



## Commentary

## Six questions about the hygiene hypothesis

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**Abstract**

According to the hygiene hypothesis, the increased incidence of allergic and autoimmune diseases in developed countries is explained by the decrease in infections. Several questions remain unsettled, concerning notably the nature of protective infections, the mechanisms of protection, the spectrum of diseases concerned by the hypothesis, the difference between triggering and protective infections and lastly the strategies which could be devised to mimic the effect of infections.

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**1. Introduction**

It has been proposed that improved hygiene, which partially explains the reduced rate of infections in western countries, is at the origin of increased incidence of allergic and autoimmune diseases [1,2]. This interesting hypothesis (which warrants rigorous confirmation because of its numerous applications) poses a number of questions that we shall address in the following pages.

(1) What is the evidence for a causal relationship between the decline of infections and increase of autoimmune and allergic diseases? It is not sufficient to observe a concomitant inverse evolution between the incidence of infections and of immune disorders to extrapolate about their causal relationship.

Three sets of data support such a causal relationship.

- The incidence of autoimmune and allergic diseases is directly correlated with the level of sanitary conditions at the national level and the individual level [2]. There

is a North–South gradient of the incidence of autoimmune diseases in Europe which correlates with socio-economic level.

- Prevention or cure of some specific infections increases the risk of allergic diseases. Thus, vaccination against *Streptococcus pneumoniae* in South Africa led to increased incidence of atopy [3]. Similarly, systematic anti-helminth treatment of infested children led to increased incidence of atopy in Venezuela and Gabon [4,5].
- Animal models of spontaneous or experimentally induced autoimmune diseases have shown increased disease incidence when the animals are bred in specific pathogen-free conditions and the diseases are prevented by a whole spectrum of bacterial, viral or parasitic infections [2].

(2) What are the components of hygiene improvement?

Epidemiological studies indicate that several factors could contribute to the so-called hygiene effect. Individual hygiene probably matters, as suggested by the correlation observed between the socio-economic level of individual families, and incidence of atopic dermatitis [6] and Crohn's disease [7]. The incidence of type 1

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diabetes, atopic diseases or Crohn's disease is higher in first-born children (who are not exposed in their infancy to the infections of siblings) the more so when they do not attend day care [8–10].

On the other hand, one may assume that collective hygiene is also very important, perhaps more so than individual: the sanitary quality of drinking water and food (cold chain), vaccination (as mentioned above) or the excessive usage of antibiotics, which affect gut flora.

(3) What are the mechanisms of the protective effect of infections on autoimmune and allergic diseases?

Several mechanisms have been proposed to explain the protective role of infections on immune disorders. Most of the evidence supporting these various hypotheses is derived from the study of animal models of autoimmune and allergic diseases.

Two main mechanisms have been proposed, both involving competition or immunoregulation. Competition between anti-infectious immune responses and other responses, whether they are directed to allergens, autoantigens or specific bacteria may be considered at several levels:

- competition for antigen processing by phagocytic cells
- competition for binding of antigen peptides to MHC molecules
- competition for cytokines essential to lymphocyte differentiation and homeostasis.

In the last case, one may hypothesize that lymphoid cells proliferating in response to pathogens compete with homeostatic signals, notably MHC-self peptide recognition and cytokines (including IL-7). This possibility has been recently reported for mycobacteria in the NOD mouse where it has been shown that complete Freund's adjuvant inhibits the proliferation of unrelated T cells [11]. It is tempting to discuss this observation in the context of the report by Stockinger that T cell proliferation in response to colitis and related antigens can inhibit the development of colitis in SCID mice reconstituted with CD45RB<sup>hi</sup> T cells, presumably through competition for homeostatic signals [12].

