OCCASIONAL REVIEW

Does environmental endotoxin exposure prevent asthma?

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Thorax 2002;**57**:86–90

The evidence as to whether exposure to environmental airborne endotoxin plays a protective or an inducing role in the development of asthma is reviewed. Studies of endotoxin and atopy, endotoxin and asthma, and farming and asthma are considered and, in each instance, a distinction is made between evidence of primary causation and evidence of secondary causation. It is concluded that, although it is plausible that bacterial endotoxin may protect against the development of asthma, there is considerable reason for caution regarding this hypothesis.

It has recently been suggested that exposure to environmental endotoxin might protect against the development of atopy and asthma.[1–5] This hypothesis has been prompted by various recent studies that reported a reduced risk of atopy (determined by skin prick tests, IgE serology or questionnaire), hay fever and, to a lesser extent, asthma in farmers' children and adolescents[6–10] and in first year university students with a farming background.[11] It was indicated that contact with livestock reduced the risk.[7 8 10] This is consistent with previous observations that pigs and cattle in the home in Guinea-Bissau[12] and Nepal,[13] respectively, and the presence of pets in the home early in life in Europe[14 15] were negatively associated with atopy. Although no specific protective factors were determined in these studies, it has been speculated that respiratory exposure to endotoxin (particularly in livestock farming) may play an important role[2 3] since it is well known, especially from occupational studies, that keeping animals is associated with strongly increased exposures to bacterial endotoxin.

The suggestions by Thomas[16] that endotoxins are "read by our tissues as the very worst of bad news" and that in response to these molecules "we are likely to turn on every defence at our disposal" elaborate well the toxic potential of these macromolecules.[17] Endotoxin is composed of lipopolysaccharides and is a non-allergenic cell wall component of Gram negative bacteria with strong pro-inflammatory properties. It is commonly present in many occupational environments[18] and also in the general environment, particularly in house dust, as was first demonstrated by Peterson *et al*[19] and more recently by others.[20–22] There is ample evidence, mainly from studies in the work environment, that very high levels of exposure to endotoxin occur in farming, particularly livestock farming.[23] Increased endotoxin levels were also found in homes where children had regular contact with farm animals[2] and in those where pets were present.[22]

It therefore seems plausible that bacterial endotoxin may account for the lower risk of atopy and perhaps asthma itself in farmers' children and, more generally, in the wider population. This hypothesis is appealing since it would not only account for the striking finding in farmers' children, but is also consistent with the "hygiene hypothesis"[1 24] and would offer potential practical methods of asthma prevention. However, there is considerable reason for caution. In particular, although endotoxin exposure may protect against the development of atopy, at most only 50% of asthma cases appear to be attributable to mechanisms involving atopy.[25] Moreover, it is well known from occupational health studies that exposure to endotoxin may induce asthma rather than protect against it (see below). Finally, there are many other factors that may explain the reduction of atopy in farmers' children—for example, high allergen exposure that may reduce the risk of sensitisation, infant infections, diet, and other lifestyle factors. These two aspects of the (potential) effects of endotoxin—namely, the protective effect with regard to atopy and endotoxin as an inducer of (occupational) asthma—have often not been carefully considered in discussing these issues. In this review we therefore consider the evidence as to whether environmental airborne endotoxin exposure plays a protective or inducing role in the development of asthma. In doing so, we distinguish between studies of endotoxin and atopy, endotoxin and asthma, and farming and asthma. Furthermore, in each instance we distinguish between evidence of primary causation and evidence of secondary causation. For clarity, we will only focus on the role of respiratory endotoxin exposure and will not discuss the potential immunomodulating role of gastrointestinal endotoxin. The latter may, however, be significant since it has been suggested that the intestinal microflora may be a very important source for microbial pressure potentially enhancing Th1 type responses.[26]

DEFINITIONS

In this review we consider "atopy" as IgE mediated sensitisation to "common allergens" such as house dust mite, pets, and various other indoor and outdoor allergens. Asthma is defined as a chronic inflammatory disorder of the airways involving airflow limitation that is at least partly reversible and which results in recurrent episodes of symptoms such as wheezing, breathlessness, chest tightness, and cough. Allergic asthma represents asthma with an underlying atopic airway inflammation involving IgE sensitisation and eosinophils whereas non-allergic asthma is characterised by non-atopic airway inflammation (not involving IgE and eosinophils).

