The hygiene hypothesis and atopy: Bring back the parasites?

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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.[1,2] The hygiene hypothesis suggests that lack of exposure to infectious agents during childhood results in a balance of T helper 1 (Th1) and T helper 2 (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.[3-5] Infections that have been negatively associated with atopy include geohelminths in warmer climates and hepatitis A, *Helicobacter pylori*, and toxoplasma in temperate climates.[6] Mycobacteria have been hypothesized to play a role in a range of climates.[7]

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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.[8] 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.[9-11] 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.[12] Similarly, a questionnaire-based study in Hanoi, Vietnam, found a positive relationship between allergic conditions and pig ownership.[13] 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.[14] 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.[15] 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.[16] 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.[17] 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.[18]

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.[19] 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.[20] Although most current evidence suggests no association between childhood vaccination and allergic disease,[21] a questionnaire-based Danish study of 9744 children found that the incidence of atopic dermatitis increased after measles, mumps, and rubella vaccination.[22]

There is evidence that endotoxin exposure in early life is negatively associated with atopic manifestations. Specifically, it may down-regulate airway eosinophilia and hyperreactivity.[23] 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.[24]

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.[25] 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.[26]

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.[27] 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.[28] Animal data suggest that strong maternal Th2 immune responses influence the neonatal response, favoring a Th2-type immune response to a novel antigen.[29]

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.[30]

Maternal helminthic infection can affect both the humoral and cellular responsiveness of newborn children, with production of both Th1- and Th2-type cytokines.[31] 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.[32]

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.[33] Thus, prenatal exposure and neonatal exposure can produce very different immune responses.

PREVALENCE OF GEOHELMINTHIC INFESTATION

More than 3.5 billion people worldwide are infested with intestinal worms, and the worm burden correlates with poor sanitation and hygiene.[34] 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.[35]

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*.[36] Mixed infections with *A lumbricoides* and *Trichuris trichiura* are also common in parts of Africa.[37] *Trichiura* whipworm infestation is thought to affect 800 million people throughout tropical and temperate areas.[38]

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.[39] 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-4-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 the presence of normal IL-12 with production of a Th1 response.[40]

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*.[41] 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.[42]

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.[43] 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).[44] 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.[45] In mice, an effective immune response to *Trichinella spiralis* also requires a threshold level of infection.[46] 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.[47]

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.[48] In sheep, immunity induced by trickle infection results in

significant differences in adult female worm length and egg counts.[49] 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 infestations after a challenge dose of 4000 ova.[50] In mice, immunization with trickle doses of subcutaneous *Wuchereria bancrofti* larvae was effective in inducing a protective immune response against *W bancrofti*.[51] 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.[52,53] Cows with an existing chronic *F hepatica* infection that are subject to further trickle infection demonstrate tolerance to the organism and moderate superinfection, suggesting that the prevailing immune response was a nonprotective Th2 response.[54]

INFESTATION WITH ONE WORM ALTERS THE IMMUNE RESPONSE TO A SECOND TYPE OF WORM, AND THE IMMUNE RESPONSE TO OTHER INFECTIOUS AGENTS

In mice, large, single-pulse laboratory infections with *Trichuris muris* are cleared, but low-level infections of fewer than 20 worms survive for long periods. The number of worms required to induce an immune response is significantly higher in mice concurrently infected with *Nematospiroides dubius*, allowing trickle infections to survive to the point of egg production.[55] In pigs, trickle 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.[56]

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.[57]

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.[58]

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 ($\geq$3564 IU/mL total serum IgE or the presence of anti-*A lumbricoides* IgG4 antibodies) were negatively associated with allergen skin test reactivity.[59] 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.[60]

Probably the strongest evidence for an association is the demonstrated effect of antihelminthic treatment on atopy or asthma risk.[61] In rural Latin America, low socioeconomic level, overcrowding, and geohelminth infection were found to be independently protective against atopy among school-age children.[62] 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.[63]

In Gambia, there is an inverse association between atopy and intestinal helminth infection.[64] 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.[65] 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 aeroallergen-specific IgE was higher in uninfected group.[66]

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.[67] 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.[68] As both helminth infections and allergic diseases are characterized by a Th2 response, compensatory down-regulation triggered by helminth infection could decrease the incidence of allergic diseases.[69] Another population-based study of clustering of allergic manifestations suggested that exposure to helminth infections may override genetic influences that are associated with positive allergen skin tests.[70]

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.[71] Recall bias may have affected the results. Among Malaysian children, serologic evidence of *Toxocara* exposure was associated with a higher incidence of bronchial asthma.[72] 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.[73] It may be that atopy exists in human beings because it confers protection against parasitic infection.

