Mycobacteria, genes and the 'hygiene hypothesis'

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Purpose of review

The 'hygiene hypothesis' suggests that a relationship exists between improved hygiene and an increase in allergic diseases. As an underlying mechanism for this hypothesis it is proposed that due to the lack of microbial stimulation either a misbalance in T helper type responses or a misbalance in regulatory mechanisms develops. As yet, however, a specific infectious factor responsible for the hygiene hypothesis has not been found.

Recent findings

Animal models have lent support for mycobacteria as important candidates in the hygiene hypothesis. These animal studies have also suggested that mycobacterial treatment generated regulatory mechanisms which restored the immune balance. In contrast, the relationship between mycobacterial infection or treatment and the development of allergy and asthma in humans is unclear and highly controversial.

Summary

Mycobacteria have been found to unambiguously reduce allergic and asthmatic manifestations, suggesting that mycobacteria perhaps can be used as an 'anti-asthma' vaccine. Conflicting results in humans, however, confirm that the complex and multifactorial interactions between the environment and the genetic background of the individual contribute to the development of allergic disease. Therefore, the hygiene hypothesis should involve the genetic and the environmental background of the individual.

Keywords

allergy, asthma, mycobacteria, hygiene hypothesis

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Abbreviations

BCG	bacillus Calmette-Guérin
Th1	T helper type 1
Th2	T helper type 2
TLR	toll-like receptor

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Introduction

Allergic diseases, such as allergic asthma, are steadily increasing in developed countries [1]. Whereas allergic diseases were a rare disorder restricted to the privileged class in the nineteenth century, at present almost a quarter of the population in some Western countries demonstrate sensitization to one or more common environmental allergens. In countries like Britain and Australia, a quarter of the children under the age of 14 years have asthma and a fifth have eczema [2]. The cause of the rise in allergic diseases must be sought in environmental factors, such as increases in allergen exposure due to housing isolation or breast feeding [3], increases in environmental pollution [4], and changes in the diet and the gut flora [5]. A lot of studies, however, have drawn the conclusion that a change in the level and the kind of early childhood infections could be the major factor influencing the development of allergic diseases. Already in 1976, Gerrard and colleagues [6] demonstrated lower IgE levels in rural living Metis Indians in Canada when compared with the local white community. They stated 'atopic disease is the price paid by some members of the white community for their relative freedom from diseases due to viruses, bacteria and helminths' [6, p. 99]. In 1989, Strachan [7] denoted the suggested relationship between hygiene and allergic disease the 'hygiene hypothesis'. Basically, this hypothesis states that improved hygiene in industrialized societies, together with improved public health measures and the use of vaccines and antibiotics have reduced the incidence of infections that normally stimulate the immune system in some way which protects against development of allergic disease [8].

The epidemiological data that underlie the hygiene hypothesis are extensive, but nonetheless highly controversial. Several studies show that a large family size or early placement in day care settings, and therefore a presumed high exposure to infectious agents, protects against the development of allergic disease [9]. Similarly, exposure to farm animals early in life reduces the likelihood of developing atopic sensitization or asthma, possibly due to higher bacterial endotoxin levels [10]. So far, a specific factor responsible for these observed effects has not been identified. It was suggested that *Mycobacterium tuberculosis* [11], hepatitis A [12], or measles [13] infection during childhood prevents the development of allergic disease later in life. The inverse relationship between these infectious agents and development of allergic asthma, however, has not been confirmed in other studies [14–16]. Therefore, the

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relationship between infection and the development of asthma still remains controversial and indefinite.