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Accepted 19 September 2001

Thorax: first published as 10.1136/thorax.57.1.86 on 1 January 2002. Downloaded from http://thorax.bmj.com/ on March 28, 2024 by guest. Protected by copyright.

ENDOTOXIN AND ATOPY

It has been proposed that bacterial endotoxin drives the response of the immune system—which is known to be strongly skewed in an atopic Th2 direction during fetal and perinatal life—in a Th1 direction and away from its tendency to develop atopic immune responses.[1] A more detailed overview of the proposed mechanisms is given elsewhere.[27 28] Briefly, bacterial endotoxin can induce a significant production of interleukin (IL)-12 in antigen presenting cells which inhibits the atopic Th2 response by promoting and enhancing the Th1 response. Endotoxin also induces interferon (IFN)γ production which downregulates the maturation of T cells into Th2 cells. Furthermore, it has been shown that atopy (assessed as high serum IgE levels) is associated with a genetic polymorphism for CD14, the lipopolysaccharide (endotoxin) receptor on monocytes and other inflammatory cells.[29] However, to date only one small study has produced direct in vivo evidence that endotoxin exposure may protect against the development of atopy by enhancing Th1 responses. In this study it was shown that, of 61 infants with a high risk for developing asthma, allergen sensitised infants had significantly lower house dust endotoxin levels than non-sensitised infants.[4] They further showed in a subset of these infants that endotoxin levels correlated with IFNγ producing T cells (Th1) but not with IL-4, IL-5, or IL-13 producing cell proportions (Th2). Finally, one study in ovalbumin sensitised rats found that airborne endotoxin exposure early in the sensitisation process (1 day before or within 4 days after intraperitoneal ovalbumin exposure) protected against the development of specific ovalbumin IgE antibodies.[30] However, in complete contrast, another study showed that mice pre-exposed to airborne endotoxin (18 hours before and 5 days during airborne ovalbumin exposure) enhanced ovalbumin IgE sensitisation.[31]

A protective effect of endotoxin on the development of atopy therefore seems plausible, but the evidence is circumstantial since most data which support this hypothesis were produced in in vitro studies. In addition, the evidence from in vivo experiments in animals is mixed. If this protective effect does exist, it appears to involve prevention of primary causation through promotion of the Th1 pathway.

ENDOTOXIN AND ASTHMA

With regard to asthma, the evidence is even more equivocal. The studies that have shown a consistent protective effect of farming exposure against atopy[6–11] have shown only a weak protective effect against asthma itself.[32]

> "Endotoxin exposure may prevent the primary causation of allergic asthma, but it may be both a primary and secondary cause of non-allergic asthma"

In fact, there is considerable evidence that endotoxin exposure may both exacerbate pre-existing asthma and induce new asthma in adults. In particular, endotoxin has been recognised as an important factor in the aetiology of occupational lung diseases including (non-allergic) asthma caused by organic dust exposure.[33] Subjects exposed to pure endotoxin in inhalation experiments experience acute clinical effects such as fever, shivering, arthralgia, influenza-like symptoms (malaise), blood leucocytosis, neutrophilic airway inflammation, asthma symptoms such as dry cough, dyspnoea and chest tightness, bronchial obstruction, as well as dose dependent impairment of lung function (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and flow-volume variables) and decreased lung transfer factor.[34–39] In addition, in naive subjects challenged with endotoxin-containing cotton dust[40] or dust from pig farms[41] the same symptoms and lung function changes (including reversible airway obstruction) were demonstrated and they were most strongly associated with exposure to endotoxin and not with exposure to dust. Inhalation studies have further shown that subjects with increased bronchial hyperresponsiveness and/or asthma were more sensitive to developing symptoms.[42–44] This has also been shown in a large epidemiological study of pig farmers.[45] Furthermore, in healthy (non-allergic) subjects a highly variable (but reproducible) airway responsiveness to inhaled endotoxin was found, suggesting that only susceptible individuals are potentially at risk.[46 47]

