

Parasites and the Hygiene Hypothesis

Regulating the Immune System?

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Abstract

The hygiene hypothesis proposes that the stimulation of the immune system by microbes or microbial products protects from the development of inflammatory diseases; therefore a reduced exposure to infectious agents may explain the rise in allergic and autoimmune diseases in industrialized countries. The contribution of studies on parasites and allergy to our understanding of the hygiene hypothesis has been two-fold. First, several studies have shown an inverse association between exposure to (*Toxoplasma gondii*) or harbouring of parasites (Schistosoma or Intestinal helminths) and allergy. Second, the mechanisms behind such protective effects have provided new insights and theories on the ability of parasite derived molecules to down-regulate immune responses and thereby to control inflammatory diseases such as allergies. In this review, recent findings and new concepts relating to the associations between parasites and allergies at the epidemiological, cellular and molecular level are discussed.

Index Entries:

Toxoplasma; schistoma; intestinal helminths; allergy; Treg; IL-10; TGF- β .

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The Hygiene Hypothesis: Does Exposure to Pathogens Protect Us From Allergy?

In a study comparing the prevalence of atopic disorders in Metis Indians and in white families in northern Canada, Gerrard and coworkers indicated that allergic diseases were less common among Metis families compared with white families. Higher degrees of infection with helminths, bacteria, and viruses were recorded, together with higher total IgE levels in Metis Indians. The authors suggested that the lower level of infection among white families might be responsible for the greater prevalence of allergic diseases in this group (1). Indeed, could lower exposure to pathogens explain the increasing prevalence of allergic disorders worldwide? The "hygiene hypothesis" was formally launched when David Strachan published a paper showing that hay fever was over-represented among first-borns, and less frequent in children from larger families. He suggested that frequent exchange of infections among siblings in large families might be responsible for the lower incidence of allergies (2). Since then, many studies have examined the relationship between infections and allergy. Some have compared distinct geographical areas where prevalence of infectious diseases or exposure to pathogens are widely different, others have correlated surrogate markers of infection with allergic disorders, whereas in another set of studies, the actual presence of pathogens was related to the prevalence of allergies.

One of the most influential studies to highlight the wide differences in the prevalence of allergic disorders in distinct geographical areas is the standardized International Study of Asthma and Allergies in Childhood (ISAAC) study, in which it was found that allergies are common in most industrialized countries but relatively rare in developing countries (3). This standardized large-scale study based on questionnaires confirms results from several stud-

ies that have used more objective measurements of allergy, such as airway function, skin-prick testing, or IgE levels, to look at differences between countries with a Western lifestyle, such as Australia, Finland, West Germany, and the United Kingdom, and those considered as developing countries or emerging economies, such as Nigeria (4), Estonia (5), East Germany (6), and Albania (7). Many believe that such studies provide support for the hygiene hypothesis, because infections are more prevalent in developing countries. Lifestyle differences associated with differential frequency of exposure to pathogens have also been considered to support the hygiene hypothesis. Day care attendance (8) and an anthroposophic lifestyle (9), both of which would be expected to result in much higher exposure to infections, have a protective role against allergic disorders. Another lifestyle that has been shown to be associated with a lower rate of atopic sensitization and allergic disorders is so-called "traditional farming." Early exposure to a farming environment and intake of farm milk have a clear protective effect on allergic manifestations (10). Again, the farming environment would be expected to present a milieu where exposure to pathogens would be very high.

However, such indirect evidence for the hygiene hypothesis does not suggest what kind of infection would protect against atopy. Clues initially came from the observation that a positive serology for food-borne and orofecal infections, such as hepatitis A, *Toxoplasma gondii* and *Helicobacter pylori* and a negative serology for airborne viruses are inversely related to atopic sensitization or diagnosed asthma among Italian military recruits (11,12). These data have been confirmed in another Italian population (13) and in a very large US general population sample (14) and recently by an independent group examining a large Danish population sample (15). A striking but retrospective observation is that in the United States, the epidemic of hay fever and asthma apparently spared the subset of the population

that had evidence of hepatitis A virus infection (14).