NEGATIVE HEALTH CONSEQUENCES OF GEOHELMINTH INFESTATION

Most human geohelminthic 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.[74] Among women in endemic areas, whipworm and hookworm infestation are significant causes of anemia.[75] 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.[76] In the Philippines, helminthic infection has been associated with poor physical and mental development.[77] 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.[78]

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.[79]

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 helminthic 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.[80] 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.[81]

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.[9] 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.[82]

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 affluence 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.

REFERENCES

1. Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? Immunology 2004;112:352-63.
2. Ndiaye M, Bousquet J. Allergies and parasitoses in sub-Saharan Africa. Clin Rev Allergy Immunol 2004;26:105-13.
3. Flohr C, Pascoe D, Williams HC. Atopic dermatitis and the 'hygiene hypothesis': too clean to be true? Br J Dermatol 2005;152:202-16.
4. Eder W, von Mutius E. Hygiene hypothesis and endotoxin: what is the evidence? Curr Opin Allergy Clin Immunol 2004;4:113-7.
5. Ramsey CD, Celedon JC. The hygiene hypothesis and asthma. Curr Opin Pulm Med 2005;11:14-20.
6. Sheikh A, Strachan DP. The hygiene theory: fact or fiction? Curr Opin Otolaryngol Head Neck Surg 2004;12:232-6.
7. Smit JJ, Folkerts G, Nijkamp FP. Mycobacteria, genes and the 'hygiene hypothesis'. Curr Opin Allergy Clin Immunol 2004; 4:57-62.
8. Matricardi PM, Bouygue GR, Tripodi S. Inner-city asthma and the hygiene hypothesis. Ann Allergy Asthma Immunol 2002; 89:69-74.
9. Wells RW, Blennerhassett MG. The increasing prevalence of Crohn's disease in industrialized societies: the price of progress? Can J Gastroenterol 2005;19:89-95.
10. Danese S, Sans M, Fiocchi C. Inflammatory bowel disease: the role of environmental factors. Autoimmun Rev 2004;3:394-400.
11. Feillet H, Bach JF. Increased incidence of inflammatory bowel disease: the price of the decline of infectious burden? Curr Opin Gastroenterol 2004;20:560-4.
12. Merchant JA, Naleway AL, Svendsen ER, Kelly KM, Burmeister LF, Stromquist AM, et al. Asthma and farm exposures in a cohort of rural Iowa children. Environ Health Perspect 2005; 113:350-6.
13. Chai SK, Nga NN, Checkoway H, Takaro TK, Redding GJ, Keifer MC, et al. Comparison of local risk factors for children's atopic symptoms in Hanoi, Vietnam. Allergy 2004;59:637-44.
14. Cohet C, Cheng S, MacDonald C, Baker M, Foliaki S, Huntington N, et al. Infections, medication use, and the prevalence of symptoms of asthma, rhinitis, and eczema in childhood. J Epidemiol Community Health 2004;58:852-7.
15. Cardoso MR, Cousens SN, de Goes Siqueira LF, Alves FM, D'Angelo LA. Crowding: risk factor or protective factor for lower respiratory disease in young children? BMC Public Health 2004;4:19.
16. Gibbs S, Surridge H, Adamson R, Cohen B, Bentham G, Reading R. Atopic dermatitis and the hygiene hypothesis: a case-control study. Int J Epidemiol 2004;33:199-207.
17. Marshall AH, Owen V, Jones NS. More siblings, less hay fever: more evidence. Clin Otolaryngol 2002;27:352-8.
18. Cullinan P, Harris JM, Newman Taylor AJ, Jones M, Taylor P, Dave JR, et al. Can early infection explain the sibling effect in adult atopy? Eur Respir J 2003;22:956-61.
19. Williams LK, Peterson EL, Ownby DR, Johnson CC. The relationship between early fever and allergic sensitization at age 6 to 7 years. J Allergy Clin Immunol 2004;113:291-6.
20. Shaheen SO, Aaby P, Hall AJ, Barker DJ, Heyes CB, Shiell AW, et al. Measles and atopy in Guinea-Bissau. Lancet 1996; 347:1792-6.
21. Koppen S, de Groot R, Neijens HJ, Nagelkerke N, van Eden W, Rumke HC. No epidemiological evidence for infant vaccinations to cause allergic disease. Vaccine 2004;22:3375-85.
22. Olesen AB, Juul S, Thestrup-Pedersen K. Atopic dermatitis is increased following vaccination for measles, mumps and rubella or measles infection. Acta Derm Venereol 2003; 83:445-50.
23. Douwes J, Le Gros G, Gibson P, Pearce N. Can bacterial endotoxin exposure reverse atopy and atopic disease? J Allergy Clin Immunol 2004;114:1051-4.
24. Gehring U, Bischof W, Borte M, Herbarth O, Wichmann HE, Heinrich J, LISA. Study Group. Levels and predictors of endotoxin in mattress dust samples from East and West German homes. Indoor Air 2004;14:284-92.
25. Prescott SL, Macaubes C, Yabuhara A, Venaille TJ, Holt BJ, Habre W, et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. Int Arch Allergy Immunol 1997;113:75-9.
26. Delespesse G, Yang LP, Ohshima Y, Demeure C, Shu U, Byun DG, et al. Maturation of human neonatal $CD4^+$ and $CD8^+$ T lymphocytes into Th1/Th2 effectors. Vaccine 1998;16:1415-9.
27. Prescott SL, Taylor A, King B, Dunstan J, Upham JW, Thornton CA, et al. Neonatal interleukin-12 capacity is associated with variations in allergen-specific immune responses in the neonatal and postnatal periods. Clin Exp Allergy 2003; 33:566-72.

