protection proposed by the hygiene hypothesis, and to find strategies to modify the environment without causing harm to susceptible individuals.

Keywords

asthma, atopy, childhood, endotoxin, hygiene

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Abbreviation

Th T-helper

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Introduction

Although there has been a change in perception and the procedures for diagnosing asthma and allergies over the past decades, there is evidence of an increase of these diseases in high-income countries [1]. During the last century improved healthcare, less crowded living conditions, and more cleanliness in homes have led unequivocally to a dramatic decrease in infectious diseases in children. It was a thought-provoking idea when in 1989, Strachan [2], after finding a strong inverse association between hay fever and the number of siblings in his study population, suggested that 'infections in early childhood transmitted by unhygienic contact with older siblings or acquired prenatally from a mother infected by contact with her older children' might actually prevent allergic diseases. Since then, studies in different populations have consistently confirmed the inverse association between the number of siblings and atopy [3–8], implying a strong underlying determinant for these immune responses. The sibling effect is stronger for the number of older siblings than for the number of younger siblings, but it is still unclear whether this effect has its origin in fetal life [7,9,10], or whether it is a consequence of the enhanced exposure to siblings with infectious diseases in infancy.

Daycare attendance represents another surrogate for exposure to a crowded and more infectious environment in early life, and it is also inversely related to the development of atopic sensitization, hay fever, and asthma [6,11]. Subsequent studies to identify infections as the explanatory protective factor for the sibling and daycare attendance effect have shown conflicting results. Some studies found no effect of common specific and non-specific infectious diseases in early life [12,13], whereas others showed a strong inverse relationship between serological markers of previous infections with food-borne or oro-fecal pathogens, such as hepatitis A, and *Toxoplasma gondii* and the prevalence of atopy [14,15,16•]. As those diseases are more easily spread under crowded and more unhygienic conditions, they represent not only exposure to infectious diseases, but also generally higher exposure to bacterial products that may confer the protective effect stated by the hygiene hypothesis. A variety of microbial molecules are potential candidates but so far only endotoxin, a group of lipopolysaccharides on the surface of Gram-negative bacteria, has been studied extensively in this context.

Endotoxin in the context of the hygiene hypothesis

It is a provocative idea to consider endotoxin as potentially protective against the development of asthma and allergies, because its adverse health effects have been studied for years in industrial workers exposed chronically to high concentrations [17], and who develop severe respiratory diseases, including byssinosis and mill fever. Endotoxin has strong pro-inflammatory properties, and when inhaled it elicits bronchoconstriction, neutrophilic airway inflammation and systemic responses, such as blood leukocytosis with neutrophilia, in otherwise healthy individuals [18]. Furthermore, patients with asthma are hypersensitive to endotoxin compared with non-asthmatic individuals, and the severity of the disease is positively correlated with the amount of endotoxin in their home environment [19]. The inflammatory responses to endotoxin are mediated by the interaction between its lipid A component and receptors of the innate immune system, which leads to a strong T-helper (Th) 1-type immune response [20•]. It was only recently that apart from the harmful effect in chronically exposed adults or asthmatic individuals, the Th1-promoting effect of endotoxin was considered to be potentially protective, if the exposure happens to occur very early in life and before the inception of asthma and allergies. In early life when the immune system is still maturing, the induction of a strong Th1-type response might favor immune responses away from Th2-type responses, such as asthma and allergies [21]. This idea was strongly supported by in-vitro experiments that described a counteracting mechanism between Th1 and Th2 immune responses. In support of this contention, endotoxin in house dust samples was found to be higher in environments previously described to be protective against the development of asthma and allergies in children, such as in house dust samples from large families [22•], in daycare centers [23•], and also in the homes of farmers [24,25••] and pet-owners [22•,26]. However, only a few studies have related endotoxin exposure early in life to the frequency of asthma and allergies.

Exposure to house dust endotoxin is inversely related to atopy in children

Cross-sectional surveys have shown a dose-dependent inverse relationship between exposure to house endotoxin and atopy. One was carried out in a rural population in Austria, Bavaria (Germany), and Switzerland in school-aged children [25••]. House dust endotoxin was found to be significantly inversely related to the frequency of atopic asthma, hay fever, and allergic sensitization, but no such association was seen for the non-atopic form of asthma. In the same study, an inverse relationship was found between the level of endotoxin exposure and the capacity of peripheral-blood leukocytes

to produce IFN- γ , TNF- α , IL-10, and IL-12 after stimulation with lipopolysaccharide, indicating a generally downregulated immune response in children with heavier exposure compared with children with less exposure.

