

EDITORIAL

The endotoxin paradigm: a note of caution

Introduction

Endotoxin is a term often used loosely to describe a group of lipopolysaccharides, found on the cell surface of gram-negative bacteria.

For years they were considered as purely pathogenic and detrimental to health.

Inhalation of high levels of endotoxin has been blamed for a large number of respiratory diseases, which range from byssinosis, humidifier fever, mill fever and grain fever (illnesses with a characteristic 'Monday' effect, thought to be due to a tolerance effect of endotoxin on repeat exposure) to almost every respiratory symptom seen on exposure to organic dusts (non-specific work related symptoms, toxic pneumonitis, organic dust toxic syndrome).

Early epidemiological work in the home environment demonstrated a relationship between reservoir concentration of endotoxin and severity of asthma in patients sensitized to dust mite allergens [1].

In the late 1990s the possibility of a beneficial role of endotoxin in promoting the Th1 phenotype and therefore protecting from atopic disease was first considered. Studies initially in farming communities [2–6] and to a lesser extent within different urban or mixed communities [7, 8], including work presented in the current journal [9], have led to the belief that endotoxin exposure at higher levels is associated with less atopic sensitization and might be the saviour from the growing epidemic of asthma in the developed world. So much so one can almost imagine the stampede of the hasty and possibly unwise (researchers and parents of at-risk children) to the door of endotoxin production plants, wishing to expose infants in order to prevent the development of atopic disease. Bearing in mind the clear pro-inflammatory potential of endotoxin, such a response might represent an excessive or premature reaction.

Lessons from occupational settings

Despite over 30 years of research, a burden of proof of a cause and effect relationship between endotoxin exposure within organic dusts and respiratory disease remains elusive. Although there is strong correlation between exposure and disease, uncertainty arises because, where endotoxin abounds so do a large number of other agents (including gram-positive bacteria and their toxins, fungi with both allergenic and toxic potential and thermophilic organisms) and the basic organic dust (containing other contaminants with physiological potential such as histamine, tannins and other chemotactic chemicals) [10, 11]. The finding of epidemiological cohorts with both the exposure (endotoxin separable from other agents) and statistical potential to elucidate the aetiological role of endotoxin from that of the other agents is likely to prove unachievable.

Adverse effects of endotoxin exposure

The potential of high concentration exposure to endotoxin to cause adverse effects have been undeniably demonstrated by *in vitro*, animal and human exposure studies. High concentration exposure to endotoxin causes bronchial hyper-responsiveness, bronchospasm, pyrexia and ultimately neutrophilic airway inflammation (with measurable systemic neutrophilia) [12, 13]. An unusual feature is the tolerance to repeated exposure to endotoxin demonstrated from *in vitro*, animal and human challenge studies. The tolerance could conveniently explain the temporal features of the 'Monday' (or first dose) diseases (byssinosis, humidifier fever, grain fever and mill fever) [14, 15].

The release of specific cytokines on exposure to endotoxin has been demonstrated for IL-8, IL-10 and IL-12 [16, 17]. CD14 receptors (in conjunction with Toll-like receptors) are the central receptors responsible for the effects of endotoxin [17]. CD14 receptor activity has been shown to increase on immediate exposure to endotoxin and has been measured at higher levels during acute attacks of asthma in children than at recovery [18]. The constitutive level of CD14 has been shown to be able to predict the magnitude of the neutrophilic inflammatory response on exposure to endotoxin [19]. Such data may lead to an explanation to individual susceptibility to the adverse effects of endotoxin, which are apparent in both occupational studies and asthma subjects [20].

Endotoxin and the domestic environment

It has been known for some time that endotoxin is present in reservoir dust samples (and to a lesser extent air samples) collected from homes, admittedly at exposure levels log doses below that experienced in the working environment. Studies of existing asthma sufferers have variably implicated increasing levels of endotoxin exposure, resulting in an increased severity of asthma [1].

Studies of the indoor environment have similar problems to those performed in occupational settings. The home environment is also a complicated milieu of allergens, pollutants and toxins. Endotoxin may just be a convenient assayable toxin with minimal health effect potential (at the relevant exposure levels). A toxin derived from fungi (1-3-beta-D-glucan), was identified as being related to peak flow variability in asthmatic children, while endotoxin was not significant when the effect of glucan was accounted for [21]. A study of adults demonstrated relationship between symptoms and exposure to glucans in adults with no demonstrable effect of endotoxin [22].

Endotoxin challenge studies have demonstrated a synergistic response of mixed endotoxin and allergen exposure. In a planned allergen challenge study with ragweed, the allergen

extracts were inadvertently contaminated with endotoxin. Challenge with this combination resulted in over-riding neutrophilic airway reaction that occurred in subjects, who responded with eosinophilic allergen response when challenged with extract free of endotoxin [23]. The cellular responses were similar to those seen in subjects with chronic uncontrolled asthma rather than acute allergic asthma. A study of cellular responses following nasal challenge showed a greater response when allergen and endotoxin were combined in challenge compared to allergen or endotoxin alone [24].

Endotoxin as a preventor of sensitization

The possibility of a protective effect of low-dose exposure to endotoxin against the initial development of asthma, atopy and the Th2 phenotype, originated from initial views on the hygiene hypothesis [25] and the findings of reduced risks of atopic disease in a wide variety of farming populations [2–6]. A further study from Australia has suggested this protective effect is limited to animal farming and does not extend to arable farming [26]. Exposure to endotoxin will occur in both types of farming, though the dose and temporal (seasonal) cycle of exposure to endotoxin may vary significantly.