28. Gabrielsson S, Soderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in in vitro activated cord blood mononuclear cells. Clin Exp Immunol 2001;126:390-6.
29. Herz U, Ahrens B, Scheffold A, Joachim R, Radbruch A, Renz H. Impact of in utero Th2 immunity on T cell deviation and subsequent immediate-type hypersensitivity in the neonate. Eur J Immunol 2000;30:714-8.
30. Early EM, Reen DJ. Antigen-independent responsiveness to interleukin-4 demonstrates differential regulation of newborn human T cells. Eur J Immunol 1996;26:2885-9.
31. Soboslay PT, Geiger SM, Drabner B, Banla M, Batchassi E, Kowu LA, et al. Prenatal immune priming in onchocerciasis-*Onchocerca volvulus*-specific cellular responsiveness and cytokine production in newborns from infected mothers. Clin Exp Immunol 1999;117:130-7.
32. Pit DS, Polderman AM, Schulz-Key H, Soboslay PT. Prenatal immune priming with helminth infections: parasite-specific cellular reactivity and Th1 and Th2 cytokine responses in neonates. Allergy 2000;55:732-9.
33. Malhotra I, Ouma J, Wamachi A, Kioko J, Mungai P, Omollo A, et al. In utero exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. J Clin Invest 1997;99:1759-66.
34. Luong TV. De-worming school children and hygiene intervention. Int J Environ Health Res 2003;13(Suppl):S153-9.
35. Olsen A, Samuelsen H, Onyango-Ouma W. A study of risk factors for intestinal helminth infections using epidemiological and anthropological approaches. J Biosoc Sci 2001;33:569-84.
36. Chandrasekhar MR, Nagesha CN. Intestinal helminthic infestation in children. Indian J Pathol Microbiol 2003;46:492-4.
37. Legesse M, Erko B, Medhin G. Comparative efficacy of albendazole and three brands of mebendazole in the treatment of ascariasis and trichuriasis. East Afr Med J 2004;81: 134-8.
38. Cooper ES, Bundy DA. *Trichuris* is not trivial. Parasitol Today 1988;4:301-6.
39. Palmas C, Gabriele F, Conchedda M, Bortoletti G, Ecca AR. Causality or coincidence: may the slow disappearance of helminths be responsible for the imbalances in immune control mechanisms? J Helminthol 2003;77:147-53.
40. Schopf LR, Hoffmann KF, Cheever AW, Urban JF Jr, Wynn TA. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. J Immunol 2002;168:2383-92.
41. Betts J, deSchoolmeester ML, Else KJ. *Trichuris muris*: $CD4^+$ T cell-mediated protection in reconstituted SCID mice. Parasitology 2000;121:631-7.
42. Turner J, Faulkner H, Kamgno J, Else K, Boussinesq M, Bradley JE. A comparison of cellular and humoral immune responses to trichuroid derived antigens in human trichuriasis. Parasite Immunol 2002;24:83-93.
43. Maxwell C, Hussain R, Nutman TB, Poindexter RW, Little MD, Schad GA, et al. The clinical and immunologic responses of normal human volunteers to low dose hookworm (*Necator americanus*) infection. Am J Trop Med Hyg 1987;37:126-34.
44. Bancroft AJ, Else KJ, Humphreys NE, Grencis RK. The effect of challenge and trickle *Trichuris muris* infections on the polarization of the immune response. Int J Parasitol 2001;31: 1627-37.
45. Sutherland IA, Leathwick DM, Green R, Brown AE, Miller CM. The effect of continuous drug exposure on the immune response to *Trichostrongylus colubriformis* in sheep. Vet Parasitol 1999;80:261-71.