A mechanism for the hygiene hypothesis: T helper type 1 and 2, and regulatory T cells

The first proposed mechanism for the hygiene hypothesis was based on the T helper type 1 and 2 (Th1 and Th2) dichotomy in specific murine and human immune reactions [17]. Th2 cells, by secreting mediators such as IL-4 and IL-5, are important in initiating and sustaining the allergic and asthmatic response by regulating the production of IgE and the growth, differentiation and recruitment of eosinophils [18]. Th1 cells, secreting IFN-γ and involved in the immune response to a number of intracellular pathogens, dampen the activity of Th2 cells [17]. The immune system in newborns is weakly Th2-biased [18]; infection in infants would lead to stimulation of this Th2-biased immune system to mature and to develop more Th1-biased immune responses. For this reason, a lack of infections would lead to less frequent stimulation of Th1-biased responses and, thereby, facilitate development of Th2-biased immune responses [19,20]. In contrast with this initially widely accepted mechanism is the observation that a massive Th2-dominated immune reaction as seen in helminth infections is associated with protection against the development of allergy and asthma [21,22]. Moreover, the incidence of Th1-mediated autoimmune diseases such as type 1 diabetes and multiple sclerosis is increasing as well [23,24].

It is important therefore to realize that the connecting link between allergic and autoimmune diseases is the fact that both represent poorly regulated and hence exaggerated immune responses. Most of the regulation in the immune system is performed by various regulatory T cells [25]. These cells prevent the activity of effector T cells and other inflammatory cells, by secreting inhibitory cytokines such as IL-10 and transforming growth factor-β, but also by direct contact with effector T cells or antigen presenting cells [26]. Since regulatory T cells control immune reactions to commensal and pathogenic bacteria, microbial exposure could have a huge impact on regulatory T-cell development [27•,28] and therefore on the development of allergic or autoimmune disease. This was elegantly illustrated in experiments using germfree mice in which it was impossible to elicit tolerance to an allergen [29]. Similarly, germfree rats were highly sensitive to arthritis induction compared with specific pathogen free or conventional rats [30]. The hygiene hypothesis may not be explained by a mechanism which involves a deregulated Th1/Th2 balance, but may involve a mechanism employing a balance between regulated and deregulated immune responses instead (Fig. 1) [8,23]. Allergic disease is caused by a deregulated Th2 response, while in autoimmune diseases a deregulated Th1 response is involved.

Mycobacteria

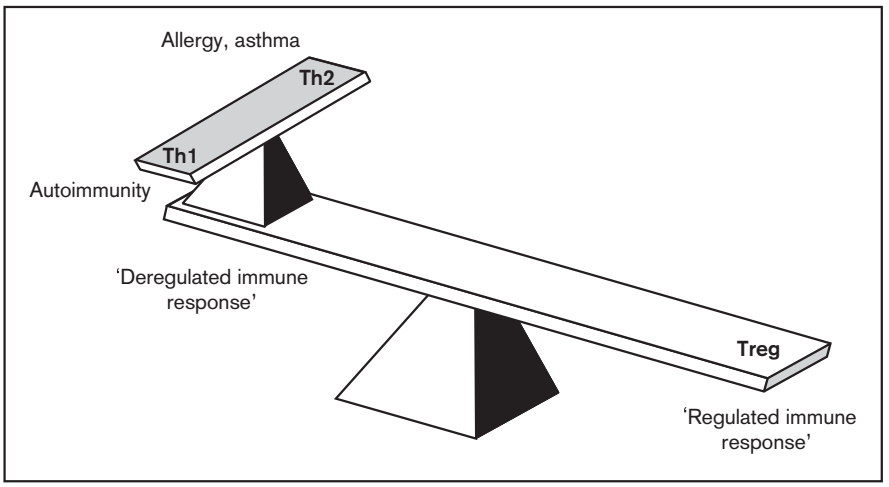
If the increase in diseases by a faulty immune regulation is due to decreased exposure to certain microorganisms, one may speculate that certain bacterial species can be used as protective or therapeutical vaccines against diseases such as allergic asthma. In this respect, mycobacterial infections or vaccines are the subjects most studied. Mycobacteria are in general saprophytes, present in soil, water and dust in a natural environment. Since the exposure to these bacteria is regulated by lifestyle, the exposure to mycobacteria may be hugely reduced in Western societies. Mycobacterial infections usually have a very strong capacity to elicit Th1 responses [31,32]. As a result, the mycobacteria attracted much attention in the hygiene hypothesis in which initially the concept of Th1–Th2 balancing immune regulation was applied. In addition, the impact of mycobacteria on the immune system is indicated by the existence of CD1-restricted T cells which were first described to recognize only mycobacterial components [33,34].