Farming studies

Endotoxin levels have repeatedly been demonstrated as being greater in farming than urban communities and that the level of endotoxin is statistically related to lower levels of atopic disease expressed by skin prick testing, atopic symptoms including eczema [27] and the production of certain cytokines TNF- α , IFN- γ , IL-10 and IL-12 [28]. Recently, farmers' children were shown to express higher levels of CD14 and a 'Toll-like receptor 2' in peripheral blood cells. Bearing in mind the evidence of the modulation of CD14 in endotoxin exposure in occupational environments, the postulation was made that repeated exposure to endotoxin and other microbiological agents could be responsible for influencing the immune response specifically to allergens, reducing the risk of sensitization by inducing a Th1 phenotype response [29].

Unfortunately it remains unproven that it is specifically endotoxin of all the possible microbiological exposures experienced by farming children that is responsible for the protection afforded against atopic sensitization. As in the occupational setting it could be that endotoxin is just one of a complicated microbiological milieu responsible for the immune response differences and that the endotoxin is just a convenient, measurable marker of the environmental exposures experienced in these communities.

Factors that have been strongly statistically linked (with or without measured correlation to endotoxin exposure) to the development of atopic sensitization within the farming communities include early exposure to livestock and particularly stables, drinking unpasteurized milk [5, 30], and levels of indoor animals including cattle in a Nepalese study [31]. While protective potential of endotoxin in farming communities is a plausible explanation, it is equally feasible that early high exposure to allergens, alteration in gut flora induced by drinking unpasteurized milk, other dietary differences between farming and non-farming children and even the ecological or survivor effects on

the gene pool of farming communities in part or in whole, might explain the difference in atopic sensitization illnesses between farming and non-farming children.

Urban studies

In urban communities, links between levels of endotoxin exposure and fungal allergens [21], house dust mite, animal ownership [8, 32], reduced ventilation and cleaning [33] and use of indoor storage of organic waste have all been reported [34].

Studies of the relationship between endotoxin and atopic sensitization in urban (non-farming) populations are variable. In a US child cohort study Gereda et al. published data suggesting lower levels of endotoxin in those children found to be skin test-positive [8]. Unfortunately this study needs interpreting with caution as the skin prick-positive (atopic) group numbered only 10 (small by any epidemiological standard). A larger study from Boston found the opposite association [35]. Higher levels of endotoxin were positively related to episodes of respiratory illness in the first year of life. This study can also be criticised, as it is accepted that respiratory illness in the first year of life does not correlate well with subsequent asthma or atopic status.

The results of the study by Böttcher et al. in the current issue are a welcome addition to this confusing data [9]. Lower levels of atopic disease at 2 years of age were found in the population from Estonia where endotoxin exposures are measurably higher than in the comparison population of Sweden. Endotoxin levels in the family carpet was the only environmental factor predictive of atopic symptoms at 2 years of age, after accounting for atopic hereditary, pet ownership, allergen levels and home dampness. The study has some weaknesses and the number of atopic children in Estonia included in the study was only eight. It is also evident that different sampling methods were performed in the Estonia compared to the Sweden arm of the study.

More studies of comparable populations, including regional birth-cohort studies and international comparative studies are needed, with other indoor pollutants and social factors accounted for to investigate and clarify a possible causal effect of the levels of endotoxin present. In addition, those factors responsible for higher levels of endotoxin require further elucidation.

Experimental exposure studies

A limited amount of experimental *in vitro* and animal data does support a protective mechanism afforded by exposure to endotoxin.

Tulic et al. demonstrated that pre-exposure and early post-exposure to endotoxin in rats in an intraperitoneal ovalbumin sensitization model, subsequently reduced risk of production of specific IgE, bronchial responsiveness and airway inflammation [36]. However, Wan et al. had demonstrated a contradictory effect in mice pre-exposed to airborne endotoxin [37]. Antibody responses to inhaled ovalbumin were increased, higher eosinophil levels were witnessed at 2 weeks and higher levels of IL-10 mRNA were expressed. Differences in these two studies exist in the persistence of exposure at intervals for up to 10 days in

the Tulic study, but a single pre-exposure only was used by Wan et al.

In a chronic mouse model exposure study to endotoxin, without allergen challenge, subsequent histological analysis demonstrated persistent peribronchial inflammation and perivascular lymphocyte aggregates (CD4⁺ CD8⁺ and CD19⁺) [38].

Interpretation of endotoxin exposure and respiratory disease

Superficially it might seem implausible for an agent like endotoxin with such potent pro-inflammatory potential, to act in a protective way. The explanation requires events in the level and timing of exposure to act with factors associated with the specific period of development of allergen sensitization to be critical.

It is unlikely that endotoxin levels will change over the first years of life. It has been postulated to explain all the data, that the response to endotoxin by the developing infant might be bi-phasic.

The studies of farming communities give convincing evidence that, whatever the agent, the exposure needs to be within the first year of life. Braun-Fahrlander demonstrated that the protection from exposure to stables only offered protection if it occurred in the first year of life [2].

More studies of exposure levels to endotoxin in urban birth cohorts are required. In addition the factors responsible for higher levels of endotoxin in indoor environments need to be clarified.

It is possible that a burden of proof will remain elusive. The same weaknesses that have dogged the occupational literature potentially abound in the domestic setting. Primarily the question arises, does endotoxin just represent a convenient measurable contaminant of the environment and act as a pseudo-marker of some other environmental exposure or risk factor? Or is it directly responsible for a protective effect against respiratory sensitization to allergens?

It seems a little too early to test such a hypothesis by an intervention study involving increased exposure to standard endotoxin in neonates. The potential adverse effects of the pro-inflammatory action of endotoxin are of too great a concern to make this ethical at this stage and the exposure level required at a personal dosing level is immeasurable.

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