Commentary

The coming-of-age of the hygiene hypothesis

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Received: 22 December 2000
Revisions requested: 23 January 2001
Revisions received: 27 February 2001
Accepted: 5 March 2001
Published: 2 April 2001

Respir Res 2001, **2**:129–132

(Print ISSN 1465-9921; Online ISSN 1465-993X)

Abstract

The hygiene hypothesis, as originally proposed, postulated an inverse relation between the incidence of infectious diseases in early life and the subsequent development of allergies and asthma. New evidence from epidemiological, biological and genetic studies has significantly enlarged the scope of the hypothesis. It now appears probable that environmental 'danger' signals regulate the pattern of immune responses in early life. Microbial burden in general, and not any single acute infectious illness, is the main source of these signals. The latter interact with a sensitive and complex receptor system, and genetic variations in this receptor system may be an important determinant of inherited susceptibility to asthma and allergies.

Keywords: atopy, CD14, endotoxin, genetics, hygiene

Introduction

There is now convincing evidence indicating that the prevalence of allergic diseases in general, and of asthma in particular, is on the rise in high income societies [1]. Many hypotheses have been proposed to explain these increases, but the most widely discussed and the most controversial is the so-called 'hygiene hypothesis' [2,3]. This hypothesis was first enunciated in quite straightforward terms: the Western lifestyle has succeeded in markedly decreasing the incidence of infections in early life, and these infections may have a protective effect on the subsequent development of allergies.

Initially, the hypothesis was mainly based on epidemiologic evidence of an inverse relation between indirect markers of increased infectious burden and prevalence of allergic diseases and allergic sensitization (reviewed in [4]). Concomitant studies on the development of the immune system in early life seemed to provide a biological basis for the hypothesis' main postulate. It has been reported that mononuclear cells obtained from cord blood showed markedly decreased cytokine responses to nonspecific stimuli [5]. This included both responses that characterize the T-helper (Th) 1 type (ie IFN-γ) and the Th2 type (ie IL-4). When studied both in cord blood and during the first year of life [6,7], however, Th1-like responses were particularly decreased among children with a family history of allergies and among those who would subsequently become sensitized to aeroallergens. Since IFN-γ is known to downregulate Th2-type responses, and these responses are essential for IgE synthesis by B cells, it was suggested that the development of IFN-γ responses could be stimulated by exposure to infectious agents postnatally [3,8], and that this could be the mechanism by which these infections protected against the development of allergic diseases.

Infectious diseases versus microbial burden

Presented in this fashion, the 'hygiene hypothesis' was tested in relation to several infectious diseases. The results were contradictory: whereas markers of a previous infection with foodborne pathogens appeared to be associated with decreased risk of subsequent allergic sensitization,

LPS = lipopolysaccharides; Th = T-helper.

this was not the case for respiratory pathogens [9] or was confined to certain respiratory viruses [10]. Moreover, while some authors reported that contagious diseases such as measles were associated with decreased likelihood of developing allergic conditions [11], other workers were unable to confirm these observations [12]. The finding of an inverse relation between responses to tuberculin test and asthma and allergies in Japan was interpreted by some authors as indicating that infection with *Mycobacterium tuberculosis* could protect against allergies [13]. Other workers, however, contested that the association was more simply explained by a reduced Th1-type response to tuberculin in atopic subjects.

But perhaps the greatest challenge to the 'hygiene hypothesis', expressed simply in terms of an inverse relation between incidence of infectious diseases and allergies, has arisen from studies of children of farmers [14–17]. These studies have consistently found that growing up on farms confers significant protection against the development of atopy (as assessed by skin test reactivity to local allergens), allergic rhinitis and (to a lesser extent) asthma. A more detailed analysis of several of these studies showed that the factor that best explained the difference in the prevalence of allergies among children living on farms and those living in the same rural villages but not on a farm was having contact with livestock and poultry [16].

These results suggested that substances produced by farm animals, which could presumably also be abundant in homes located close to these animals quarters, could play a role in the prevention of allergies. In a study by von Mutius *et al*, dust collected from homes of children living on farms had markedly higher levels of endotoxin than that from homes of children living in the same rural communities but away from animal farms [18].

These findings suggest a broader approach to the understanding of the environmental factors that may influence the development of the immune system and, through this mechanism, decrease the likelihood of the development of allergies. Endotoxins are lipopolysaccharides (LPS) that form part of the outer structure of the cell wall of Gram-negative bacteria. An exquisitely sensitive mechanism that detects the presence of LPS is present in vertebrate immune systems. This receptor system is expressed mainly in antigen presenting cells, and constitutes the first, nonadaptive response to external microbial stimuli. The system in question is in fact made up of pattern-recognition receptors that are capable of detecting the presence of different structures present in Gram-negative and Gram-positive bacteria, mycobacteria, fungi, and even viruses [19]. This receptor system activates a complex intracellular signaling mechanism that will not be discussed in the present article in detail, but that results in the production of a set of cytokines and immune mediators by antigen presenting cells.

The finding that exposure to environmental bacterial products, that do not directly cause specific diseases in those exposed, may influence the pattern of immune responses in humans provides an entirely new framework for the understanding of the 'hygiene hypothesis'. The influence of potential infectious agents on the risk of allergies is thus not confined to those that directly produce infectious diseases in humans, but may comprise a much broader set of agents, including those to which the individual is exposed in the home, in schools, in daycare, etc. These agents may act through the respiratory system but also through the intestinal track [9], modulating the development of the immune system during the first years of life.

