Atopy has been increasing in prevalence in the industrialized world for at least two decades, but the same increase has not been noted in the developing world. The hygiene hypothesis suggests that lack of exposure to infectious agents during childhood results in a balance of T helper 1 (Th1) and Th2 type immune responses that favor atopy. Typical Th1 cytokines include IL-12 and interferon (IFN)-γ, whereas Th2 cytokines include IL-4, IL-5, IL-9, and IL-13. Most antimicrobial responses are Th1 responses, whereas atopy is associated with a Th2 pattern of cytokines.

The hygiene hypothesis has gained significant credibility, but is not universally accepted. It should be noted that most of the evidence in favor of the hypothesis is in the form of population-based studies of incidence and association, but there is currently no proof of causation. Studies have varied in their methods, with some relying on historic data, and some using skin test positivity rather than clinical disease manifestations as a measured outcome.

There are prospective data supporting an inverse relationship between atopic dermatitis and endotoxin exposure, early day care, and animal exposure, but other data suggest a positive association between infections in early life and atopic dermatitis. Infections that have been negatively associated with atopy include geohelminths in warmer climates and hepatitis A, Helicobacter pylori, and toxoplasma in temperate climates. Mycobacteria have been hypothesized to play a role in a range of climates.

Universal acceptance of the hygiene hypothesis would require proof from prospective clinical trials, and explanations for some apparent paradoxes. First is the apparent paradox that although helminthic infections are potent stimuli for Th2 responses, many populations with high endemic levels of helminth infections have a low incidence of atopy. Another paradox is the increase in asthma among inner-city populations in the United States, an observation that appears to contradict the hygiene hypotheses, as these populations presumably live in a less sterile environment than more affluent populations. In addition, the prevalence of Crohn’s disease, widely regarded a Th1-dominant inflammatory disease, is increased in industrialized societies, and parallels the increase in Th2-dominant atopic responses. The incidence of insulin-dependent diabetes mellitus, also mediated by a type 1 response, has increased in parallel with that of childhood asthma.

In this article, I review recent evidence for and against the hygiene hypothesis and examine the role of helminthic pathogens, as they may represent a plausible link. Immune responses to early infection are complex, and the timing and intensity of exposure to pathogens appear to influence the immune response, and the risk of atopy. These factors may explain some of the apparent paradoxes presented by the hygiene hypothesis.

EVIDENCE FOR AND AGAINST THE HYGIENE HYPOTHESIS

Children who grow up on farms have a lower incidence of atopy and other allergic manifestations as compared with children who do not grow up on farms, but there is a paradoxically high prevalence of asthma. The prevalence of asthma is highest among children living on farms that raise swine and those that add antibiotics to animal feed. Similarly, a questionnaire-based study in Hanoi, Vietnam, found a positive relationship between allergic conditions and pig ownership. It is possible that the beneficial immune priming of a farm environment is outweighed by the addition of antibiotics to animal
feed. Other evidence also suggests an association between antibiotic administration to children early in life and an increased risk of asthma. The association between pig farming and asthma deserves further study.

A case control study of 313 pairs of children living in Sao Paulo, Brazil, suggests that household crowding increases the risk of acute lower respiratory infection, and is inversely associated with asthma. Epidemiologic evidence supports a birth order effect, and an increased risk of atopy in children born into small, affluent households. A case control study of 602 children in the United Kingdom found a reduction in atopy with increasing birth order, but no specific measure of infection reduced the odds significantly. A questionnaire-based study of 34,362 individuals from 26,100 households found a 19.3% incidence of hay fever. The odds of having hay fever were less for individuals with two or more siblings. Recall bias could have influenced the results. In a European cohort study, atopy, defined by prick tests to common aeroallergens, was less common among those from larger families. A higher number of brothers appeared to offer the best protection. (Having grown up with two brothers, I can appreciate a plausible link with the hygiene hypothesis.) In this study, gastrointestinal infections had a strong negative association with atopy, but the protective effect did not correlate with either hepatitis A or Helicobacter pylori infection, or with the total number of documented infections or antibiotic prescriptions.

