

The hygiene hypothesis: does it function worldwide?

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Purpose of review

This article intends to be a systematic review of papers published during 2003–2004 quoted in a Medline search for 'hygiene hypothesis'. The worldwide perspective of the article does not refer just to a geographical concept, but tries also to address the question of whether the consensus on the hypothesis is global or whether it applies to all types of allergic or immunologic disease, to any age sample or infectious agent. Furthermore, the article outlines those clinical and experimental studies which, in the authors' opinion, may represent significant contributions to a better understanding of the hygiene hypothesis and may guide further investigations on the subject.

Recent findings

The association between a reduced exposure to infectious agents (as a part of a changed lifestyle) and a higher prevalence of atopy seems now to be confirmed by consistent evidence. Mechanisms underlying this association, however, are not yet completely clear (immune deviation or immune regulation).

Summary

Further experimental and clinical studies are needed, with special reference to the time, duration and intensity of exposure to any specific infectious agent which is related to well-defined allergy outcomes. The background information for using microbial products in allergy prevention and treatment is still limited.

Keywords

allergic diseases, hygiene hypothesis, immune modifiers, infections, microbial products

Introduction

The hygiene hypothesis is based on the postulate that a reduced exposure to infections during infancy is associated with an increased risk of developing allergic diseases later in life.

Studies on the hygiene hypothesis may be ascribed to three major areas of investigation, which also represent subsequent historical phases of how the hypothesis developed following its introduction by David Strachan [1].

The first group of investigations includes epidemiological studies of the association between direct or indirect evidence of exposure to infectious agents and the prevalence of allergic diseases in different population samples. These studies encompass Strachan's original paper, showed an effect of family size and birth order on the risk of allergy, as well as studies on the association between allergic diseases and day-care, socio-economic status, diet, lifestyle, animal ownership, development levels in different countries and particular environmental settings [2]. In addition to these studies, which used surrogate markers of different degrees of exposure to infections, particularly during early life, this area of investigation also includes studies which measure exposure to specific infectious agents, such as tuberculosis [3], measles [4], and hepatitis A [5] through history-taking or serology.

The second area of investigation refers to studies addressing potential mechanisms responsible for the negative association between allergic diseases and infections, emerged from epidemiological studies. They are based on experimental evidence that infectious agents direct the immune response to a Th1-phenotype profile, and therefore that the lack of infectious burden may be responsible for an immune deviation to a Th2-cytokine profile and the rising prevalence of allergic diseases in Westernized countries [6,7]. More recently, the immune deviation hypothesis (based on a Th1/Th2 imbalance) has been integrated by the immune regulation hypothesis (based on the role of T-regulatory cells, acting on both Th1 and Th2 responses) [8••]. Within this area of investigation, special relevance should be given to studies on farmers showing endotoxin or LPS to be a major trigger of the cascade of events leading to a protective immune response (or to an allergic response in the lack of it) [9].

Finally, the third area of investigation refers to intervention studies which aim to demonstrate a preventing effect

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on allergic diseases of immune stimulation with immune modifiers such as mycobacteria, CpG or other bacterial products [10^{••}].

Well, where are we now and what was new and relevant with reference to the hygiene hypothesis?

This article intends to be a systematic review of papers published during 2003–2004 quoted in a Medline search for 'hygiene hypothesis' and their contribution to accumulated evidence that the hygiene hypothesis may be proven as true. The worldwide perspective of the article will not only refer to a geographical concept, but will also try to address the question of whether the consensus is global or whether it applies to any allergic or immunologic disease, to any age sample or to any infectious agent. The article will also outline those clinical and experimental studies which, in the authors' opinion, may represent significant contributions to a better understanding of the hygiene hypothesis and may guide further investigations on the subject.

The methodology used for selecting the papers of this systematic review certainly does not allow the analysis of all papers which might be related to the hygiene hypothesis, particularly the vast literature covering the relationship between innate immunity and the adaptive response to allergens or the use of microbial products to modulate the allergic response.

Does everybody agree on the hygiene hypothesis?

The critical evaluation of the 111 papers selected for the authors' systematic review shows that the number of favorable opinions largely exceeds the number of contrary ones, although there is still not a unanimous consensus. As shall be discussed below, however, the heterogeneity of population samples considered, of markers used to prove infections and of outcome measures for documenting disease prevents any possible meta-analysis or grading of evidence for the hygiene hypothesis. Moreover and surprisingly enough, the majority of recent papers on the hygiene hypothesis are review articles or speculative papers addressing hypotheses on potential mechanisms responsible for a cause–effect relationship rather than original studies aimed at proving or disproving associations reported, or prospective cohort studies or, finally, well-designed and focused experimental studies.

