Microbial Manipulation of Immune Function for Asthma Prevention

Inferences from Clinical Trials

Jennifer Yoo¹, Haig Tcheurekdjian¹, Susan V. Lynch², Michael Cabana³, and Homer A. Boushey¹

¹Division of Allergy and Immunology, Department of Medicine, ²Department of Anesthesia and Perioperative Care, and ³Department of Pediatrics, University of California, San Francisco, San Francisco, California

The “hygiene hypothesis” proposes that the increase in allergic diseases in developing countries reflects a decrease in infections during childhood. Cohort studies suggest, however, that the risks of asthma are *increased* in children who suffer severe illness from a viral respiratory infection in infancy. This apparent inconsistency can be reconciled through consideration of epidemiologic, clinical, and animal studies. The elements of this line of reasoning are that viral infections can predispose to organ-specific expression of allergic sensitization, and that the severity of illness is shaped by the maturity of immune function, which in turn is influenced by previous contact with bacteria and viruses, whether pathogenic or not. Clinical studies of children and interventional studies of animals indeed suggest that the exposure to microbes through the gastrointestinal tract powerfully shapes immune function. Intestinal microbiota differ in infants who later develop allergic diseases, and feeding *Lactobacillus casei* to infants at risk has been shown to reduce their rate of developing eczema. This has prompted studies of feeding probiotics as a primary prevention strategy for asthma. We propose that the efficacy of this approach depends on its success in inducing maturation of immune function important in defense against viral infection, rather than on its effectiveness in preventing allergic sensitization. It follows that the endpoints of studies of feeding probiotics to infants at risk for asthma should include not simply tests of responsiveness to allergens, but also assessment of intestinal flora, immune function, and the clinical response to respiratory viral infection.

Keywords: asthma; gastrointestinal; lactobacilli; microbes; prevention

The purpose of this review is to sketch the rationale for a theory of asthma’s pathogenesis. The proposed theory is that asthma is caused, at least in part, by infection, especially in infancy, by a respiratory virus, most likely a human rhinovirus (HRV). The development of asthma may thus be a function of the “asthmagenic” pathogenicity of the strains of RV causing asthma in infancy and of the efficacy of antiviral immune function at the time of infection. This review notes how the validity of this theory might be examined by adding additional outcomes to ongoing trials of feeding a “probiotic,” like *Lactobacillus casei*, to infants as a primary prevention strategy for allergic disease. Space constraint does not permit more than a brief, partial review

of the evidence supporting the multiple elements to the rationale for this theory (Table 1), but some of the evidence, like that showing that exposure to products of intestinal flora reduces the risk of allergic disease, are at least indirectly addressed by other articles in this issue of *PATS*.

THE “HYGIENE HYPOTHESIS” FOR THE INCREASE IN PREVALENCE OF ALLERGIC DISEASE

The proportion of people affected by allergic diseases, including allergic rhinitis, eczema, and asthma, has increased dramatically over the past 50 years (1). The increase has been most marked in children, and was first noted in developed, “Westernized” countries (1). These diseases remain relatively uncommon in poor rural populations (2), but have increased sharply in such populations on migrating to urban areas or to regions of high prevalence (3, 4). Multiple different theories have been proposed to account for this phenomenon, with many focusing on differences in diet or in childhood exposure to allergens, like house dust mite, cockroach, and molds trapped in indoor air by Western patterns of housing (5, 6). The theory that has held up best so far, the “hygiene hypothesis,” was first put forward by Strachan when he noted an inverse association between family size and the rate of allergic disease, with the greatest protection being associated with the number of older siblings (7). Reasoning that older children likely pass on infections to their younger siblings, Strachan proposed that the development of allergic disease is prevented by infections in early childhood, so that the rise in prevalence is a consequence of the smaller family size and greater hygiene of modern Western societies. As Strachan noted in a review written 10 years after he first proposed the theory, it has held up well. Several subsequent studies have confirmed a negative association between the risk of developing allergic disease and sibling rank, and have further shown a positive association with higher socioeconomic status (8).