A cohort study of 835 children from suburban Detroit, Mich, found that those with fevers before age 1 year had a lower incidence of atopy. Each additional febrile episode in the first year was associated with reduced odds for allergic sensitization. Upper respiratory tract infections showed a particularly strong effect. In a population-based study in Guinea-Bissau, measles infection was negatively associated with house dust mite skin-prick test positivity, even after adjustment for variables such as breast-feeding. Although most current evidence suggests no association between childhood vaccination and allergic disease, a questionnaire-based Danish study of 9744 children found that the incidence of atopic dermatitis increased after measles, mumps, and rubella vaccination.

There is evidence that endotoxin exposure in early life is negatively associated with atopic manifestations. Specifically, it may down-regulate airway eosinophilia and hyperreactivity. Endotoxin levels in dust from infant mattresses in East German homes are significantly higher than those in the West. The levels correlate with pet ownership, close contact with pets, and the number of persons living in the home.

THE PRENATAL AND NEONATAL IMMUNE RESPONSE

The neonatal immune response is weighted toward a Th2 response, and the observation that cord blood mononuclear cells proliferate in response to food and inhalant allergens suggests that T-cell priming already occurs in utero. The Th2 bias of neonatal T cell–dependent responses is heavily dependent on the level of CD28 costimulation. When CD28 cosignal is minimal, IL-12 can prime neonatal CD4+ T cells to produce both IL-4 and IFN-γ. In contrast, strong CD28 costimulation results in production of high levels of type 1 (IL-2, IFN-γ, and tumor necrosis factor beta) and low levels of type 2 (IL-4 and IL-13) cytokines by neonatal T cells. Maternal peripheral blood mononuclear cells synthesize greater amounts of IL-12 than neonatal cells, and reduced IL-12 production in the perinatal period is associated with stronger neonatal Th2 responses in the postnatal period. There is also evidence of a Th2 bias after in vitro stimulation of cord blood mononuclear cells from children with a family history of atopy. Animal data suggest that strong maternal Th2 immune responses influence the neonatal response, favoring a Th2-type immune response to a novel antigen.

Newborn CD4/CD45RA+ T cells are highly responsive to IL-4. IL-4 plus anti-CD2 monoclonal antibodies, and IL-4 plus phytohemagglutinin. In contrast, adult CD4/CD45RA+ T cells are unresponsive to the same stimulation. This sensitivity to IL-4 could also promote a Th2 immune response in the newborn.

Maternal helminthic infection can affect both the humoral and cellular responsiveness of newborn children, with production of both Th1- and Th2-type cytokines. Proliferative responses of umbilical cord mononuclear cells to helminthic antigens generally parallel those of maternal peripheral blood mononuclear cells. In the setting of maternal infection, neonate’s cells do not present a dominant expression of immunity of either Th1 or Th2 in response to helminthic antigens.

A study of cord blood lymphocytes from neonates from a highly endemic area for schistosomiasis, filariasis, and tuberculosis in Kenya showed that the cord blood cells mimicked the response by maternal peripheral blood mononuclear cells. Both produced helminthic antigen-specific IL-5 and/or IFN-γ and purified protein derivative-induced IFN-γ, but little or no IL-4 or IL-5. In contrast, no
Ag-specific IL-4, IL-5, or IFN-γ release was detected from cord blood cells of children in the United States. These findings suggest that prenatal exposure does not lead to immune tolerance, but rather primes the immune response in the same fashion as exposure in adults. Thus, prenatal exposure and neonatal exposure can produce very different immune responses.

PREVALENCE OF GEOHELMINTHIC INFESTATION

More than 3.5 billion people worldwide are infected with intestinal worms, and the worm burden correlates with poor sanitation and hygiene. In addition to the presence of latrines and sewage systems, the practice of washing with soap and water is independently associated with a lower risk of helminthic infestation.

In a study of 1000 children in India, the incidence of helminthic infection was 68.0%. In urban children, the rate of infestation was 56.8%, compared with 79.2% in the rural group. In both populations, Ascaris lumbricoides was the single predominant species. The most common mixed infestation was a combination of A lumbricoides and Trichuris trichiura. Mixed infections with A lumbricoides and Trichuris trichiura are also common in parts of Africa. Trichiura whipworm infestation is thought to affect 800 million people throughout tropical and temperate areas.

