



The hygiene hypothesis in allergy and asthma: an update

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Purpose of review

It has been hypothesized that increased cleanliness, reduced family size, and subsequent decreased microbial exposure could explain the increases in global asthma prevalence. This review considers the recent evidence for and against the 'hygiene hypothesis'.

Recent findings

Recent evidence does not provide unequivocal support for the hygiene hypothesis: the hygiene hypothesis specifically relates to atopic asthma, but some of the protective effects (e.g. farm exposures) appear to apply to both atopic and nonatopic asthma; asthma prevalence has begun to decline in some western countries, but there is little evidence that they have become less clean; Latin American countries with high infection rates have high asthma prevalence and the hygiene hypothesis relates to early-life exposures, but exposures throughout life may be important.

Summary

There is a considerable body of evidence which warrants scepticism about the hygiene hypothesis. However, these anomalies contradict the 'narrow' version of it in which microbial pressure early in life protects against atopic asthma by suppressing T-helper 2 immune responses. It is possible that a more general version of the hygiene hypothesis is still valid, but the aetiologic mechanisms involved are currently unclear.

Keywords

allergy, asthma, epidemiology, farming, intestinal microbiota, microbial exposure, protective factors

INTRODUCTION

The last few decades have seen a dramatic increase in the global prevalence of asthma and allergies [1]. This has been particularly apparent in the western world, although similar increases have been observed in low-income countries [2]. The reasons for this increase (and reported recent decreases in some regions) have not been entirely clear. Genetic changes are unlikely to be responsible due to the short timeframe involved. The increase in allergy contrasts strongly with a reduced prevalence of infectious diseases, a result of improved public health measures, treatment, vaccination programmes and hygiene. This has also led to a corresponding decreased exposure to noninfectious microorganisms, which has been hypothesized as a possible explanation for the allergy and asthma epidemic [3]. This is commonly referred to as the 'hygiene hypothesis'.

THE HYGIENE HYPOTHESIS

The original 'hygiene hypothesis' was prompted by evidence that overcrowding, unhygienic conditions

and larger family size were associated with a lower prevalence of atopy, eczema, hay fever and asthma [1,4–7]. Increased infections and exposures to microorganisms and/or their components, particularly early in life, have been proposed as an explanation for these findings [8]. More recently, it has been suggested that the hygiene hypothesis may also be relevant for autoimmune conditions [9–11].

INFECTIONS

Several studies have shown associations between infections (e.g. hepatitis A, measles) or *Bacillus Calmette-Guerin* (BCG) immunization and a lower

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KEY POINTS

- The hygiene hypothesis has been proposed to explain the increases in asthma and allergy prevalence observed over the last few decades but is unlikely to be the sole explanation for this increase.
- Protection against allergy and asthma has been associated with microbial exposures in several studies, but recent evidence suggests that diversity of microbial exposure may be particularly protective.
- Interaction between the immune system and intestinal microbiota in particular has been shown to protect against allergic inflammation in murine models.
- Farming exposure also appears to protect against nonatopic asthma, providing further evidence that the 'narrow' interpretation of the hygiene hypothesis, in which early-life microbial pressure protects against atopic asthma by suppressing TH₂ immune responses, is oversimplistic.
- Although the hygiene hypothesis goes some way to explaining recent global changes in allergy and asthma prevalence, further aetiological theories which are consistent with the epidemiological evidence for both allergic and nonallergic asthma are required.

prevalence of atopy and allergies [12–15]. However, findings have been inconsistent, particularly for viruses (measles, mumps, rubella and chickenpox) and BCG vaccination [16–19]. It is, therefore, possible that protection is specific to certain infections, at particular times of life. For example, one study [10] found that croup [odds ratio (OR)=0.3; 95% confidence interval (CI), 0.12–0.72] and repeated ear infections (OR=0.58; 0.35–0.98) in the first 12 months of life were inversely associated with atopy, whereas bronchiolitis was positively associated with asthma (OR=2.77; 1.23–6.22). Furthermore, interplay between some early-life respiratory infections and allergic sensitization may increase the risk of asthma development [9].