Mycobacteria express a whole range of ligands that trigger signalling through pattern recognition receptors such as the mannose receptor, toll-like receptor (TLR) 2 and TLR4 on host phagocytes and other immune cells. This interaction results in activation of these cells and the release of pro- but also antiinflammatory mediators. The predominant antigen on mycobacteria is the glycolipid lipoarabinomannan. While slow-growing mycobacteria such as bacillus Calmette-Guérin (BCG) and *M. tuberculosis* express mannose-capped lipoarabinomannan, fast-growing mycobacteria like *M. vaccae* probably express arabinofuransyl-terminated lipoarabinomannan. The lipoarabinomannan molecules differ in the velocity and intensity of cytokine release from phagocytes [35]. In addition, mycobacteria express heat shock proteins [36], which are important in the regulation of the immune system during inflammation [37]. Both CD14 and the macrophage mannose receptor serve as lipoarabinomannan receptors, whereas TLR2 and -4 bind lipoarabinomannan [35] and heat shock protein. Activation of the TLR leads to the induction of inflammatory responses and to the development of adaptive immunity, including regulatory T-cell development [38].

The experimental data in animal models of allergic asthma supporting the candidature of mycobacteria in the hygiene hypothesis are quite convincing; vaccination with mycobacteria was found to suppress the development of several allergic and asthmatic manifestations in the mouse [39–41] and in the rat [42]. Treatment of mice with heat-killed *M. vaccae* during allergen immunization

Figure 1. The balance between regulated and deregulated immune response

If regulatory activity is low, deregulated immune response will develop. The nature of this deregulated response depends on the balance between T helper type 1 (Th1, autoimmune disease) or T helper type 2 (Th2, allergy and asthma). (Adapted from Rook and Brunet [23].)



or provocation reduced airway hyperresponsiveness and eosinophilia and Th2 type responses (IgE and Th2 cytokine levels), although timing and dose of *M. vaccae* were critical for effectiveness [43]. Interestingly, no induction of allergen-specific Th1 responses was observed, as demonstrated by other investigators as well [39]. Therefore, mechanisms other than mere changes in the balance between Th1 and Th2 are responsible for the observed effects of mycobacterial treatment. Zuany-Amorim and co-workers [44••] provided evidence that the downregulatory effect of *M. vaccae* was probably mediated through the induction of specific regulatory T cells. The downregulatory effect was dependent on production of IL-10 and transforming growth factor-β by these cells, and not by induction of Th1 responses. This was the first experimental proof that regulatory T cells (elicited by mycobacteria) are implicated in the hygiene hypothesis.

It remains to be investigated whether the induction of regulatory T cells is the only mechanism by which mycobacteria can inhibit the allergic response. Interestingly, many of the observed effects of *M. vaccae* bear a resemblance to the inhibition of allergic asthma symptoms after glucocorticoid treatment. It has been suggested that (myco)bacterial infections directly, or indirectly, via neural pathways, activate the hypothalamus–pituitary gland–adrenal gland to secrete glucorticoids [45,46], which subsequently may reduce allergic inflammation.

Mycobacteria and allergy in humans

In contrast, the relationship between mycobacterial infection and the development of allergy and asthma in humans is highly controversial. It was shown that *M. tuberculosis* infection rates were significantly inversely correlated with the prevalence of allergic [47] or asthmatic [11] manifestations. Whether immunization with the commonly used BCG vaccine is associated with protection against development of allergic disease is still a matter of debate. The first epidemiological study showed that within a population of BCG-vaccinated Japanese children, a positive tuberculin reaction at 6 and 12 years of age correlated with a reduced incidence of allergic manifestations compared with children with negative tuberculin reactions [48]. A similar study in Guinea-Bissau confirmed that development of atopy was lower in BCG-vaccinated children [49]. In contrast, several retrospective studies in a variety of countries failed to demonstrate a negative correlation between BCG vaccination (as indicated by tuberculin responses) during early childhood and the subsequent development of atopy or asthma (summarized in [15,50•]). Studies using BCG [51] or heat-killed *M. vaccae* [52] therapeutically in established asthma or atopic dermatitis [53], however, showed beneficial effects of mycobacterial treatment, although the effect of *M. vaccae* in asthma patients was not confirmed [54]. These contrasting results might be explained by the fact that a lot of variables were different in these studies, such as the age at and the frequency of vaccination with mycobacteria, the BCG strain used and the varying natural exposure to mycobacteria (including *M. tuberculosis*) or allergens [55]. Moreover, the measurement of the tuberculin response might not be a good reflection of successful mycobacterial immunization [56]. Even more important might be a genetic relationship in the possible inverse correlation between (mycobacterial) infection and the development of allergy and asthma.