THE IMMUNE RESPONSE AGAINST GEOHELMINTHIC INFESTATION

Helminths, unlike most bacteria and viruses, selectively stimulate Th2 function and are among the most potent stimulators of mucosal Th2 responses. In general, induction of type 2 cytokine responses imparts resistance to intestinal nematodes, whereas type 1 responses can exacerbate the infection. In a study of IL-10-, IL-10/IL-4-, IL-10/IL-12-, IL-4-, and IL-12-deficient mice infected with Trichuris muris, the IL-10-, IL-4-, and IL-10/IL-12-deficient mice were most susceptible to infection. IL-10-deficient mice demonstrated an increase in morbidity and mortality. The increased susceptibility was attributed to a marked increase in type 1 cytokine production. In contrast, those mice deficient in both IL-10 and IL-12 were completely resistant to the infestation. The doubly deficient mice mounted a highly polarized type 2 cytokine response. This suggests that the increased susceptibility of the IL-10-deficient mice was dependent on the presence of normal IL-12 with production of a Th1 response.

Resistance to murine larval Trichuris infestation is mediated by development of a strong Th2 response. A humoral response is not required for effective immunity. The immune response appears to be directed against the worm itself and is unlikely to involve killing of infected gut epithelial cells, as Fas and Fas ligand-deficient mice (which are unable to mount a CD4-mediated cytotoxic response) can readily expel Trichuris muris. In human Trichuris trichiura infections, excretory and secretory products of the worms preferentially induce IL-10 and tumor necrosis factor-alpha, whereas IL-4 and IL-13 responses are noted with whole worm extracts. Similar responses are seen with Trichuris muris antigens, suggesting similarities in the response to different species of geohelminth.

THE IMMUNE RESPONSE TO MASSIVE HELMINTH INFESTATION DIFFERS FROM THE RESPONSE TO TRICKLE (REPEATED LOW-DOSE) INFESTATION

In general, low-level worm infestations tend to be tolerated, and an effective immune response against the worms requires a threshold dose. In human beings, low-dose hookworm exposure results in maturation to egg formation. Patients who are infected develop marked blood eosinophilia but low-level infection does not induce much in the way of T cell– and B cell–dependent immune responses. In a mouse model, antigen dose also affected the outcome of infection. Low antigen doses favored type 1 responses and susceptibility to the infestation, whereas high antigen doses favored a type 2 response and resistance. High-level challenge infection could only be established after priming of the immune response by a low-level infection. Once type 2 responses were initiated, it was impossible to induce a type 1 response, even using IL-12 (a potent stimulus of type 1 responses). In a lamb model, challenge doses of Trichostrongylus colubriformis result in increased antibody titers and eosinophil numbers in control animals but not those that had received previous trickle infection. In mice, an effective immune response to Trichinella spiralis also requires a threshold level of infection. Active down-regulation of the immune response may occur with trickle infections. In a cattle model that found trickle infection with Fasciola hepatica not to be protective against infection, gamma delta + T cells appeared to down-regulate alpha beta + T-cells.

In some animal models, repeated low-level infection can produce an immune response. In a mouse model, frequently infected mice demonstrated greater protective immunity against Heligmosomoides polygyrus than mice infected with the same total number of larvae in fewer doses. In sheep, immunity induced by trickle infection results in...
significant differences in adult female worm length and egg counts. In a porcine model, strong resistance to reinfection was induced by trickle inoculations with *Trichuris suis* (250 infective eggs twice weekly for 4 weeks). After induction of immunity, there was a 96% reduction in the mean number of established infections after a challenge dose of 4000 ova. In mice, immunization with trickle doses of subcutaneous *Wuchereria bancrofti* larvae was effective in inducing a protective immune response against *W bancrofti*. In cattle experimentally infected with *F hepatica*, both single dose and trickle infection promote a type 2 response. Single-dose infection resulted in greater humoral and cellular immunity than trickle infection, but the mean number of flukes recovered was similar in the two groups.

Clinical infection with either *Oesophagostomum dentatum* or *A suum* did not influence total worm burdens after challenge infection with the other species, but an effect on worm length was demonstrated.

In mice, persistent infection with *Taenia crassiceps* cysticerci affects the immune response to subsequent *Trypanosoma cruzi* infection. In the early stages of the helminth infection, there is a delay in the onset of *Trypanosoma cruzi* parasitemia, early specific production of IFN-γ, and high specific production of IL-4. Increased susceptibility to *Trypanosoma cruzi* was observed only when mice were coinfected in late stages of the helminth infection when the immune response against it is predominantly a Th2 type response. The response to *Trypanosoma cruzi* antigens was then characterized by low levels of both IFN-γ and IL-4.