In this contest, perhaps the most interesting new information comes from studies on new surrogate markers of infections, such as fever before the age of 1 year [11] or tonsillar hyperplasia [12] or crowding [13]. On the other hand, it has also been shown that some 'classical' surrogate markers of infection, such as family size and sibling

effect, are not necessarily associated with an increased documentary and serological evidence of infections [14].

Does the hygiene hypothesis apply to any allergic disease?

There is a confusing heterogeneity in outcome measures used to evaluate whether exposure to infectious agents may affect allergy. In fact, some papers use sensitization as outcome measure, others hay fever, or asthma, or atopic dermatitis. It is widely recognized that allergic diseases are polyfactorial and that any specific allergic disease is due to distinct genetic and environmental factors also related to the target organ involved. Accordingly using 'allergy' or 'allergic disease' as an outcome measure makes for imperfect association studies. This possibly explains some reported inconsistencies. Endotoxin for instance, while preventing allergy [9] may trigger bronchial obstruction when used in challenge studies of asthmatics [15,16]. Furthermore, sensitization itself undergoes a polyfactorial control depending on the sensitizing allergen. In fact, the authors showed [5] that exposure to hepatitis A is negatively associated with a sensitization to any allergen except cockroach, which, on the contrary, is positively associated with the presence of antibodies to hepatitis A and represents one of the major etiological factors in asthma in US inner cities [17]. Interestingly enough, in the same paper, it was shown that the negative association with exposure to some microbes was more evident for allergic rhinitis than for asthma [5].

Does the hygiene hypothesis extend to diseases other than allergy?

Strachan's [1] original description of the hygiene hypothesis refers to allergic diseases. The hygiene hypothesis, however, has also been invoked to explain the rising trends of other immunologic diseases, such as Th1-type I diseases [18[•],19], multiple sclerosis [20[•]], inflammatory bowel disease [21] or glomerular disease [22].

Immune regulation may certainly help to support the concept that a reduced exposure to infectious agents may protect from both Th2 (allergic) and Th1 (autoimmune) diseases [23,24,25[•]]. It is very difficult, however, to evaluate whether the reduced exposure to infectious agents is as a causal protective factor or one of the many variables of a changing lifestyle.

Does the hygiene hypothesis function in any world region and population sample?

The hygiene hypothesis was originally invoked to justify the rising prevalence of allergic diseases in Westernized countries. The increased prevalence of allergic diseases, however, now seems to have reached a plateau in industrialized countries [26]. Accordingly, it has been suggested that the negative association between infections

and atopy should be, at present, better observed in developing countries, although this was not the case in all studies [27,28]. Extending the hygiene hypothesis worldwide is made troublesome, however, by the different infectious agents which are prevalent in the region (parasites, mycobacteria, viruses), as well as by the degree of exposure and by the different ages of studied populations. In fact, whether the protective effect of infectious agents does not persist throughout all life, but is effective at birth or at a specific period after birth, association studies can be highly influenced by the age of cohorts in which outcome is measured [29].

Does the hygiene hypothesis apply to any infectious agent?

The influence on immune response by bacteria, viruses and parasites is quite specific, depending on the infectious agent as well as on the time, degree and duration of exposure. In fact, it seems very simplistic to refer to associations between 'allergy' and 'infections', since not only 'allergy' but also 'infections' are polyfactorial and heterogeneous entities.

The hygiene hypothesis, which originally referred to the protective effect of respiratory viruses on allergic diseases, can be more consistently proven for food-borne microbes than for air-borne agents [30]. In fact, respiratory viruses have been reported as being associated with a higher risk of atopy [31,32^{••},33]. Moreover, differences should also be stressed within the same group of infectious agents. Seropositivity for hepatitis A, *Helicobacter pilory* and *Toxoplasma gondii* was associated with a lower prevalence of atopy in a Danish population, while seropositivity to *Clostridium difficile*, *Campylobacter jejuni* and *Yersinia enterocolitica* was associated with a higher prevalence of atopy [34]. Similarly, while endotoxin has been reported as protective for atopy [35], Staphylococcal enterotoxin was shown to have no protective effect but to be a potent IL-5 inducer and a cofactor in the pathogenesis of allergic diseases [36[•]].

Special attention has recently been devoted to the role of parasites [37,38^{••},39,40,41^{••}]. Certainly, the immune deviation theory cannot be invoked to explain the protective effect on atopy (also reported for parasitic infections), since parasites are potent inducers of a Th2 response. Alternative explanations have therefore been put forward to justify the data observed [42].