ASTHMA AS A TRANSMISSIBLE DISEASE

A theory, seemingly in direct contradiction to the hygiene hypothesis, is that allergic diseases, including asthma, are the result of exposure to a transmissible agent (9). As for the hygiene hypothesis, epidemiologic evidence can be cited in this theory’s support. On the simplest level, the fact that nearly all children with asthma are allergic, but only a small proportion of allergic children have asthma, at least raises the possibility that some additional factor is involved. That this additional factor might be a transmissible agent is also suggested by the similarity between the gross epidemiologic patterns of children with paralytic poliomyelitis in the 1950s and children with asthma currently. Both are more common among children of small, well-off families and among children migrating to urban areas from rural ones. On a smaller

(Received in original form February 9, 2007; accepted in final form March 8, 2007)

Supported in part by National Heart, Lung, and Blood Institute grant HL 80074 (M.C.), National Institute of Allergy and Infectious Disease grant AI057506 (H.A.B.), by fellowships from the Glazer Foundation (H.T.) and the American College of Asthma, Allergy, and Immunology (J.Y.), and by the Sandler Family Foundation (S.V.L.).

Correspondence and requests for reprints should be addressed to Homer A. Boushey, M.D., Box 0130, 1292-M, University of California, San Francisco, 505 Parnassus Avenue, San Francisco, CA 94143-0130. E-mail: homer.boushey@ucsf.edu

Proc Am Thorac Soc Vol 4, pp 277–282, 2007

DOI: 10.1513/pats.200702-033AW

Internet address: www.atsjournals.org

TABLE 1. RATIONALE FOR MICROBIAL MANIPULATION FOR ASTHMA PREVENTION

1. A transmissible agent is involved in the pathogenesis of asthma.
2. This agent is a respiratory virus, most likely an HRV.
3. Some strains of HRV are more "asthmagenic" than others.
4. The effectiveness of immune function determines whether infection with an "asthmagenic" strain induces asthma.
5. Effectiveness of immune function increases during infancy, but more slowly in children predisposed to atopy.
6. The rate of maturation of immune function is affected by microbial exposure in infancy, especially through the GI tract.
7. Early microbial exposure can be achieved safely by feeding infants "probiotics."

Definition of abbreviations: GI = gastrointestinal; HRV = human rhinovirus.

scale, the pattern of asthma's penetration into native populations also seems consistent with exposure to a transmissible agent. Among the Fore people of New Guinea, asthma appeared first in adults returning to villages after working in a European-influenced city. Only thereafter did it appear in children (10). Similarly, in a Xhosa village in South Africa, asthma first appeared in students educated in Cape Town, and increased in prevalence among the other villagers sharply after their return (11). Supporting this idea are analyses of the rates of asthma in genetically unrelated family members. One small study has found its prevalence to be increased in nonallergic, family history-negative spouses of affected adults (12, 13), and another has found the rates of asthma to be nearly as high in adopted children of mothers with asthma as in natural children (14).

SEVERE RESPIRATORY VIRAL INFECTION IN INFANCY: ASSOCIATION WITH ASTHMA

An early prospective study noted that the first evidence of allergic sensitization in children with an atopic family history was noted shortly after they had contracted a viral upper respiratory infection (15), but it was later studies, reporting higher rates of asthma in children who had had severe bronchiolitis from infection with respiratory syncytial virus (RSV) in infancy, that attracted most attention (16). Subsequent studies have been somewhat inconsistent. One follow-up of children hospitalized for severe RSV bronchiolitis in infancy has shown not only a higher rate of asthma, but also a higher rate of allergic sensitization (17). On the other hand, another study of children who had had a lower respiratory infection from RSV before age 3 years, showed that, by age 13 years, the increase in the odds ratio (OR) for recurrent wheezing was no longer greater than in children with no such history (18).

Especially powerful epidemiologic data are provided by prospective cohort studies, and this design has been used to examine the relationship between viral infection in infancy and the risk of asthma in childhood. One such study of more than 1,300 children both supports and challenges the hygiene hypothesis (19). This study had parents record all episodes of viral respiratory illness over their children's first year of life, and found that the greater the number of "colds" confined to the upper airway, the less likely the child was to have positive skin tests, recurrent wheezing, or a diagnosis of asthma at age 7 years. The greater the number of episodes of illness affecting the lower respiratory tract, however, the *more* likely was a later diagnosis of asthma.