Most areas of the world with high rates of chronic helminthic infection also have a high incidence of mycobacterial diseases. Low efficacy of BCG has been described in these regions. The immunomodulatory effects of trickle infection may offer an explanation for this phenomenon. Mice with subclinical *Schistosoma mansoni* infection show an impaired ability to induce an effective Th1 immune response against mycobacteria.

**EVIDENCE SUGGESTING A NEGATIVE ASSOCIATION BETWEEN GEOHELMINTHIC INFESTATION AND ATOPIC MANIFESTATIONS**

Geohelminths infestation during childhood may contribute to the low prevalence of allergic disease reported from rural areas of the tropics. In a study of 2865 school-aged Ecuadorian children, active geohelminth infection (eggs in stool) and chronic geohelminth infection (≥3564 IU/mL total serum IgE or the presence of anti-*A lumbricoides* IgG4 antibodies) were negatively associated with allergen skin test reactivity. In a study of 4433 school-aged children in a rural tropical area of Ecuador, the presence of geohelminth infections was negatively associated with allergen skin test reactivity and exercise-induced wheezing. A dose-response relationship was less clear. The intensity of infection with *A lumbricoides* or *Trichuris trichiura* had a negative association with allergen skin test reactivity but not with allergic symptoms. The incidence of symptoms was low, but the authors could not attribute the low incidence to a dose-response relationship with geohelminth infestation.

Probably the strongest evidence for an association is the demonstrated effect of antihelminthic treatment on atopy or asthma risk. In rural Latin America, low socioeconomic level, overcrowding, and geohelminth infection were found to be independently protective against atopy among school-age children. In a randomized controlled trial in 317 Gabonese schoolchildren with a high prevalence of intestinal helminths, long-term antihelminthic treatment was shown to result in increased allergic sensitivity to house dust mites. Therapy with praziquantel and mebendazole correlated with a significant increase in the rate of skin sensitivity, despite decreasing total IgE levels.

In Gambia, there is an inverse association between atopy and intestinal helminth infection. In a study of 520 schoolchildren in an area of Gabon highly endemic for parasites, the prevalence of schistosome and filarial infections increased with age, whereas allergen prick test reactivity tended to decline. Of the children, 47% had mite-specific IgE antibodies, but only 11% had a positive prick test.
Mite sensitization as measured by IgE was highest in children infected with schistosomes, filariae, or both, whereas skin test reactivity was lowest in the same group. Multiple logistic regression analysis showed that the risk of a positive skin test was 8-fold higher with increasing levels of mite-specific IgE but was reduced by 72% by infection with blood stage helminths. Those heavily infected by S mansoni were compared with those who were uninfected in an endemic area of Brazil. Infection was negatively associated with skin test positivity to allergens. The odds of skin test positivity were 5 times higher in the uninfected group. Total IgE was higher in the infected group, but allergen-specific IgE was higher in uninfected group.

Modulation of the immune system by helminth infection may reduce the levels of various allergic responses. In a mouse model, experimental infection with S mansoni reduces the incidence of anaphylactic responses. Both B cells and IL-10 are responsible for the protection. A population-based study in human beings found a negative association between schistosome infection and allergen skin test positivity. IL-10 levels were negatively associated with mite skin-test reactivity. As both helmith infections and allergic diseases are characterized by a Th2 response, compensatory down-regulation triggered by helminth infection could decrease the incidence of allergic diseases. Another population-based study of clustering of allergic manifestations suggested that exposure to helmith infections may override genetic influences that are associated with positive allergen skin tests.

EVIDENCE AGAINST A PROTECTIVE EFFECT OF HELMINTH INFESTATION

In a study of 2164 rural Chinese children between the ages of 8 and 18 years, a history of Ascaris infestation or a positive stool examination was associated with increased risk of asthma and an increased number of skin tests positive to aeroallergens. Recall bias may have affected the results. Among Malaysian children, serologic evidence of Toxocara exposure was associated with a higher incidence of bronchial asthma. In a Venezuelan study designed to evaluate the influence of atopy on the antiparasitic response, the prevalence of infection was similar between individuals with and without atopy, but the intensity of A lumbricoides infection was considerably higher in the children who were nonatopic. This observation supports the concept that atopy confers a selective evolutionary advantage. It may be that atopy exists in human beings because it confers protection against parasitic infection.