The evidence so far supports the hypothesis that changes in the acquisition of food-borne and oro-fecal infections, most pronounced with Westernization, have contributed to the epidemic trend of allergy and asthma (12). However, the exact nature (viral, bacterial, parasitic) of the food-borne and oro-fecal infections possibly contributing to protection from atopy is still unclear (14).

The putative etiologic role of food-borne and oro-fecal viral infections in protection from allergy and asthma has been investigated only recently (16), whereas more studies have been published on the potential role of the gut microflora in the inception of atopic diseases (17,18). Some preliminary studies suggested that there might be differences in the rate (19) as well as the type of bacteria (20) that colonize the gut of infants who are at higher risk of developing allergies.

The importance of the interaction of the gut with microbes for a normally functioning immune system has been demonstrated in germ-free animals (21). In these animals, it is not possible to establish oral tolerance, a phenomenon characterized by systemic hyporesponsiveness to orally administered proteins and characterized immunologically by high production of interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) by regulatory T cells (T_{reg}) (22). Thus, oral tolerance is an important mechanism for control of inflammatory reactions, and it appears to be dependent on sufficient microbial exposure. The fact that microbial products play an important role was shown by the ability of lipopolysaccharide (LPS) to restore the capacity to induce oral tolerance in germ-free animals (21). Thus, repeated exposure to an infection that enters via the gut or involves an intestinal phase could contribute to the establishment of a tolerogenic response.

Nevertheless, there have been only a few studies in which direct measurement of viral

or bacterial pathogens or pathogen-derived molecules have been associated with the prevalence of allergy when viral or bacterial infections are considered; in contrast, there are abundant studies involving parasites. Indeed, clear associations between direct pathogen measurements and allergies come from studies involving parasitic infections. For example, studies carried out by Lynch and coworkers in the 1980s and 1990s showed that chronic presence of intestinal helminths protected from atopic reactivity (23,24). This observation has been confirmed in studies in Ethiopia (25), in the Gambia (26), and in Ecuador (27). Another class of helminths, the schistosomes, also protects against atopy, as shown in studies in Gabon (28) and Brazil (29). However some other studies have shown that helminth infections are risk factors for atopic disorders (23,30). We and others have proposed (31–33) that this discrepancy may be explained by the burden and the chronicity of helminth infections. In areas where high transmission intensities lead to heavy and chronic infections, helminths protect against allergies, whereas in regions where helminth infections occur occasionally and are transient in nature, they potentiate allergic reactivities.

Despite a large number of epidemiological studies that show a negative association between high pathogen exposure and allergies, it is still not clear whether there is a direct link between the presence of pathogen and protection from atopic disorders. To prove a direct link, a pathogen has to either be introduced or removed and the effect of such intervention has to be assessed on atopy and clinical allergy. Interestingly, it has been shown that anti-helminth treatment results in increased atopic reactivity to house dust mite in Venezuelan and Gabonese children (24,34).

How Would Parasites Protect Against Allergies?

Intestinal helminths (23–27), schistosomes (28,29), and *T. gondii* (12,14,15) are parasites