A later prospective cohort study, the Childhood Origins of Asthma Study, was designed to examine the relationship between the severity of viral respiratory infections during infancy and the development of asthma in children at risk for atopy. This study also identified the viral causes of infection by polymerase chain reaction (PCR)-based analysis of nasal aspirates ob-

tained at the time of infection (20). A total of 285 children with a parent with history of respiratory allergies were enrolled. All colds reported by parents were assessed by standardized questionnaire. Whether the children had "recurrent wheezing" was assessed at age 3 years. The surprising finding of this study was that simply having a moderate or severe illness, with or without wheezing, from a viral respiratory infection in the first year of life was associated with a greater than threefold increase in the OR for recurrent wheezing at age 3 years. Even more surprising was the importance of developing a moderate or severe illness with wheezing from RV infection during infancy. To have this response to RV infection was far more strongly predictive of persistent wheezing at age 3 years (OR, 10) than was having the same response to RSV infection (OR, 3.0), and far more strongly predictive than passive exposure to cigarette smoke (OR, 2.1) (20).

Supporting this surprising finding of the Childhood Origins of Asthma Study is a 7-year follow-up study of 100 children who had been hospitalized for a wheezing illness before the age of 2 years (21). In 66 of these children, it was possible to assess whether they had asthma at ages 9–10 years (by history and exercise testing), and also to analyze by PCR testing nasal aspirates that had been kept frozen since hospitalization 7 years earlier. The only virus found to be associated with asthma at ages 8–9 years was RV. The OR for asthma among those with RV was 4.0. It was not increased among RSV⁺ or enterovirus⁺ cases.

PERSISTENT EFFECTS OF RV INFECTION IN INFANCY

The strength of the association between moderate or severe clinical illness, especially with wheezing, from infection with HRV in the first year or two of life can, of course, be interpreted in various ways. One interpretation is that HRV simply happens to be of the right "potency" in causing severe illness with wheezing, so that these clinical manifestations occur only in infants strongly predisposed to asthma, whereas RSV causes severe illness with wheezing even in children not so disposed. An alternate interpretation is that some strains of RV can cause permanent, persistent changes in airway function, especially if the infection occurs before antiviral host defense is well developed.

The idea that asthma might result from a persistent alteration in bronchial function or structure from viral respiratory infection has been nicely reviewed by Holtzman and colleagues (22). He interprets his finding that signal transducer and activator of transcription-1 expression is constitutively up-regulated in bronchial epithelial cells from patients with asthma as suggesting that asthma is associated with aberrant activation of epithelial immune-response genes for antiviral defense (22). A study of primary human bronchial epithelial cells infected with RV *in vitro* has shown that up-regulation of signal transducer and activator of transcription-1 expression is at least an acute response (23). Holtzman and colleagues also offer a "hit and run" theory for virus effects on airway structure and function to explain their finding that a single, transient paramyxoviral infection results in bronchial hyperreactivity and goblet cell hyperplasia that persists long after the virus is cleared (22).

Experimental study of these, or indeed any, theories of RV involvement in the initiation—as distinct from exacerbation—of asthma is hampered by RV's noninfectivity of any species other than humans. Furthermore, RV's resistance to culture has meant that even observational studies of RV's importance in humans have only become feasible with the development of PCR tests for detecting RV (24). The application of these tests have by now established that RVs are the major lower respiratory pathogens in infants (25), and chronic RV infection of the lower respiratory tract can occur in immunosuppressed patients, such

as lung transplant recipients (26). However, until an animal model susceptible to RV infection is produced or a highly effective and selective treatment for RV is developed, evidence of RV playing a role in asthma causation will necessarily remain circumstantial.

VARIATIONS IN PATHOGENICITY AMONG RVs

The idea that virulence may vary among RVs has gained currency as the broad variety of clinical consequences of RV infection has become recognized (27). More than 100 serotypes of this single-strand RNA virus have been identified, and only two regions of the 7,200 base genome, the VP4/VP2 and 5' untranslated regions, have been sequenced for all serotypes. Phylogenetic cluster analysis of variations in these regions has defined three groups of RV: a large "HRV-A" group, a smaller "HRV-B" group, and a third group with a single member resembling an enterovirus, RV 87.