Another cross-sectional study [27••] also measured house dust endotoxin in a group of 5–10-year-old children, half of whom had either a diagnosis of asthma or were atopic, as assessed by a positive skin-prick test or any specific IgE of 0.35 kU/l or greater to common inhalant allergens, and half of whom had no asthma or atopy. In these children, an increase of two geometric standard deviation values of house dust endotoxin was associated with a decreased odds ratio for sensitization against any of the allergens tested. The effect of endotoxin was of borderline significance when using the lowest cut-off value of 0.35 kU/l for specific IgE as the definition of sensitization, and was increasingly strengthened using the higher cut-off levels of 0.70 and 3.5 kU/l, respectively. No relationship was found between asthma and hay fever, but as discussed by the authors, the number of cases of these diseases was too low to yield sufficient statistical power to detect an association.

Cross-sectional studies are limited in their capacity to describe the chronological order of the manifestation of the phenotype in relation to exposure and to rule out reverse causation. A prospective study addressed this issue and compared the incidence of allergic sensitization and allergic diseases in relation to exposure to house dust endotoxin in Swedish and Estonian children. The Estonian population is known to have lower prevalences of atopic sensitization and allergic diseases than the Swedish population [28,29], and the Estonians are assumed to live a similar lifestyle to that seen in western Europe 30–40 years ago, before the marked increase in asthma and allergy was observed. Endotoxin levels in the Estonian households were higher than in the Swedish households, but only in the Swedish children was an inverse relationship between the levels of house dust endotoxin in the first year of life and the development of asthma, atopic dermatitis, and sensitization at the age of 2 years found [30•]. It is unclear whether this discrepancy can be explained by variations in the genetic background, by a maximum environmental effect of endotoxin in the Estonian children, by differences in co-exposure to allergens, or by endotoxin as a marker for exposure to two qualitatively different microbial environments in the two populations.

Exposure to endotoxin may already influence the immune system prenatally. In a prospective birth cohort study [31], endotoxin measured from the mother's mattress was inversely related to IgE in cord blood among those with detectable concentrations of IgE,

supporting the idea of an influence of house dust endotoxin on fetal T cells. The same study group showed on a cellular level that this early endotoxin exposure was positively associated with the proportion of Th1 cells at the age of 2 years [26]. However, atopic sensitization to inhalant allergens, defined as specific IgE of 0.35 kU/l or greater, was found in 5% of the children by the age of 2 years and showed no association with exposure to endotoxin [32••]. It seemed rather that children with atopic parents were even more likely to produce specific IgE when heavily exposed. However, it might be premature to conclude that early endotoxin exposure has no protective effect against atopy in this population. The production of specific IgE to inhalant allergens increases sharply with age, and those children sensitized against common inhalant allergens by the age of 2 years might represent a group with a strong genetic predisposition that responds differently to endotoxin to those without a strong atopic genotype.

Exposure to house dust endotoxin is positively associated with wheezing in infancy

The protective effect of siblings, daycare attendance, and endotoxin is more consistent for atopic sensitization and atopy-related diseases than for all forms of asthma. Asthma itself is a heterogeneous disease with differences in the genetic and environmental determinants, and only a part of the asthma phenotype is attributable to atopy [33•]. Wheezing in the first years of life is unrelated to atopy in the majority of children, and endotoxin seems to be a risk factor. In a birth cohort study [34] there was a clear positive association between exposure to house dust endotoxin and wheezing in the first year of life in 500 children of parents with asthma or allergy. A similar result was seen in another cohort study including more than 1800 children [35], in which the odds ratio for wheezing was significantly higher in the highest quintile of endotoxin compared with the lowest quintile throughout the first year of life, despite a decreased odds ratio for atopic dermatitis at age of 6 months. A recent longitudinal study in 226 children of parents with asthma or allergy [36••] confirmed high endotoxin as a risk factor for wheezing in the first year of life, but over the follow-up period the risk rapidly decreased, and by the age of 4 years the proportion of children with wheezing episodes was no longer higher in children heavily exposed to endotoxin compared with those not heavily exposed. Over the next few years these ongoing longitudinal studies will answer the open questions about the long-term effects of exposure to house dust endotoxin in wheezing infants, and whether these children or a subgroup of them might benefit from a potential protection from allergic sensitization later in life.

The role of lipopolysaccharide on allergic sensitization in murine experiments

Although murine experiments are limited in the extent to which the results may be relevant to the human organism, they have revealed a valuable insight into the role of the timing and dosage of lipopolysaccharide in the process of allergic sensitization.