Acute airway effects found in experimental inhalation studies have been confirmed in many field studies conducted in farming and various other industrial environments.[33] As long ago as 1961 it was proposed that inhalation of bacterial endotoxin was a causal factor in the development of occupational respiratory diseases in cotton workers.[34] Since then, numerous studies have been conducted that indicated a causal role for endotoxin exposure in the pathogenesis of both reversible (asthma) and chronic airway obstruction, respiratory symptoms (symptoms of asthma, bronchitis and byssinosis), and increased airway responsiveness (summarised in table 1). This was consistently observed in various occupational environments characterised by different exposure levels and different compositions of the bioaerosol exposures. Several of these studies reported clear exposure-response relationships between endotoxin exposures and lung function effects and respiratory symptoms.[55 56 58 63 64] Interestingly, one study by Zock *et al*[55] showed that acute airway obstruction was already apparent at very low exposure levels of about 50 EU/m^3 (~5 ng/m^3), indicating that adverse respiratory effects (including asthma) may occur at very low levels.

Based on this evidence, "no effect levels" have been calculated and legal exposure standards for airborne endotoxin levels in the work environment have been proposed.[69–71] These levels range from 50 endotoxin units (EU)/m^3 (~5 ng/m^3) to several hundreds of EU/m^3, and are very low and thus highly prevalent in farming environments, particularly in those involved in livestock farming.

Several studies[20 22 66–68] have also shown that endotoxin in house dust is associated with exacerbations of pre-existing asthma in children and adults (see table 1). One cross sectional study performed in Belgium showed that domestic endotoxin levels in 69 adult patients with asthma were significantly correlated with a decrease in FEV_1 and an increase in symptoms and daily need for oral and inhaled asthma medication.[66] These results were confirmed in a Brazilian study in 10 asthmatic and 10 control children.[67] Douwes *et al*[22] have shown that endotoxin levels in house dust were positively associated with peak flow variability in asthmatic children but not in non-asthmatic children. After adjusting for the presence of pets in the home (which was significantly associated with high levels of endotoxin), the association with endotoxin disappeared. Interestingly, dust mite allergen levels were not associated with symptoms or lung function in these studies. Finally, one recent birth cohort study in 499 infants with a familial predisposition to asthma or allergy showed that early exposure to indoor endotoxin was associated with an increased (rather than a decreased) risk of repeated wheeze during the first year of life (RR=1.6, 95% CI 1.03 to 2.38).[68]

There is therefore consistent evidence that endotoxin can induce (at least in occupationally exposed populations) and exacerbate asthma. However, the underlying pathology is different from that observed in "classic" allergic/atopic asthma and does not include sensitisation and eosinophil involvement. The exact pathophysiology is not clear, but it is well established that it is mediated by an acute inflammatory response involving various cytokines including IL-1, IL-6, IL-8 and tumour necrosis factor (TNF)-α, and the subsequent massive recruitment and activation of neutrophils in the lower and upper airways.[37 38 72 73] The inflammatory reactions are

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Table 1 Overview of epidemiological studies indicating adverse respiratory effects related to environmental endotoxin exposure