Genes and the hygiene hypothesis

Besides the above-mentioned environmental factors, genetic factors undoubtedly play a role in the pathogen-

esis of asthma as well. A recent study suggested that 73% of asthma susceptibility is due to genetic factors [57]. The complexity of the genetic contribution to the development of asthma and other atopy-associated phenotypes is reflected by the linkage of susceptibility to allergy and asthma with several chromosomal regions on chromosome 5, 6, 11, 14 and 12, which contain numerous candidate genes. The chromosomal part 5q31 is particularly important because it contains a large number of candidate genes, including the genes for IL-12p40, IL-9, β-adrenergic receptor and the IL-4 cytokine cluster, containing the genes for IL-4, IL-5 and IL-13 [58]. Nonetheless, the significant increase in the prevalence of allergic diseases over the past few decades cannot be explained by mere changes in gene frequencies. It is more likely that various predisposing genetic factors interacting with changes in the environment, such as a decline in childhood infections, have caused an increase in the percentage of the population that is susceptible to allergic disease.

Studies investigating the interactions between genes and microbial factors in allergy and asthma are scarce and hampered by ethnic differences in the immune response to infections. Nevertheless, some interesting candidate genes that interact with the link between infection and allergic disease have appeared recently. First, *Tim1*, a mouse homolog of the human gene encoding the receptor for hepatitis A, a virus associated with protection from allergy and asthma [59], influenced Th2 responses and the development of airway hyperresponsiveness in allergic mice [60]. Second, a genetic variation in the regulation for CD14, the endotoxin receptor, is strongly correlated with IgE responses [61,62]. Interestingly, the expression of both CD14 and TLR2 was higher in children from a farm (an environment believed to lead to a lower prevalence of allergic disease) compared with the children of nonfarmers [63•]. Finally, other candidate genes in this respect are the genes for IL-12B [64], nitric oxide synthase 1 [65], tumor necrosis factor-α [66] and IFN-γ [67], all important mediators of both resistance to infection and players in the pathogenesis of allergic disease [62]. Therefore, since the ability to mount a response to mycobacterial antigens is highly heritable [68,69], a genetic contribution to the inverse relationship between mycobacterial infection and the development of allergy and asthma is very plausible. *Nramp1*, a gene that determines resistance to mycobacteria [69], is associated with atopy in mice [70] and humans [71] and with autoimmune disease in humans [72,73]. In fact, this gene strongly affected efficacy of mycobacterial treatment in mice [74•]. These findings have important implications for the possible future use of mycobacteria and their components in the prophylaxis or treatment of allergic disease.

Conclusion

Although several studies have shown that the level of infection may affect the development of allergy and asthma, a specific infectious factor responsible for this effect has not been found as yet. In the search for such a factor, the relation between mycobacterial infection and allergic disease has attracted a lot of attention and discussion. Experiments in rodents unambiguously have demonstrated that mycobacterial infection or mycobacterial treatment is able to prevent allergic and asthmatic manifestations. In contrast, in humans, the protective effect of mycobacterial infection and of other infections is still challenged and a matter for debate. Differences in study design, mycobacterial preparations and background exposure to mycobacteria may explain the contradictive results so far. In our opinion, however, the genetic background of the individual may strongly influence the outcome of the interaction between the (infectious) environment and the development of allergic disease. Genetic variations that evolved to improve resistance to infections may very likely be misdirected to promote allergic inflammation in the absence of infection in Western societies. Studies investigating this interaction should take into account and elucidate the complex interaction between genes, the environment and allergic disease.

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