BALB/c mice are known to be genetically determined to have a high Th2 response, but systemic lipopolysaccharide administration before ovalbumin sensitization reduced total and specific IgE serum levels, Th2 cytokine production, and the extent of airway eosinophilia compared with those sensitized without previous lipopolysaccharide administration [37]. The same study group showed that an enhanced Th2-mediated immune response was the outcome when mice were pretreated with repeated low-dose inhalation of lipopolysaccharide instead of systemic administration [38]. The effect of administering the antigen together with different levels of lipopolysaccharide on the inflammatory response was recently shown in another study [39•]. Intranasal sensitization in mice by using lipopolysaccharide-free ovalbumin showed no airway inflammation after challenge with inhaled ovalbumin as did previous exposure to lipopolysaccharide alone. However, when mice were sensitized with ovalbumin together with a low dose of lipopolysaccharide, a Th2-mediated inflammation with high levels of locally produced IL-5 and IL-13, eosinophilic airway infiltration, and the production of ovalbumin-specific IgE and IgG1 was seen. In turn, using a high dose of lipopolysaccharide during intranasal ovalbumin priming resulted in a Th1-associated response, with the production of IFN- γ , neutrophilic inflammation of the bronchial mucosa, and the production of ovalbumin-specific Th1 isotype antibodies.

Another murine experiment used the cat antigen Fel d 1 for sensitization, and a strong elevated Th2 and Th1 isotype antibody response was achieved when the mice were primed simultaneously with Fel d 1 and lipopolysaccharide [40•]. When the immune system was introduced to lipopolysaccharide before the combination dose of lipopolysaccharide and Fel d 1, the response was skewed in the direction of a weaker Th1 and a slightly elevated Th2-dependent antibody response, and lipopolysaccharide given alone before or subsequent to Fel d 1 given alone did not alter the antibody response.

These experiments led to the speculation that the ratio rather than the individual level of allergen exposure and lipopolysaccharide exposure determines the direction of the immune response. This idea is supported by the finding that the children of farmers are not only found to be exposed to considerably higher levels of endotoxin than their non-farming peers, but also to significantly

higher levels of house dust mite, yet they are not more often sensitized [25•,41]. Given that exposure to endotoxin in a steady environment is fairly constant, the finding of a positive association between high exposure to birch pollen during the first year of life and subsequent atopic sensitization against this allergen and atopic diseases would reflect this concept in a similar way [42].

Lipopolysaccharide activates T regulatory cells

In the context of the hygiene hypothesis, the Th1/Th2 paradigm has been a useful model and has influenced our concept of the pathogenesis of asthma and allergic diseases over the past few years. The paradigm stated that the Th1 and Th2 immune responses counteract each other, and thus the stimulation of a strong Th1 response during the development of the immune system would be able to modify responses away from those of the Th2 type.

Recent findings have suggested that this model, initially derived from in-vitro and murine experiments, is too simplistic in humans, and instead of a Th2-type immune response as a result of lacking a strong Th1 stimulation, asthma and allergic diseases are rather the result of a dysregulation of the immune system [43]. Central in this new concept are T regulatory cells balancing both Th1 and Th2 responses. Initially, these cells have been studied in the context of autoimmune diseases and self-tolerance, but distinct subsets of T-regulatory cells secrete IL-10 and transforming growth factor beta, and both cytokines are related to the suppression of allergic airway inflammation [44,45]. In a murine model, T-regulatory cells were found to express Toll-like receptor 4, which is part of the endotoxin receptor complex, and exposure to endotoxin promoted T-regulatory cell survival and proliferation and enhanced their suppressive functions [46•]. On explanted nasal mucosa from non-atopic children, the local application of lipopolysaccharide not only induced a Th1-type immune response, but also the activation and proliferation of T cells, carrying surface proteins characteristic for immunoregulatory T cells [47•]. Such studies strongly supported the concept that exposure to microbial molecules such as lipopolysaccharide, interacting with the innate immune system, modify the allergic responses by promoting T-regulatory cells.

A variety of other microbial cell components also have powerful immune modulatory properties, but little is known about their role in the development of asthma and allergies in children. Natural exposure to endotoxin is correlated with exposure to other microbial products such as beta(1→3)-glucan from mold cell walls [48] and bacterial DNA [49•], and each of them can represent a

marker for the general microbial load of the environment from which the dust sample was collected. It might also be that different microbial molecules interacting with the innate immune system at a critical timepoint during the maturation of the immune system may lead additively or synergistically to a commonly defined phenotype.

Conclusion

Epidemiological studies have suggested that exposure to house dust endotoxin is inversely associated with atopic sensitization against common aeroallergens, hay fever and atopic asthma. No such relationship was seen for the non-atopic form of asthma. The lack of an inverse association between asthma independently of atopic sensitization raises the possibility that endotoxin protects against allergic sensitization but not against asthma. It also appears that the endotoxin effect is more strongly related to the severity of sensitization than to the prevention from any sensitization. The underlying molecular mechanisms for the observed relationship are still unclear and need further elucidation. Bearing in mind that no prospective studies on the long-term effects of endotoxin exposure on healthy or wheezing infants are available, caution in recommendations is clearly indicated. The challenge for the next few years will be to identify other factors that might explain the protective effect proposed by the hygiene hypothesis.

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