Very interesting research is coming from the area of experimental studies on intestinal microflora alterations and the role of fungi in promoting allergic disease [43^{••}].

It is of practical relevance to note that vaccination does not influence the prevalence of allergic diseases [44^{••},45].

The hygiene hypothesis: immune deviation, immune suppression ... or something else

The mechanism by which a reduced exposure to pathogenetic or non-pathogenetic microbes results in a higher prevalence of allergic diseases is still controversial [8^{••},46–48].

An exciting and new molecular genetic option for explaining the hygiene hypothesis has derived from data of the very strong and consistent negative association between hepatitis and atopy [5,49,50^{••}]. In fact, the discovery, cloning and polymorphism of *TIM-1* gene has opened a new key of interpretation for a direct effect of microbial agents in preventing atopy [51]. *TIM-1* functions as a receptor for hepatitis A virus and T-cell activation through *TIM-1* may affect directly the development of a Th2-driven allergic inflammatory response.

With reference to mechanisms, it may be interesting to note that the hygiene hypothesis might also be looked at the other way around, i.e. that a pre-existing atopic status might influence a higher prevalence of viral respiratory disease because of the Th1 deficit associated [33] or that parasitic antigens and concurrent Th2 responses may ameliorate Th1-mediated diseases [52].

The interventional impact of the hygiene hypothesis

One of the major translational impacts of the hygiene hypothesis is represented by the possibility of using microbial products to modulate Th2-allergic inflammation, either through immune deviation (Th1 shift) or immune suppression (T-regulatory cells) [45,53,54].

It is not clear yet, however, whether this approach is only useful for preventing sensitization and disease or whether it can be applied to revert an ongoing allergic inflammatory process.

Several substances have been used in both experimental and clinical studies [10^{••}].

Among recent papers included in the authors' systematic review, special relevance has been given to the study of Hayashi *et al.* [55^{••}], who reported that the administration of Toll-like receptor ligands, via the activation of innate immunity, induces high levels of indoleamine 2,3-dioxygenase (the rate-limiting enzyme of tryptophan catabolism) and inhibits Th-2 driven asthma in an experimental model.

Other interesting experimental approaches were based on the use of CpG DNA (alone or in combination with immunotherapy) [56[•]] or *Mycobacterium vaccae* [57]. Interestingly enough, in this last model, the modulatory effect of *M. vaccae* on airway hyperresponsiveness, airway

eosinophilia, Ag-specific IgE, IL-4 and IL-5 production after OVA aerosol allergen challenge was shown to be dependent upon susceptibility or resistance to intracellular bacteria regulated by *Nramp1* (*S1c11ac*) genes, thus suggesting the importance of genetic–environmental interactions in response to immune modifiers [58**].

Conclusion

Epidemiological studies of disease should not only be confined to providing figures on prevalence, incidence, association, etc., but should also be aimed at generating hypothesis to be experimentally tested and which can hopefully serve for a better understanding of pathogenetic mechanisms and disease management. The high and increasing number of investigations and the discussion, which stemmed from Strachan's first publication, show that the hygiene hypothesis fully answered these expectations. Moreover, it stressed the need for a comprehensive vision of the multiple genetic–environmental interactions involved in the immune response, where reciprocal influences of concurrent or subsequent stimuli (such as allergens and infectious agents) cannot be neglected by a sectorial specialist approach.

From the review of previous and recent studies following Strachan's observation, it is possible to state that the hygiene hypothesis – intended as the influence of hygiene as a part of changing lifestyle on the changing prevalence of allergic and immunologic diseases – is now confirmed. Accordingly, further association studies are not needed and cannot add substantial new information.

A different experimental approach is now needed to explain inconsistencies observed and to better understand how exposure to infectious agents may modulate allergic diseases. This approach must be more specific with reference to the time, degree and duration of the exposure to a given microbial stimulus, as well as to the outcome measures of 'allergy'.

The potential translational implications of the hygiene hypothesis represent an exciting area of investigation. Additional experimental evidence and well-designed controlled studies are, however, needed for all microbial products which have been proposed for prevention or treatment of allergic diseases.

Perhaps the best summarizing statement of the progress made by the hygiene hypothesis in the 15 years following its introduction is made by Strachan himself [2]: 'The hygiene hypothesis remains a credible but non specific explanation for observed variations over time, place and person at risk for developing atopic allergic disorders. More prospective studies are needed to unravel which infectious agents exert a protective effect and the time period of importance for sensitisation. The clinical

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