A protective effect may not be limited to only microbial or viral exposure. Some helminth infections, such as *Schistosoma mansoni*, are associated with a strong protective effect against atopy [11]. This may be related to intensity of the infection, as parasite load was inversely associated with SPT and HDM-specific immunoglobulin E [20]. Furthermore, helminth eradication programmes are associated with an increase in atopic sensitization [11,21,22]. For example, in a large study [23] examining the effect of chronic helminth infection on allergy/atopy in school-age children, antihelminthic treatment was associated with a doubled

prevalence of allergen skin test reactivity, and increased prevalence of eczema symptoms, without observed increases in asthma or rhinoconjunctivitis. Interestingly, other parasites such as *Ascaris* and *Trichuris* may not be protective [24]. It remains unclear as to what is the most important factor in protection from particular manifestations of atopy and asthma: dose, route (oral–faecal versus airborne), type of infection (viral versus bacterial versus parasitic) or location (upper versus lower airways or digestive tract).

NONINFECTIOUS MICROBES

Environmental exposures to proinflammatory microbial agents (such as bacterial endotoxin) have also been suggested to be protective [25–27]. Studies [28–30] in both rural and nonrural environments have reported a significant inverse association between indoor endotoxin levels and atopic sensitization, hay fever and atopic asthma. Recent work from International Study of Asthma and Allergies in Childhood phase II supports this, that is, combined across countries, household endotoxin levels were inversely related with asthma ever {OR=0.53 [0.29–0.96] and current wheeze [0.77 (0.64–0.93)]}. However, the relationship with atopy was much weaker after adjustment for sex, parental allergies and exposure to cat and house dust mite allergens [31]. Overall, the available data regarding endotoxin are inconsistent. For example, one birth cohort found that early endotoxin exposure was associated with an increased risk of atopy at the age of 2 years [32], whereas two similar birth cohort studies found a protective effect on atopy in 2-year-olds [28] and asthma symptoms in 4-year-olds [30]. Endotoxin exposure has also been shown to be a risk factor for asthma as demonstrated in inner city urban environments [33,34].

Exposure to other microbial components, such as bacterial CpG, peptidoglycans [35], extracellular polysaccharides and fungal 1–3 β -d-glucans [36] may also be protective, but epidemiological evidence remains limited. There are, however, numerous studies showing a protective effect of microbial components against the development of allergic inflammation in murine models. For example, Toll-like receptor (TLR) 9 agonists inhibit the development of asthma-like inflammation [37,38], and intranasal administration of TLR2/4 ligands has been tested as a potential protective vaccine against allergic airway inflammation [39]. Protection may not be elicited through only microbial components. Short-chain fatty acids (SCFAs), produced by bacterial fermentation of dietary fibre, may play a role in gut immunoregulation and reduce allergic

airway inflammation in the mouse through SCFA interaction with the G-protein-coupled receptors 41 and 43 [40].

Exposures to different types of nonpathogenic bacteria may have differential effects on atopy and asthma. One birth cohort study [41] showed that exposure to both Gram-positive and Gram-negative bacteria was inversely associated with asthma, whereas only Gram-negative bacteria were associated with allergic sensitization. More recently, studies have suggested that diversity of microbial species, rather than exposure to one particular microbial component, may be particularly protective (reviewed by Heederik and von Mutius [42]). For example, Ege *et al.* [43[¶]] showed that the diversity of environmental microorganisms to which individuals were exposed explained a substantial fraction of the inverse association between farm upbringing and asthma. Also, house dust obtained from homes in Russian Karelia (a low-allergy area) contained more Gram-positive bacteria (probably of animal origin) than neighbouring high-allergy Finnish Karelia in which samples contained more Gram-negative and proteobacteria [44]. Furthermore, daycare centres – known to be associated with a lower prevalence of allergies and asthma in attending children – have been shown to be associated with considerable bacterial diversity [45] as have households with daycare attendees or with cats and dogs [46]. Interestingly, a recent study [47] suggested that diversity of exposure may not be limited to microorganisms; in particular, it showed that atopic individuals may also have lower diversity of plant species around their homes, as well as reduced diversity of skin gamma proteobacteria, compared with nonatopics. The mechanisms through which diversity confers protection remain unclear, but one possibility may be that increased diversity simply represents exposure to higher levels of pathogen-associated molecular patterns (PAMPs).