Reference	Population	N	Exposure*	Health effect
Occupational studies:				Acute respiratory effects:
48	Pig farm workers	40	180 ng/m^3	Cross-shift decline in FEV_1 and MEF_{25}
49	Slaughter house workers	23	20–1500 ng/m^3	Cross-shift decline in FEV_1 and FVC; increased prevalence of respiratory symptoms
50	Animal feed workers	119	29 ng/m^3	Cross-shift decline in MMEF and MEF_{50}; cross-week decline in FEV_1, MEF_{25}, MMEF and MEF_{50}
51, 52	Fibreglass workers	130	0.4–759 ng/m^3	Cross-shift decline in PEF and FEV_1; increased amplitude of PEF; increased prevalence of respiratory symptoms and symptoms of fever, joint pains, and influenza-like symptoms
53	Potato processing workers	61	21–56 EU/m^3	Cross-shift decline in FEV_1 and MMEF
54	Potato processing workers	97	534 EU/m^3	Cross-shift decline in PEF; increased prevalence of respiratory symptoms
				Chronic respiratory effects:
55	Cotton mill workers	443	2–550 ng/m^3	Decline in FEV_1; increased prevalence of chronic bronchitis and byssinosis
56	Pig farm workers	183	130 ng/m^3	Decline in FEV_1 and FVC; increased prevalence of respiratory symptoms
57	Cotton mill workers	253	9–126 ng/m^3	Decline in FEV_1 and FVC; increased prevalence of respiratory symptoms
58	Animal feed workers	315	25 ng/m^3	Decline in FEV_1, FVC, PEF, MEF_{75}, MEF_{50}
59	Cotton mill workers	34	20–320 ng/m^3	Increase in bronchial hyperresponsiveness
60	Pig farm workers	54	11332 EU/ m^3	Decline in FEV_1 and FVC; increased prevalence of cough and chronic bronchitis
61	Grain workers	410	2859 EU/ m^3	Decline in FEV_1; increased prevalence of respiratory symptoms
62†	Farm workers (pig farms/others)	168/127	588/410 EU/m^3	Longitudinal decline in FEV_1 and MMEF
63†	Pig farm workers	171	105 ng/m^3	Longitudinal decline in FEV_1
64†	Grain and animal feed workers	140	3.6–99.0 ng/m^3	Longitudinal decline in FEV_1 and MMEF
65†§	Cotton mill workers	366	~3200 EU/m^3	Longitudinal decline in FEV_1 and FVC
Indoor studies:				
20	Adult asthmatic patients	28	2.59 ng/mg	Decline in FEV_1 and FEV_1/FVC; increase in asthma medication and symptoms
66	Adult asthma (40)/rhinitis (29) patients	69	1.78 ng/mg	Decline in FEV_1, and FEV_1/FVC; increase in asthma medication and symptoms
67	Children (50% with asthma)	20	1–100 EU/mg	Increase in asthma medication and symptoms in asthmatic children
22	Children (50% with airway symptoms)	148	24.9 EU/mg	Increased PEF variability in atopic children with asthma symptoms‡
68†	Infants	499	100 EU/mg	Increased prevalence of wheeze during first year of life

FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; MEF_{25}, MEF_{50}, MEF_{75} = maximum expiratory flow rates at 25%, 50% and 75% of the vital capacity; MMEF = maximum mid expiratory flow; PEF = peak expiratory flow.
*Exposure is expressed as the mean exposure (or range of (mean) exposures if no overall mean is given) in ng or endotoxin units per m^3 or per mg of house dust; one endotoxin unit is approximately 0.1 ng (the exact conversion factor varies depending on the source of endotoxin for calibration).
†Longitudinal study (all other studies were cross sectional studies)
‡Association between endotoxin exposure and PEF variability disappeared after adjusting for pets in the home.
§15 year follow up of study by Kennedy *et al* in cotton mill workers.

orchestrated by alveolar macrophages that carry specific endotoxin binding receptors (CD14) which play a crucial role in the activation of these cells and the subsequent inflammatory processes.[74 75] There is thus consistent evidence that endotoxin is both a secondary and primary cause of asthma and that this occurs through non-atopic—that is, non-IgE mediated—mechanisms.

FARMERS AND ASTHMA

A brief review of studies of farmers and their families seems appropriate here since it is primarily in this context that the hypothesis regarding the possible protective effects of endotoxin has arisen. As noted above, several studies have suggested a moderately decreased risk of asthma in farmers' children, adolescents, and students with a farming background.[6–11] On the other hand, various studies have found a substantially increased risk of respiratory morbidity and mortality among adult farmers and farm workers[23] despite their lower prevalence of smoking compared with the general population.[76 77] In the European Community Respiratory Health Survey which investigated occupational asthma in 15 637 randomly selected people aged 20–44, the highest risk of asthma was found for farmers (OR 2.6, 95% CI 1.3 to 5.4) and agricultural workers (OR 1.8, 95% CI 1.0 to 3.2).[78] An increased risk of asthma morbidity and mortality in farmers has also been reported in several other studies.[79–81] Interestingly, in a Norwegian study of 8482 farmers and their spouses it was shown that subjects involved in animal production had a higher risk of asthma (OR 2.2, 95% CI 1.1 to 4.2) than those not involved in animal production.[82] However, some studies have reported no additional risk or even a lower risk for asthma in farmers[83 84] or young farmer students.[85]