The broader application of PCR testing for RV has suggested that some strains may be particularly virulent. One example is an outbreak in a long-term care facility in Santa Cruz, California, in 2003 (28). Among the residents, the attack rate was 60%. The isolates could not be passaged well in culture, but were characterized as RV 82 by analysis of sequence data. Another example of possibly heightened virulence for an RV is the finding of a novel RV isolate in respiratory secretions from patients suffering from a flulike illness in New York State in 2004 (29). Attempting to relate variations in RV serotype or genotype to variations in patterns of clinical presentation will be difficult, however, for there is great diversity in the RV strains circulating in a community over even a short period of time (30). That certain strains of RV might be particularly "asthmagenic" is thus plausible, but will be hard to establish by classic epidemiologic approaches.

DEFENSE AGAINST VIRAL RESPIRATORY INFECTION

Only an extremely sketchy and incomplete review of the mechanisms of defense against viral respiratory infection is possible here, but highlighting a few of the mechanisms will be sufficient for advancing the argument of this review: that manipulation of antiviral immune function might be a possible strategy for asthma prevention. The main point to be highlighted is that Th1 cytokines are indispensable for effective, cell-mediated immunity, necessary for eradication of intracellular pathogens, including viruses (31). In mice genetically engineered to overproduce Th1 cytokines, herpes simplex infection is less severe (32), as is the clinical course of RSV infection in infants who produce high levels of Th1 cytokines in response to the infection (33). The importance of Th1 responses in limiting viral respiratory infection is further confirmed by the inverse correlation between RV-induced IFN- γ secretion from peripheral blood mononuclear cells (PBMCs) *ex vivo* and peak RV shedding after experimental RV inoculation of allergic adults (34). Closer to the heart of the argument is the finding that, in infants, a decrease in phytohemagglutinin-induced IFN- γ secretion from cord blood mononuclear cells was associated with increased number of viral illnesses in the first year of life (35).

AGE, ATOPY, AND DEFENSE AGAINST VIRAL RESPIRATORY INFECTION

Infants are born with a "Th2 bias" in immune function, and robust, cell-mediated immunity, necessary for effective defense against intracellular pathogens, like viruses, requires production of Th1 cytokines (36). The capacity to induce protective Th1 immune responses develops early in life, primarily over the first

year (37), but appears to be delayed in children predisposed to atopy (38). In fact, Th2 polarization appears to be more prominent and to persist until a greater age in such children (39, 40). If age-dependent development of antiviral immune function is important in shaping the clinical response to infection with respiratory viruses, then differences in the month or season of birth would be expected to be associated with differences in the risk of severe respiratory illness in infancy. This is because the seasons in which respiratory viral infections are most common (in the Northern Hemisphere) are the autumn and winter. Specifically, the rates of bronchiolitis—indisputably a consequence of viral respiratory infection—and of asthma—possibly a consequence of viral respiratory infection—would be expected to be higher in infants born in the autumn. This expectation is supported by a large cross-sectional study by Strachan, the father of the hygiene hypothesis, which showed hospitalization for RSV bronchiolitis to be three times more common in infants born in autumn months, and hospital admissions for asthma to be significantly more common among children and young adults born during those months (41).

MICROBIAL COLONIZATION AND RISK OF ATOPY

Knowing that Th1-dependent immune function matures through infancy, and that the maturity of this function may affect the risk of developing severe or persistent consequences of viral respiratory infection, invites speculation as to how maturation of immune function might be accelerated. Clues as to a possible approach can be inferred from close consideration of the hygiene hypothesis. Explanations other than a lowered frequency of viral infections can be offered for the observation that the risk of asthma and allergy is greater in first-born children than in younger siblings (8). A reduction in frequency of viral infections seems an unlikely explanation for the additional observations that the risk of asthma and allergy is lower in children raised in households with a pet dog (42), on farms with domestic animals (43), or in bedrooms with high levels of endotoxin (44). Especially compelling is the finding that the risk of asthma and allergy is lower in adults with serologic evidence of infection with microbes transmitted by the "orofecal" route (45). Taken together, these observations suggest a protective effect not of viral infection, but of microbial exposure in a broader sense, including nonpathogenic microbes.