FARMING AND ANIMAL EXPOSURE

Several studies have found consistently low prevalences of allergies and asthma in farmers' children in both high-income and low-income countries [48–56]. These protective effects have also been observed in adult farmers [49,52,57–59] despite increased risks of other respiratory conditions such as chronic obstructive pulmonary disease [60]. The observed protective effects of farming on allergies and asthma have been particularly strong for animal contact [50,55,61,62] or unpasteurized farm milk consumption [54,55,63,64]. It remains unclear which specific factors are most important, but microbial exposures may play a role either through

ingestion (lactobacilli, particularly through raw milk) or inhalation (endotoxin and other microbial components; see above). Protection conferred by farm residence may be dependent upon the type of farming conducted. For example, Ege *et al.* [65] found that atopic asthma was inversely associated with animal exposures and raw milk consumption, but an effect for nonatopic asthma was only observed with silage exposure and agricultural farming.

Exposure to domestic animals may also confer protection. For example, pet ownership has been shown to be inversely associated with atopy and asthma in children and adults [66–68]. This effect may be particularly strong with multiple exposures; in one birth cohort study [69], children of atopic parents who owned both a dog and a cat were less likely to be atopic at 13 years than those owning only one pet. The evidence of pets protecting against asthma is, however, mixed with some studies reporting no association, or a positive association, despite showing an inverse association with atopy [70]. In other parts of the world (Guinea-Bissau and Nepal), it has been shown that pigs and cattle in the home are associated with less atopy [15,71]. This is consistent with observations that animal contact in farmers' children may confer protection, and as hypothesized for the farmers' children, increased microbial exposures may play a role [72].

INTESTINAL MICROBIOTA

Ingestion of lactobacilli associated with raw milk consumption may be important as they can colonize the human gut [73] and may be involved in immunomodulation during development [74–81]; however, various microbes of the gut flora or alterations in total gut microbiota may play a role as well [82–84]. Reduced intestinal biodiversity during infancy has been associated with increased allergy at school age [85[¶]]. Furthermore, an assessment of gut microbiota in infants using pyrosequencing found that low diversity (reduction in members of Bacteroidetes and Proteobacteria phyla), especially in the first month of life, was associated with atopic eczema [86]. Similar findings of low-gut microbial diversity in allergic individuals have also been reported [87–89], and altering intestinal flora with antibiotics in murine models increases susceptibility to allergic airway inflammation [90]. Furthermore, it has been suggested that the composition of maternal intestinal flora may be related to wheeze in infants, possibly through indirect exposure, or maternal transfer [91[¶]]. In a mouse model of such prenatal exposure (albeit respiratory), maternal exposure to the

cowshed bacterium *Acinetobacter lwoffii* F78 protected offspring from subsequent development of allergic inflammation through an epigenetic process [92] and was totally dependent upon the presence of maternal (not offspring) TLR signalling [93].

Studies in germ-free (gnotobiotic) mice have indicated that exposure to commensal microbiota is critical for appropriate immune development [94]. Germ-free mice had significantly increased allergic airway inflammation compared with specific pathogen-free (SPF) mice. Although regulatory T-cell populations and regulatory cytokine levels were unaltered, germ-free mice exhibited an increased number of basophils and decreased numbers of alveolar macrophages and plasmacytoid dendritic cells (DCs), suggesting that absence of commensals leads to alterations in allergen-presenting cell populations [95[¶]]. A study [96] assessing the effect of colonizing germ-free mice at different time points with different microbiota found that expression of hundreds of genes was affected, particularly those involved in type-1 interferon (IFN) induction, showing that altering the commensal microbiota leads to alterations in immune-associated gene expression.