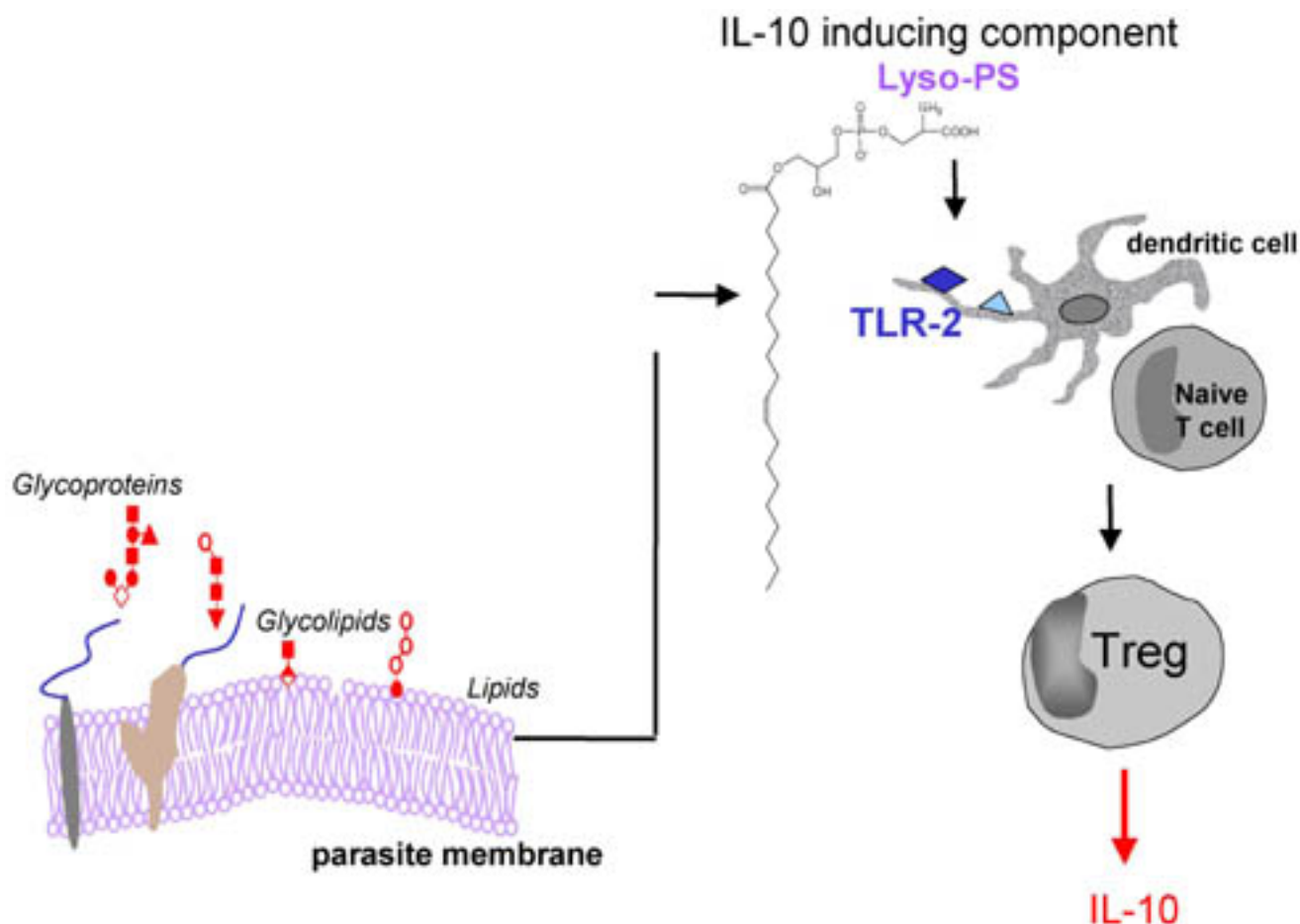


Fig. 1. Lipids isolated from *Schistosoma mansoni* can interact with maturing dendritic cells (DC) and alter their phenotype in such a way that upon coculture of DCs with naive T cells, regulatory T cells are induced. The active fraction of schistosome lipid contains lysophospholipids, which interact with DC via Toll Like Receptor-2 (TLR-2). As not all ligands that stimulate TLR-2 on DC, induce the same phenotype, involvement of another co-receptor may have to be postulated.

that have been negatively associated with allergic disorders. Each of these parasites can establish a chronic infection, which highlights two important issues: (1) the presence of these metabolically active pathogens means that the immune system is relentlessly challenged with foreign antigens; this continuous immune reactivity if uncontrolled could lead to severe pathology; and (2) for long-term survival within an immunocompetent host, throughout evolution these pathogens may have developed strategies to evade the immune system.