This idea, that exposure to nonpathogenic microbes might play a role in preventing the development of asthma and other allergic diseases, was greatly advanced by studies of stool flora in infants from populations with different rates of allergic disease. Sepp and colleagues demonstrated higher counts of lactobacilli and eubacteria in stool samples from infants in Estonia (low prevalence of atopy), and an increased number of clostridia in the samples from infants in Sweden (higher prevalence) (46). That these differences in stool flora might be related to the development of atopic disease is suggested by longitudinal studies. An example is another study by Bjorksten and coworkers, who followed infants in Estonia and Sweden through the first 2 years of life, and found that the babies who developed allergy were less often colonized with enterococci at 1 month of life and less often colonized with bifidobacteria at 1 year of life, but had higher clostridia counts at 3 months (47). The study by Kalliomaki and colleagues of stool samples from 76 infants with a family history of atopy similarly found that sensitization to allergens at 12 months was associated with fewer bifidobacteria and more clostridia in stool samples at 1 and 3 months of age (48). Even more impressive is a large-scale prospective study (the "KOALA" [Child, Parent, and Health: Lifestyle and Genetic Constitution] study) of nearly 1,000 newborns. This study showed

gastrointestinal colonization by *Escherichia coli* at age 1 month to be associated with the later development of eczema, and colonization with *Clostridium difficile* to be associated with a higher risk of developing recurrent wheeze, allergic sensitization, and atopic dermatitis (49). Collectively, these studies demonstrate the importance of gut microbial community, and suggest that differences in microbial community composition and increased abundance of specific bacterial species correlate with development of atopy.

ALTERING GUT FLORA TO ALTER IMMUNE FUNCTION

With recognition of the importance of gut microbial flora in stimulating the development of immune function (50) has come the idea that intentional introduction or encouragement of specific microbes might shape the pattern of immune function developed. This stimulated interest in the use of “prebiotics” and “probiotics” (51). The former are nutrients that escape digestion in the upper gastrointestinal tract and foster the growth of bifidobacteria and lactobacilli in the colon. The latter are bacteria that not only present little or no risk of disease, but are thought to promote health. Most probiotics used in humans are yeasts and bacteria (e.g., bifidobacteria and *Lactobacillus* species) traditionally used in the dairy industry.

As treatment, probiotics have so far been used most for gastrointestinal disorders, especially antibiotic-associated diarrhea (52), but the possibility that they can alter extraintestinal immune function is suggested by the efficacy of feeding *Lactobacillus rhamnosus* in improving eczema in children (53), an effect that might be related to its induction of increased IFN- γ production by PBMCs stimulated with phytohemagglutinin or *Staphylococcus aureus* enterotoxin B (54). Other studies using the same *Lactobacillus* species have shown that feeding it to children with gastrointestinal inflammation (55) or IgE-associated dermatitis and cow's milk allergy (56) improves the clinical condition and increases the production of IL-10 and IFN- γ by PBMCs. The idea that *L. rhamnosus* feeding increases regulatory T-cell (Treg) activity, suggested by the increase in IL-10 production, has been pursued. In T-cell cultures, dendritic cells primed with *L. casei* have been shown to induce IL-10-producing Tregs (57), and, in mice, feeding the same *Lactobacillus* increased the numbers of Tregs expressing transforming growth factor- β and PBMC production of IL-10 (58).

Given the emerging evidence of the importance of Tregs in shaping immune function in infancy, it is surprising that so little is currently known about the influence of probiotics on the number and activity of these cells. As already stated, infants are born with a Th2 bias in their immune responsiveness, a pattern that persists longer in infants with atopy than in those without atopy (40, 59). Because CD4⁺CD25^{high} Tregs suppress Th2 responses (60), and because CD4⁺CD25^{high} Tregs are decreased in number and/or function in atopy (61), it is tempting to speculate that the benefits of feeding probiotics for atopic dermatitis or cow's milk allergy are mediated through stimulation of this critically important cell population.