46. Bell RG, Liu WM. *Trichinella spiralis*: quantitative relationships between intestinal worm burden, worm rejection, and the measurement of intestinal immunity in inbred mice. Exp Parasitol 1988;66:44-56.
47. McCole DF, Doherty ML, Baird AW, Davies WC, McGill K, Torgerson PR. T cell subset involvement in immune responses to *Fasciola hepatica* infection in cattle. Parasite Immunol 1999;21:1-8.
48. Brailsford TJ, Behnke JM. The dynamics of trickle infections with *Heligmosomoides polygyrus* in syngeneic strains of mice. Int J Parasitol 1992;22:351-9.
49. Sutherland IA, Brown AE, Green RS, Miller CM, Leathwick DM. The immune response of sheep to larval challenge with *Ostertagia circumcincta* and *O ostertagi*. Vet Parasitol 1999; 84:125-35.
50. Pedersen S, Saeed I. Acquired immunity to *Trichuris suis* infection in pigs. Parasitology 2001;123:95-101.
51. Rajasekariah GR, Monteiro YM, Netto A, Deshpande L, Subrahmanyam D. Protective immune responses with trickle infections of third-stage filarial larvae of *Wuchereria bancrofti* in mice. Clin Exp Immunol 1989;78:292-8.
52. Bossaert K, Farnir F, Leclipteux T, Protz M, Lonneux JF, Losson B. Humoral immune response in calves to single-dose, trickle and challenge infections with *Fasciola hepatica*. Vet Parasitol 2000;87:103-23.
53. Bossaert K, Jacquinet E, Saunders J, Farnir F, Losson B. Cell-mediated immune response in calves to single-dose, trickle, and challenge infections with *Fasciola hepatica*. Vet Parasitol 2000;88:17-34.
54. Clery D, Torgerson P, Mulcahy G. Immune responses of chronically infected adult cattle to *Fasciola hepatica*. Vet Parasitol 1996;62:71-82.
55. Behnke JM, Ali NM, Jenkins SN. Survival to patency of low-level infections with *Trichuris muris* in mice concurrently infected with *Nematospiroides dubius*. Ann Trop Med Parasitol 1984;78:509-17.
56. Helwigh AB, Christensen CM, Roepstorff A, Nansen P. Concurrent *Ascaris suum* and *Oesophagostomum dentatum* infections in pigs. Vet Parasitol 1999;82:221-34.
57. Rodriguez M, Terrazas LI, Marquez R, Bojalil R. Susceptibility to *Trypanosoma cruzi* is modified by a previous non-related infection. Parasite Immunol 1999;21:177-85.
58. Elias D, Akuffo H, Thors C, Pawlowski A, Britton S. Low dose chronic *Schistosoma mansoni* infection increases susceptibility to *Mycobacterium bovis* BCG infection in mice. Clin Exp Immunol 2005;139:398-404.
59. Cooper PJ, Chico ME, Rodrigues LC, Ordonez M, Strachan D, Griffin GE, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 2003;111:995-1000.
60. Cooper PJ, Chico ME, Bland M, Griffin GE, Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. Am J Respir Crit Care Med 2003;168:313-7.
61. Cooper PJ. Intestinal worms and human allergy. Parasite Immunol 2004;26:455-67.
62. Cooper PJ, Chico ME, Rodrigues LC, Strachan DP, Anderson HR, Rodriguez EA, et al. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004;34:845-52.
63. van den Biggelaar AH, Rodrigues LC, van Ree R, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.
64. Nyan OA, Walraven GE, Banya WA, Milligan P, Van Der Sande M, Ceesay SM, et al. Atopy, intestinal helminth infection and