NEGATIVE HEALTH CONSEQUENCES OF GEOHELMINTH INFESTATION

Most human geohelmintic infestations are asymptomatic, and some evidence suggests that helminthic infections may exert a protective effect against atopy. However, there are potential serious deleterious effects related to infestation. Eosinophilic inflammation and ascites have been reported with trichuriasis. Among women in endemic areas, whipworm and hookworm infestation are significant causes of anemia. In a study of 1996 schoolchildren in 13 Pacific Island countries, the prevalence of helminthiasis was 32.8%. Anemia prevalence was 12.4%. Those children with both helminthiasis and anemia were 8.7 times more likely to show evidence of growth retardation. In the Philippines, helmintic infection has been associated with poor physical and mental development. In India, heavy infestation with Trichuris trichiura has been shown to result in severe colitis and significant blood loss. The associated impaired cognitive functions were reversible with treatment.

There is also evidence that whipworm infestation may increase the pathogenicity of Campylobacter jejuni, a leading cause of acute diarrhea worldwide. Coinfection has resulted in toxic megacolon and acute renal failure.

In light of existing data, are we likely to use live nematodes for medical therapy?

There is no question that probiotic therapy will become part of mainstream medicine, but the most imminent clinical application is for the treatment of inflammatory bowel disease rather than atopy. Clinical trials have already been performed in the setting of inflammatory bowel disease, and these studies may serve as models for studies of helmintic pathogens in the treatment or prevention of atopic manifestations. Inflammatory bowel disease, especially Crohn's disease, represents a chronic Th1 intestinal inflammatory process. Induction of a Th2 response by intestinal helminths may diminish Th1 responsiveness and has been shown to improve inflammatory manifestations of the disease. Specifically, ingestion of live Trichuris suis eggs led to improvement in disease activity and quality of life. The benefit was temporary, but could be sustained with repeated ingestion. In a study of 29 patients with inflammatory bowel disease treated with 2500 live Trichuris suis ova every 3 weeks for 24 weeks, 23 patients (79.3%) responded and 21 (72.4%) remitted.

Interestingly, a separate hygiene hypothesis has been proposed in the gastroenterology literature, suggesting that lack of exposure to helminths results
in Th1-mediated diseases including inflammatory bowel disease in developed countries. Trials in patients with inflammatory disease indicate that helminthic pathogens can modify the immune response long after any initial priming effect. It should be emphasized that doses required for prevention and treatment may be very different. It is possible that properly dosed helminthic infection in early life could down-regulate systemic Th2 responses, whereas properly dosed infection in adulthood down-regulates gut Th1 responses. Infections may, in fact, be able to modify both Th1 and Th2 diseases. For instance, pinworm infestation has been shown to inhibit diabetes development in the nonobese diabetic mouse, and exerting a protective effect against asthma.

The data regarding the role in geohelminths in the epidemiology of atopy are mixed. Some of the apparently contradictory data may relate to these issues of timing of infection and the infective dose. As noted above, the immune response to helminthic pathogens is complex and varies significantly with age of exposure, dose, and coinfection. Much more study is needed regarding the immune response to helminths and its effect on atopy. The hygiene hypothesis represents an intriguing potential link between allergy and atopy. The ability to mount an atopic response may have had an evolutionary advantage, but in a sterilized world, the cost could be atopic dermatitis, asthma, diabetes, or inflammatory bowel disease. As the prevalence of atopic dermatitis increases in industrialized societies, the potential for nematode therapy deserves consideration. Ultimately, proof of the hypothesis will rest on prospective clinical trials.

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CONFERENCE ANNOUNCEMENT

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When: March 1-2, 2006
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This Conference will address the obstacles that have prevented the translation of the knowledge of the gene defects underlying dozens of heritable skin diseases into useful, effective molecularly-targeted therapies. The focus will be on obstacles to identification and production of useful molecules; obstacles to their delivery and immunologic barriers to their use; regulatory obstacles; and financial obstacles. Speakers will be from the Academic, FDA, Pharma, Biotech, and Venture Capital communities. The organizers are Ervin Epstein, Barbara Gilchrest, and Leonard Milstone.

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