***Toxoplasma* Infection**

Although the inverse association between allergy and a positive serology for *T. gondii* is

rather weak (12–15), the specific interaction of toxoplasma with the immune system should also be considered. *T. gondii* is a pathogen that infects humans when oocytes or undercooked infested meat is ingested. Once established in the intestine, it spreads rapidly to other tissues. The immune response that controls this infection is predominantly Th1, stimulated by toll-like receptors (tlr). These receptors signal the entry of the pathogen into the human host (35,36), but it has also become clear that other immune reactions that can help the parasite establish a successful chronic infection are initiated. The infection of macrophages and dendritic cells (DCs) by *T. gondii* results in the secretion of LTA4, an eicosinoid that has

profound antiinflammatory action (37): it downregulates IL-12 synthesis and hence can paralyze the interferon- γ (IFN- γ)-mediated pathway, which has strong antitoxoplasma activity (37). The ability of *T. gondii* to induce a suppressor immune milieu may be the mechanism whereby this pathogen induces lower allergic reactivity in infected subjects. Although at first glance this may seem counter-intuitive, because lower levels of IL-12 would predict higher levels of Th2 cytokines and thus more allergic reactions, nevertheless, the lower IL-12 levels also imply less pro-inflammatory reactivity. However, at this stage of our knowledge, this idea is mere speculation, because the relationship of the suppressor reactions in *T. gondii* infection and reactivity to third-party antigens has not been investigated and is therefore an area for future research.

Helminth Infections

The overall immune response to helminths is characterized by strong skewing towards Th2 with increased production of IL-4 by peripheral blood mononuclear cells (PBMCs) and increased total IgE in the peripheral blood (38). In the face of such pro-allergic activity, the negative association of helminths with allergies seems puzzling. Although the high levels of total IgE stimulated by helminths was considered to be able to block Fc ϵ receptors present on mast cells and thus compete for binding with any allergen-specific IgE (23,33), this "blocking hypothesis" has been questioned as a result of studies that report that Fc ϵ receptors are not saturable (39). Indeed, several studies have now confirmed that the blocking hypothesis cannot be the only mechanism that could explain the negative association of helminth infections with allergic disorders (25,28). In these latter studies, it was shown by logistic regression analysis of the data that total IgE could not explain the low prevalence of either atopy (28) or wheeze (25) in parasitized or sensitized subjects. In intestinal helminth infections (*Ascaris*, *Trichiuris*, and hookworm) as

well as in *Schistosoma mansoni* infection, there are phases in the life cycle of the parasites where they interact with the intestinal wall. As already highlighted for toxoplasma infections, the possibility that exposure of the immune system to parasite antigens via the gut mucosa would favor a tolerogenic response also applies to these pathogens.

At another level, some of these parasites have been shown to carry immunomodulatory molecules that can stimulate a suppressor immune response, which, again, can be beneficial for their long-term survival within the human host. In a series of recent studies, molecules present in schistosomes have been shown not only to induce a Th2 response (40,41) but also to stimulate suppressor responses (42,43). In experimental infections, lacto-*N*-neotetraose, a carbohydrate molecule that has also been found in schistosomes, can stimulate the production of antiinflammatory cytokines by antigen-presenting cells (APCs) (42). Such a response, when present in the organs where potential inflammatory responses would be taking place, could indeed dampen the inflammation that results from mast-cell degranulation. In another study, where the ability of schistosome products to modulate human DC function was examined, it was shown that lyso-phosphatidylserine (lyso-PS), which has acyl chain lengths not present in mammalian lyso-PS, could modify DC function, so that upon interaction with naïve T cells, T_{reg} cells capable of producing IL-10 were induced (43;Fig. 1). Interestingly, lipids with similar immunomodulatory activities have been isolated from *Ascaris lumbricoides* (van Riet, unpublished data), indicating that at least two of the helminth species that have been associated with protection from allergies carry compounds that, upon interaction with the immune system, may result in a suppressor response. The question of how T_{reg} cells can suppress allergic reactivities and whether they can indeed protect against inflammatory disease in the lung, as they have been shown to do in the gastrointestinal tract (44), has yet to be addressed.