This new set of potential exposures may be very relevant for our understanding of the marked increases in the prevalence of allergies and asthma that have taken place in past decades. The widespread availability of products and practices that promote an aseptic environment for humans in general, and for young children in particular, may have markedly decreased the exposure of our species to the myriad of 'danger' signals coming from microbes and germs that has been part of our entourage since we first became a species or even earlier [20].

The coming-of-age of a hypothesis

The author believes that, with the discovery of specific markers of environmental microbial exposure, such as endotoxin, that interact with a well-known receptor system, the hygiene hypothesis has reached a new stage of maturity. Although no-one can reasonably propose or even believe that marked decreases in the burden of microbial exposure are the only causes of the increases in asthma and allergies observed recently, there is now strong indirect evidence suggesting that those exposures play a role in the postnatal maturation of immune responses. This evidence not only comes from studies performed in rural communities like those described earlier. Gereda *et al* [21], for example, assessed the concentration of endotoxin in house dust in a group of young children living in the Denver area, and subsequently assessed sensitization to local aeroallergens and cytokine responses by peripheral blood mononuclear cells. They found that children exposed to higher levels of endotoxin were significantly less likely to become sensitized to local aeroallergens. Gereda *et al* also reported that IFN-γ responses by peripheral blood T cells were significantly increased among children exposed to higher levels of endotoxins. Other studies in which house dust is being collected during infancy and in which subsequent development of asthma and allergies is being studied are now in progress, and they may provide important new information in the near future.

The molecular mechanisms by which microbial burden in early life, and endotoxin exposure in particular, can influence asthma risk are beginning to be understood. It is now well established that early allergic sensitization is an important risk factor for the development of asthma [22]. We have suggested that the early establishment of a chronic, IgE-mediated immune response in the lungs may alter lung development and predispose to chronic airway hyperresponsiveness [3]. Our group has also shown that subjects who, by the age of 6 years, will become sensitized to *Alternaria*, the most asthma-related allergen in Tucson, Arizona, have significantly lower IFN-γ responses by peripheral blood mononuclear cells as compared with subjects who will not become sensitized to *Alternaria* [6]. It is thus possible that exposure to endotoxin, by stimulating the early development of IFN-γ responses, may prevent early allergic sensitization and, by this mechanism, prevent the development of asthma. A recent report by Tulic *et al* [23], suggesting that pre-exposure to endotoxin prevents subsequent sensitization to allergens in rats, strongly supports this hypothesis.

It is important to mention that, although exposure to endotoxin may be preventive in the development of allergies and asthma, it may be an important risk factor for more severe symptoms in subjects who have already developed the disease [24]. It is thus possible that, once an IgE-mediated response to aeroallergens has been established, endotoxin may enhance this response. Interestingly, in the Tulic *et al* report [23], exposure to endotoxin in rats after sensitization to allergens had already been established was shown to enhance the IgE-mediated response in these animals, thus providing experimental support for this contention.

Gene/environment interactions in the development of allergies

The use of endotoxin as a potential marker for microbial exposure not only provides a helpful epidemiologic tool, but it also allows the identification of a well-defined, specific biological pathway directly involved in immune responses to such exposure. It is plausible to surmise that polymorphisms in the genes that code for proteins involved in this pathway may determine, at least in part, individual susceptibility to the effects of endotoxin.

In our laboratories, we have begun the process of screening for polymorphism genes involved in the receptor system for LPS. We initiated this search with the CD14 gene, which codes for one of the main components of the endotoxin receptor system [19], and found a C→T variation at position −159 of the promoter region of the gene (CD14/−159). This polymorphism was very frequent in the population, with one-half of all chromosomes containing one or the other allele (C or T). Carriers of the T allele in homozygote form were shown to have significantly higher levels of circulating sCD14, the soluble form of the receptor. Researchers interested in genetic risk factors for myocardial infarction subsequently reported that the T allele was also associated with higher expression of CD14 on the surface of antigen presenting cells [25]. Our group found that homozygotes for the T allele had significantly lower levels of total serum IgE, especially if they were skin test positive to local aeroallergens. Moreover, atopic carriers of the T allele had significantly lower numbers of positive skin tests than carriers of the C allele. Two other research groups have reported similar findings [26,27], although not all researchers have been able to confirm our findings [28,29]. It thus appears that, at least in some populations, polymorphisms that increase the expression of CD14 may be associated with lower levels of IgE. A potential explanation for this finding could be that increased sensitivity to endotoxin and other microbial products that interact with CD14 could increase IL-12-mediated responses to these products, with increased likelihood of development of Th1-type responses and thus less likelihood of IgE-mediated immunity.

The presence of biologically meaningful polymorphisms in genes associated with the receptor system for endotoxin opens a new chapter for the assessment of the so-called hygiene hypothesis. It has now become possible to assess the role of these genetic variants as determinants of susceptibility to different allergy-related outcomes in individuals who are exposed to different levels of endotoxin in the environment. Variations in many genes involved in the response to endotoxins and other microbial products will be defined, as part of the Genome Project, in the very near future.

Conclusion

A better understanding of the biological effects of specific environmental products that are responsible for the inverse association between infectious burden and allergy and asthma will enhance our understanding of the gene/environment interactions that cause these common and burdensome diseases. Moreover, this understanding may offer new strategies for the primary and secondary prevention of allergies and asthma in the near future. It may thus be possible to design prevention strategies based on exposure to innocuous surrogates of bacterial products in individuals who may be more or less susceptible to the preventive effects of these products depending on their specific genetic background.

Acknowledgements

This work was supported by National Heart Lung Blood Institute Grants HL 66447, HL 56177, HL 61892, HL 64307, and HL 66800.

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