Suppressor mechanisms essentially involve suppressor cytokines such as IL-10 and TGF- $\beta$ , two cytokines known to play a central role in the mode of action of regulatory T cells, particularly in the experimental colitis models. These cytokines can be produced by T cells through a mechanism of bystander suppression: regulatory T cells stimulated by infectious agents produce IL-10 or TGF- $\beta$  whose suppressor effect extends to other immune responses (autoimmune and allergic, for example). The production of the two cytokines can also be induced by stimulation of Toll-like receptors, the probable explanation of the diabetes protection observed in NOD mice after treatment with various TLR agonists [13,14].

This latter hypothesis would fit with the loss of the lactobacilli protective effect on inflammatory bowel disease (IBD) noted in TLR9<sup>-/-</sup> mice [15]. Two recent observations support possible TLR involvement in the protective effect of infections on immune disorders in man. A significant correlation was observed between a polymorphism of the TLR2 gene and the propensity of developing asthma in children raised on animal farms, an environment previously shown to confer some protection from atopy [16]. Moreover, schistosome-infected children show altered cytokine production in response to TLR2 and TLR4 agonists, suggesting that helminth infection modulates TLR responses [17].

Other mechanisms could be considered (involving various receptors and ligands), as has been described for a measles virus protein [18] and the Hepatitis A virus (HAV) receptor. It has been shown that atopic diseases are less common in subjects previously exposed to HAV [19]. Recently, the possibility has emerged that HAV may act by direct action on Th2 cells which express the HAV receptor. This observation derives from the positional cloning of the asthma predisposing gene on human chromosome 5 through a complex approach using congenic mice [20]. Interestingly, in HAV seropositive subjects, the propensity of developing atopy is linked to the HAV receptor gene polymorphism [21].

(4) What are the diseases concerned by the hygiene hypothesis?

Most data supporting the hygiene hypothesis are derived from a limited number of diseases: type 1 diabetes, multiple sclerosis, Crohn's disease, and atopic diseases even though one may assume that the hypothesis applies to many other immune-related diseases. There are some very suggestive data for systemic lupus erythematosus and much more limited data for other autoimmune diseases such as rheumatoid arthritis. One may also mention the case of acute lymphoblastic leukaemia [22] and lymphomas, where the increased incidence in developed countries is related to improvements in hygiene. It is interesting to note that the incidence of Hodgkin's disease in young adults is the highest in first-born children as in the autoimmune and allergic diseases as discussed above [23].

(5) How does one explain that infections can either induce or protect from autoimmune diseases?

Autoimmune diseases may be induced by infectious agents. Thus, rheumatic fever is triggered by streptococci and Guillain-Barré syndrome appears following various infections with a special putative role for *Campylobacter jejuni*. Autoimmune diseases can also be induced in animal models by infections. Underlying mechanisms involve antigen mimicry and local inflammation increasing the immunogenicity of autoantigens. On the other hand, as discussed above, infections may also have a protective effect on immune disorders. In fact, this contradictory role of infections probably

explains the differential validity of the hygiene hypothesis according to diseases and countries. When the infection-mediated triggering role is very large, the protective role is not seen or is difficult to identify.

(6) How can one prevent the undesirable effects of improved hygiene?

The successful elimination of a large number of major infections undoubtedly represents one of the major achievements of modern medicine. Ironically, the search is now on find new therapeutic approaches which could substitute for these infections. One may think of non-pathogenic infections according to the principle of attenuated live vaccines. It is interesting to observe that various probiotics have proven capable of preventing (or improving) allergic or inflammatory bowel diseases both in experimental models and in human diseases [15,24,25]. One may also envision using bacterial extracts (as has been achieved in the NOD mouse) or synthetic TLR stimulating compounds. Because these therapies should be applied to healthy individuals, their safety should be investigated with great care. One should in particular evaluate the risk of systemic infections by lactobacilli in immuno-deficient subjects [26] and the risk of hypersensitivity or inflammation with the other products mentioned. One should lastly precisely evaluate the nature of the diseases in which this therapeutic approach will be used and at which stage of the disease.

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