Contribution of Parasites to the Hygiene Hypothesis

The hygiene hypothesis, which attempts to explain the worldwide increase in allergic disorders that we are facing, was initially focused mainly on bacteria and viruses. Several studies outlined earlier indicate that parasitic infections may also reduce the risk of developing allergies. Therefore, it is not only the reduction in bacterial and viral infections, but also the reduction in the prevalence of parasitic infections in recent decades, that may be responsible for the increase in allergic disorders in the increasingly modernized world. It is clear that parasites have a long history of being highly prevalent, even in industrialized countries, up to the early 1900s. There are epidemiological studies suggesting a decline of the prevalence rate of specific antibodies against toxoplasmosis in Westernized countries, including France (45), the UK (46), and Sweden (47). With respect to helminths, there is ample evidence that in the early 1900s, a large proportion of the world's population, including many people in the Western world, were infected with helminths. The prevalence of helminth infections declined in the United States in the first half of the 20th century, especially among the advantaged segment of the population (48), corresponding to the onset of the current allergy epidemic (14), whereas it probably declined later (not until the 1970s) among minorities (48), corresponding to the emergence of allergic asthma in inner cities of the United States (49). In the Netherlands, studies carried out in the 1930s on Friesian Islands revealed a high prevalence of *Ascaris lumbricoides* (50%) and *Trichuris trichiura* (80%) in children aged 6–10 yr (50). Thus, the increase in allergic disorders followed the decrease in the prevalence of helminth infections observed in a number of industrialized countries.

There is also an immunological contribution that parasites have made to understanding the hygiene hypothesis. Initially, the

hygiene hypothesis was explained by the Th1/Th2 see-saw, arguing that a decline in bacterial and viral infections would result in weaker Th1 responses accompanied by an increase in Th2 responses and, consequently, in allergic diseases as well (51,52). The observation that chronic helminth infections, which are the strongest natural stimulus for a Th2 response, are actually negatively associated with allergic disorders, challenges this explanation. In any case, an increase in Th2 responses could not be the sole explanation for the increase in allergic disorders, because in many areas where the population exhibits strong Th2 responses, few allergic disorders are observed. Indeed, a Th1/Th2 see-saw would not explain the parallel increase in industrialized countries of allergic disorders with Th2 responses, and of autoimmune disorders with mostly Th1 responses (53). The concurrent increase in Th1 and Th2 disorders would imply common underlying mechanisms in a deregulated immune system. Could this imbalance be provided by the induction of suppressor immune responses in people with chronic parasitic infection, as outlined earlier?

For a careful examination of the immunological mechanisms that may explain the hygiene hypothesis, it is important to highlight the fact that allergic disorders can be divided into two phases, the sensitization phase and the effector phase (54). The sensitization phase is considered to be the stage when Th2 responses are first induced, preparing the way for increased downstream allergic inflammation. The effector phase encompasses the reactions that follow the expansion of the initial Th2-mediated reactions. In this phase, allergen reacts with IgE on mast cells, eosinophils become involved, and pro-inflammatory mediators are released, attracting additional inflammatory cells into the affected organs; some of these mediators act on surrounding tissues to enhance blood flow and they can increase smooth-muscle contraction and mucus secretion. The effector phase is responsible for the

expression of allergic inflammation and thus allergic disease.