Atopy has not been systematically studied in farmers since no controls were included in most studies so a direct comparison with the general population was not possible. However, several studies have reported relatively low prevalences of atopy,[86–88] which suggests that the reduced risk of atopy in farmers' children continues into adult life. It therefore seems that asthma is more prevalent among adult farmers, despite the apparent lower prevalence of atopy and the slightly lower prevalence of asthma in farmers' children.

CONCLUSIONS

It is important to identify specific factors and underlying mechanisms that explain the protective effect of the farming environment on atopy, particularly in children, since this may result in novel approaches to preventing atopic diseases such as allergic asthma. Exposure to bacterial endotoxin in neonatal life has been proposed to play a role, and plausible mechanisms have been suggested. However, only limited and indirect evidence for this hypothesis is currently available, and there is consistent evidence that exposure to bacterial endotoxin may induce respiratory symptoms including (non-allergic) asthma in children and certainly in occupationally exposed adults. This apparent discrepancy in the potential role of endotoxin may be related to the timing (prenatal and neonatal versus child and adult life) and dose of exposure. A recent study by Park *et al*[89] that measured airborne endotoxin

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in 15 homes in Boston (USA) reported a mean airborne endotoxin level of 0.64 EU/m^3 (and a mean dust endotoxin level of 44–105 EU/mg), indicating that endotoxin exposures are likely to be much less than are usually encountered in the work environment (table 1). Nonetheless, occasional high(er) exposures to airborne endotoxin in the indoor environment may occur during specific activities in the home such as vacuum cleaning and, in the case of children, when playing on the floor close to dust reservoirs. Thus, if endotoxin exposure early in life indeed inhibits the development of atopy and allergic asthma, it may not necessarily reduce the prevalence of asthma in general since this effect may be counterbalanced by an increase in non-allergic asthma induced by (higher) endotoxin exposures later in life. This may be one of the reasons that atopy was clearly reduced in farmers' children whereas asthma symptoms were not (or only marginally) reduced, and asthma appears to be more common in farmers themselves.

In summary, although it is plausible that bacterial endotoxin may protect against the development of asthma, there is considerable reason for caution regarding this hypothesis. In particular: (1) a protective effect has only been established for atopy (and mainly for specific pollen sensitisation) and for hay fever; (2) the prevalence of asthma was only marginally reduced in children with frequent contact with animals, and it is not established that endotoxin is the aetiological factor; and (3) it is well established that endotoxin exposure itself may induce respiratory symptoms including (non-allergic) asthma symptoms. It therefore appears that endotoxin exposure may prevent the primary causation of allergic asthma, but it may be both a primary and secondary cause of non-allergic asthma. Further research is clearly needed, particularly of the timing and dose of endotoxin exposure. Several prospective birth cohort studies in both general and farming populations are currently in progress across Europe, the USA, and Brazil which focus on allergy, asthma, and early (including prenatal) endotoxin exposures and allergen exposure that may establish whether endotoxin prevents or induces asthma and at what levels of exposure. Some of those studies include a detailed characterisation of the T cell immune status (Th1, Th2 or Th3 immunity), providing important information on the causal mechanisms. Finally, in those children who develop asthma, it is interesting to determine whether they have developed allergic or non-allergic asthma since it is well known from occupational studies that endotoxin induces asthma through non-allergic mechanisms.

ACKNOWLEDGMENTS

The authors thank Bert Brunekreef for his critical comments on the draft manuscript. Neil Pearce is funded by the Health Research Council of New Zealand and Jeroen Douwes is supported by a research fellowship from the Netherlands Organisation for Scientific Research (NWO).

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