total serum IgE in rural and urban adult Gambian communities. Clin Exp Allergy 2001;31:1672-8.
65. van den Biggelaar AH, Lopuhaa C, van Ree R, van der Zee JS, Jans J, Hoek A, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. Int Arch Allergy Immunol 2001;126:231-8.
66. Araujo MI, Lopes AA, Medeiros M, Cruz AA, Sousa-Atta L, Sole D, et al. Inverse association between skin response to aeroallergens and *Schistosoma mansoni* infection. Int Arch Allergy Immunol 2000;123:145-8.
67. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 2004;173:6346-56.
68. van den Biggelaar AH, van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG, et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. Lancet 2000;356:1723-7.
69. Capron A, Dombrowicz D, Capron M. Helminth infections and allergic diseases: from the th2 paradigm to regulatory networks. Clin Rev Allergy Immunol 2004;26:25-34.
70. Wahyuni S, Sartono E, Supali T, van der Zee JS, Mangali A, van Ree R, et al. Clustering of allergic outcomes within families and households in areas endemic for helminth infections. Int Arch Allergy Immunol 2005;136:356-64.
71. Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X. *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002;165:1489-93.
72. Chan PW, Anuar AK, Fong MY, Debruyne JA, Ibrahim J. *Toxocara* seroprevalence and childhood asthma among Malaysian children. Pediatr Int 2001;43:350-3.
73. Lynch NR, Hagel IA, Palenque ME, Di Prisco MC, Escudero JE, Corao LA, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol 1998;101:217-21.
74. Hong ST, Lim HS, Kim DH, Kim SJ. A case of gastroenteritis associated with gastric trichuriasis. J Korean Med Sci 2003; 18:429-32.
75. Gilgen D, Mascie-Taylor CG. The effect of anthelmintic treatment on helminth infection and anemia. Parasitology 2001; 122:105-10.
76. Hughes RG, Sharp DS, Hughes MC, Akau'ola S, Heinsbroek P, Velayudhan R, et al. Environmental influences on helminthiasis and nutritional status among Pacific schoolchildren. Int J Environ Health Res 2004;14:163-77.
77. Easton A. Intestinal worms impair child health in the Philippines. BMJ 1999;318:214.
78. Ananthakrishnan S, Nalini P, Pani SP. Intestinal geohelminthiasis in the developing world. Natl Med J India 1997;10: 67-71.
79. Shin JL, Gardiner GW, Deitel W, Kandel G. Does whipworm increase the pathogenicity of *Campylobacter jejuni*? A clinical correlate of an experimental observation. Can J Gastroenterol 2004;18:175-7.
80. Summers RW, Elliott DE, Qadir K, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am J Gastroenterol 2003;98:2034-41.
81. Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. Gut 2005;54:87-90.
82. Gale EA. A missing link in the hygiene hypothesis? Diabetologia 2002;45:588-94.