With respect to the sensitization phase of allergy, few studies examining the hygiene hypothesis have reported changes in IgE antibody levels over time. It is very likely that throughout the period when helminth infections were highly prevalent in the presently industrialized world, the infected population had strong Th2 responses. However, serial serum samples obtained some time during that period and later when these infections had disappeared in that part of the world are not available, so it is not possible to determine whether and how antibody responses to allergens might have changed in response to the disappearance of the parasites. Nevertheless, some data are available. In a study of adolescent Japanese female students, serum IgE antibody against both mites and pollen was present in 21.4% in 1978, 25% in 1981, 35.5% in 1985, and 39.4% in 1991 (55). Similarly, in a study carried out in Greenland with sera collected in 1987 and again in 1998, a twofold increase in prevalence of IgE sensitization to allergens, most frequently to grass pollen, was recorded over that period (56). The level of exposure to this allergen is not likely to have increased significantly over this 11-yr period, as has been argued for exposure to house dust mite. These results point to a clear change in how the immune system recognized this allergen over the period of a decade. Therefore, an important question is whether the immune system of these individuals was able to recognize these allergens at all, but then not with an IgE response. It was recently proposed that, in the case of cat allergy, a modified Th2 response to the allergen may be associated with lower allergic reactivity in those individuals with high exposure to cats (57), and this idea may be relevant to the aforementioned studies. Did the Greenlanders make anti-grass IgG or IgG4 rather than IgE in 1987? If the answer turns out to be positive, then there could still be a role for the antiinflammatory response to explain

the increase in IgE reactivity to common allergens in Western populations. For example, IL-10 has been shown to be a switch factor that enhances IgG4 production while inhibiting IgE production (58). Thus, in populations with chronic parasitic infections and a strong regulatory network characterized, among other factors by high IL-10 production, the abundance of this cytokine could bias antibody responses towards IgG4. However, if no immune responses were made to environmental allergens, not even IgG, at times prior to the beginning of the allergic march, then the role of infections in allergen ignorance by the immune system would have to be investigated.

With respect to the effector phase, the increase in allergic disorders, measured by skin-prick tests, airway responsiveness, or clinical scores of allergy needs to be mechanistically linked to lower exposure to infections. A recent report showing that IL-10 can inhibit mast-cell degranulation (59) provides a possible mechanism by which an antiinflammatory response, stimulated by parasites and characterized by increased production of IL-10, could prevent allergic reactions (28). However, suppressor cytokines such as IL-10 and TGF- β can also inhibit allergic inflammation by suppressing the influx of granulocytes into affected tissues or by inhibiting APCs, leading to down-regulation of T-cell responses (60). The question of whether these cytokines can directly inhibit smooth muscle cell contraction or goblet-cell function is as yet unanswered. In animal models of airway inflammation, it has been shown that dendritic cells that release IL-10 can suppress airway hyperreactivity (61), which would support the notion that regulatory networks stimulated by persistent pathogens could suppress allergic reactivity.

The explanation of the hygiene hypothesis through the Th1/Th2 paradigm implied initially that increases in IFN- γ should be associated with decreased allergic diseases. However, later studies showed that: (1) high levels of IFN- γ can be found in the bronchial lavage cells

of asthmatic subjects (62); (2) T cells producing IFN- γ can exacerbate airway inflammation in animal models (63); and (3) protective measures to suppress airway inflammation such as *Mycobacteria vaccae* administration are effective via mechanisms not mediated by IFN- γ (64). Thus, with regard to the effector phase of the allergic response, there is considerable evidence that the antiinflammatory network may be an important mechanism to downregulate inflammation resulting from a Th2 response.

Future Studies

With increasing urbanization, disease patterns are changing and so are immune responses. In order to establish how pathogens interact with the immune system and modulate the expression of inflammatory diseases that occur in the Western world as well as in urban centers of developing countries, it is necessary to carry out longitudinal studies involving rural-traditional vs urban-Westernized populations. It is also important to study individual pathogens and their contribution to the allergic status of these populations. Moreover, to establish unequivocally a role for helminthic pathogens in suppression of allergic diseases, additional intervention studies are needed.

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