One intriguing recent finding [97[¶]] suggests that reduced exposure to intestinal microbiota (i.e. in germ-free compared with SPF-specific mice) is associated with expansion of invariant natural killer T cells (iNKTs) cells in the colonic lamina propria and lung, which correlated with increased development of allergic airway inflammation and colitis. The introduction of commensal bacteria prenatally or at early age in previously germ-free mice reduced this risk, whereas inoculation of adult germ-free mice did not have a protective effect. The observed compartmental overrepresentation of iNKTs was associated with increased expression of CXCL16 (an iNKT chemotactic factor), which is hypermethylated in germ-free mice, and may be the epigenetic basis for the lack of effect observed in adult mice. This study suggested that interaction between microbiota and innate cells (such as iNKTs) may be important in protection from allergies and/or asthma, although it is currently unclear how these findings relate to allergies and asthma in humans.

MECHANISMS UNDERLYING THE PROTECTIVE EFFECT OF MICROBIAL EXPOSURE

Microbial exposures activate pattern-recognition receptors, such as TLRs (reviewed in Medzhitov [98]) or CD14 [99], which are specific for different microbial components (or PAMPs). This process is hypothesized to lead to downstream suppression of

T-helper-2 (TH₂) cell expansion, and therefore TH₂-mediated diseases, including allergic asthma, hay fever and eczema [100]. Originally, this was believed to function simply through altering the TH₁/TH₂ balance, that is, growing up in a more hygienic environment with less microbial exposure would enhance atopic (TH₂) immune responses, whereas microbial pressure would drive the response of the immune system – which is known to be skewed in an atopic TH₂ direction during fetal and perinatal life – into a TH₁ direction and away from its tendency to develop atopic immune responses [101,102]. This model now appears to be oversimplistic, as demonstrated by parasitic infections which are associated with powerful TH₂ responses but protect against atopy (see above). Furthermore, endotoxin exposure has been shown to be associated with reduced IFN- γ , tumour necrosis factor- α , interleukin (IL) 12 and IL-10 production by peripheral blood leukocytes, actually suggesting a reduced rather than increased TH₁ cytokine milieu [103]. There is also evidence that the exposure dose of PAMP, for example, endotoxin, may in itself alter TH₁/TH₂ balance [104].

Alternatively, altered immunoregulatory mechanisms may be important. Microbial modulation (either directly or indirectly) of regulatory T cells may result in reduced expression of both TH₁ and TH₂ immunity. In newborns exposed to farming *in utero*, an increase in cord blood FoxP3+ T regulatory cells has been described [105]. Similar findings have been observed in mice in which perinatal administration of the probiotic *Lactobacillus paracasei* NCC2461 prevented allergic inflammation in sensitized/challenged offspring, required TLR2/4 signaling and was associated with increased FoxP3 RNA expression in the lung [106].

Furthermore, it has been reported that bacterial exposure in the airways may protect against allergic inflammation through a TLR4-dependent mechanism, without induction of either T regulatory or TH₁ cells but with alterations in DC activation status [107].

Microbial exposure may also protect against nonatopic asthma

Although current data support the idea that microbial exposure is associated with reduced risk of allergies and allergic asthma, there is also evidence that it may be protective against nonallergic asthma. When assessing the effect of farm exposure upon asthma and allergy, Riedler *et al.* [54] found a reduced risk of asthma (1%; 3/241) in nonatopic children regularly exposed to farm stables compared with 4% (17/399) in those who were not ($P = 0.034$).

A subsequent study [108^{*}] reproduced this finding, showing that farming was associated with protection against nonatopic wheeze (adjusted odds ratio 0.45, 95%CI 0.32–0.63).

Early-life bacterial exposure may also modulate immunity to more adequately respond to infectious agents. In a murine model of *Streptococcus pneumoniae* infection, early-life exposure to microbial components was associated with airway colonization by nonpathogenic bacteria (such as lactobacilli), increased airway expression of TLR2, 4 and 9 and decreased neutrophil recruitment [109]. Most importantly, early-life exposure was associated with prolonged survival of pneumonia (with increased expression of IFN- γ , IL-4 and monocyte chemoattractant protein-1) following infection. Thus, colonization may be responsible for increased pathogen resistance and protection against pathogen-mediated nonallergic asthma, in addition to a less inappropriate response to allergens.

Although the lower airways in humans have long been thought sterile, recent studies have shown considerable bacterial colonization, with asthma associated with a preponderance of proteobacteria compared with the Bacteroidetes found in healthy individuals [110]. There is also evidence that some asthmatics are colonized with *Mycoplasma* and *Chlamydial* species [111]. Such chronic bacterial infections may be directly responsible for at least a fraction of nonallergic asthma, although it remains to be seen whether they are cause or effect of the asthma process.

PROBLEMS WITH THE HYGIENE HYPOTHESIS

The hygiene hypothesis has gained considerable support from researchers world wide [100]. However, there is a considerable body of evidence warranting scepticism about the hygiene hypothesis as the primary explanation for global asthma prevalence time trends in particular.

First, it has now well been established that the proportion of asthma attributable to atopy is usually less than one-half [112], with a similar proportion attributable to eosinophilic airway inflammation, the hallmark of allergic asthma [113]. The original hygiene hypothesis suggests that decreased microbial exposure would – through enhanced atopic immune responses – increase the incidence of allergies and allergic asthma. If true, then the protective effects would be most pronounced for the atopic asthma phenotype as well as other atopic conditions such as eczema. However, there is some evidence that nonatopic asthma may have increased more than atopic asthma [114]. Also, farming exposures

may also be protective against nonatopic wheeze (see above), suggesting that microbial exposure may not be only affecting the prevalence of allergic conditions. Furthermore, in a repeated population survey among preschool children, an increase in asthma prevalence was not only found in children with the classic asthma pattern of wheeze but in all wheezing phenotypes including viral-induced wheezing [115]. Thus, the hygiene hypothesis may either not fully explain current time trends or the mechanisms through which the hygiene hypothesis mediates protective effects do not exclusively involve enhanced atopic immune responses.

Second, as noted above, asthma prevalence has begun to decline in both children [116,117] and adults [118] in western countries, but it appears unlikely that these countries have become less clean [100], and there is certainly no evidence that family size has increased. For example, although housing conditions are unlikely to have become more hygienic in US inner city populations, asthma prevalence has increased significantly in those populations, particularly among African-Americans living in poverty [119]. Furthermore, studies of specific infections and asthma risk have not consistently demonstrated a protective effect [120]. Also, the observed decline in asthma did not correspond with a decline in atopic eczema and food allergy, which continue to increase in prevalence [121].

A third anomaly is the high asthma prevalences in Latin America, which appear unlikely to have lower infection rates than European countries such as Spain and Portugal which have lower asthma symptom prevalence [122]. Despite this, the early epidemiological findings showing that daycare attendance and farm animal contact were protective of atopy and asthma have been replicated in Latin America (Chile), suggesting a similar effect to that originally observed [123].

Finally, although the hygiene hypothesis is generally explained as a protective effect of early exposures resulting in long-lasting health benefits, recent studies [49,124] suggest that exposures throughout life may be important (and that long-term continual exposure may be required to maintain optimal protection).

CONCLUSION

Ultimately, none of these anomalies is fatal for the hygiene hypothesis in general, but only for a very 'narrow' version in which early-life microbial pressure protects against atopic asthma by suppressing TH₂ immune responses. A more general version of the hygiene hypothesis may, however, remain valid for asthma. In particular, it is a very useful model to

explain the significant protective effects of farming on asthma observed in many studies worldwide [29]; the hygiene hypothesis is consistent with findings that pets in the home may protect against allergies and asthma [67] and many aspects of the hygiene hypothesis can be reproduced in murine models [125]. However, it is unlikely that the hygiene hypothesis can solely explain the large asthma prevalence increases observed or the decline in asthma prevalence observed more recently in western countries. As we have previously noted [100], it is important to consider the 'forest' of changes that occur with westernization, as well as the specific 'trees', and that the package of changes that come with westernization and increased hygiene may increase asthma risk but not necessarily exclusively through an imbalance of TH₁/TH₂ immunity. New aetiological theories of global asthma prevalence are, therefore, required that are more consistent with the epidemiological evidence and which take into account factors affecting the time trends for both allergic and nonallergic asthma.

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Conflicts of interest

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- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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