ALTERING GUT FLORA FOR PREVENTION OF ALLERGIC DISEASE

Knowing whether probiotic feeding alters Treg numbers or activity is not necessary for examining whether it prevents the development of atopic disease. Indeed, the positive findings of a prospective, double-blind study of the effects of daily feeding of *L. casei* for the first 6 months of life to 159 infants of mothers with a first-degree relative with atopic disease has stimulated tremendous interest. This intervention reduced the prevalence

of eczema by 50% at age 2 years (62). Two years later, eczema was still 50% less common in the treated group, but, curiously, the number of positive skin tests, a marker of allergic sensitization, was no different (63). This dissociation of effects on clinical expression of allergic disease from effects on markers of allergic sensitization was noted again in a study of 925 infants at risk for allergic disease and fed placebo or a mix of four probiotics or a prebiotic for 6 months (64). Assessment at 2 years of age showed that active treatment had no effect on specific IgE levels, positive skin tests, or cumulative incidence of allergic disease, but did reduce the rates of eczema and atopic eczema. A possible explanation for this dissociation is that the effect of probiotic feeding is not to alter the mechanisms of allergic sensitization, but to alter the mechanisms of heightened end-organ responsiveness, which might in turn reflect a persistent effect of viral infection in infancy, when susceptibility to infection is heightened. In other words, the protective effects of feeding a probiotic on the development of allergic disease might be due to enhancement of Th1-dependent mechanisms of antiviral defense, rather than a direct effect on mechanisms of allergic sensitization.

USE OF PROBIOTICS FOR PREVENTION AND TREATMENT OF INFECTIOUS DISEASE

The infectious diseases most often selected as targets for probiotic treatment have understandably been those affecting the gastrointestinal tract. Feeding of probiotics, especially of species of *Lactobacillus*, has been shown to reduce the frequency of diarrhea in undernourished children (65) and the duration of diarrhea due to rotavirus infection (66). Direct evidence of the effect of such feeding on antiviral defense in children is its enhancement of the immunogenicity of rotavirus vaccine (67, 68). Direct evidence of its effect on defense against viral infection outside the gastrointestinal tract comes from a study of BALB/c mice, in which infant mice fed *L. casei* before inoculation with influenza virus fared better than their control littermates. The mice fed *L. casei* had better survival (40 vs. 14%) and lower viral titers in nasal lavage fluid (69). That these better outcomes were due to enhancement of antiviral immune function was suggested by higher pulmonary natural killer cell activity and greater IL-12 production by mediastinal lymph node cells in the *L. casei*-fed mice.

Some clinical studies seem to confirm these promising findings in mice. One such study of schoolchildren showed that regular, daily ingestion of probiotics resulted in fewer lost days of school because of viral respiratory illness (70), and a double-blind, prospective, placebo-controlled study of 479 adults showed that, although daily ingestion of lactobacilli and bifidobacteria had no effect on the frequency of common colds, it reduced their mean duration by 2 days. (71).

CALL FOR ENLARGEMENT OF THE SCOPE OF CLINICAL TRIALS OF PROBIOTIC TREATMENT FOR PREVENTION OF ALLERGIC DISEASES

The unexpected but highly promising findings of the study by Kalliomaki and colleagues, showing that feeding *L. casei* dramatically reduced the rate of eczema in infants at risk for allergic disease (62), has prompted initiation of similar studies. These studies offer an opportunity to examine fundamental questions about how this promising approach to therapy might act. New methods for broad, unbiased detection of fecal microbiota could be used to determine whether the feeding of a probiotic alters the composition or diversity of stool flora (72), and might thus suggest whether the action is mediated directly by the probiotic feeding, or by its altering the gut microenvironment to allow

growth of other, actually protective bacteria. New methods for enumerating and assessing Treg number and activity (73) would reveal whether acceleration of development of this apparently critical cell population might be responsible for accelerating the maturation from an infantile Th2-biased pattern of immune responsiveness to a mature, balanced pattern of response, more effective in antiviral defense. Finally, examining the effects of probiotic feeding on the severity of clinical illness associated with respiratory viral infection, especially due to RV, would broaden the scope of the study. With this approach, potentially important actions of feeding a probiotic, other than its direct effects on allergic sensitization, would not be overlooked.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References

- Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000;105:S466–S472.
- The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998;351:1225–1232.
- Waite DA, Eyles EF, Tonkin SL, O'Donnell TV. Asthma prevalence in Tokelauan children in two environments. *Clin Allergy* 1980;10:71–75.
- Odhiambo JA, Ng'ang'a LW, Mungai MW, Gicheha CM, Nyamwaya JK, Karimi F, Macklem PT, Becklake MR. Urban-rural differences in questionnaire-derived markers of asthma in Kenyan school children. *Eur Respir J* 1998;12:1105–1112.
- Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, Woolcock AJ. House dust mite allergens: a major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;153:141–146.
- Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. *Allergy* 1998;53(Suppl 48):115–120.
- Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–1260.
- Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax* 2000;55:S2–10.
- Hussain I, Smith J. Evidence for the transmissibility of atopy: hypothesis. *Chest* 2003;124:1968–1974.
- Turner KJ, Dowse GK, Stewart GA, Alpers MP, Woolcock AJ. Prevalence of asthma in the South Fore people of the Okapa District of Papua New Guinea: features associated with a recent dramatic increase. *Int Arch Allergy Appl Immunol* 1985;77:158–162.
- Van Niekerk CH, Weinberg EG, Shore SC, Heese HV, Van Schalkwyk J. Prevalence of asthma: a comparative study of urban and rural Xhosa children. *Clin Allergy* 1979;9:319–324.
- Smith J, Knowler L. Epidemiology of asthma and allergic rhinitis. *Am Rev Respir Dis* 1965;92:16–38.
- Hippisley-Cox J, Coupland C, Pringle M, Crown N, Hammersley V. Married couples' risk of same disease: cross sectional study. *BMJ* 2002;325:636.
- Smith J, Cadoret R, Burns T, Troughton E. Asthma and allergic rhinitis in adoptees and their adoptive parents. *Ann Allergy Asthma Immunol* 1998;81:135–139.
- Frick OL. Role of viral infections in asthma and allergy. *Clin Rev Allergy* 1983;1:5–17.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501–1507.
- Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;171:137–141.
- Stein RT, Sherrill D, Morgan WJ, Holberg CJ, Halonen M, Taussig LM, Wright AL, Martinez FD. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541–545.
- Illi S, von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, Wahn U. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001;322:390–395.
- Lemanske RF Jr, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, Kirk CJ, Reisdorf E, Roberg KA, Anderson EL, *et al.* Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;116:571–577.
- Kotaniemi-Syrjaneen A, Vainionpää R, Reijonen TM, Waris M, Korhonen K, Korppi M. Rhinovirus-induced wheezing in infancy: the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;111:66–71.
- Holtzman MJ, Tyner JW, Kim EY, Lo MS, Patel AC, Shornick LP, Agapov E, Zhang Y. Acute and chronic airway responses to viral infection: implications for asthma and chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005;2:132–140.
- Chen Y, Hamati E, Lee PK, Lee WM, Wachi S, Schnurr D, Yagi S, Dolganov G, Boushey H, Avila P, *et al.* Rhinovirus induces airway epithelial gene expression through double-stranded RNA and IFN-dependent pathways. *Am J Respir Cell Mol Biol* 2006;34:192–203.
- Johnston SL, Sanderson G, Pattemore PK, Smith S, Bardin PG, Bruce CB, Lambden PR, Tyrrell DA, Holgate ST. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 1993;31:111–117.
- Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;25:680–686.
- Kaiser L, Aubert JD, Pache JC, Deffernez C, Rochat T, Garbino J, Wunderli W, Meylan P, Yerly S, Perrin L, *et al.* Chronic rhinoviral infection in lung transplant recipients. *Am J Respir Crit Care Med* 2006;174:1392–1399.
- Hershenson MB, Johnston SL. Rhinovirus infections: more than a common cold. *Am J Respir Crit Care Med* 2006;174:1284–1285.
- Louie JK, Yagi S, Nelson FA, Kiang D, Glaser CA, Rosenberg J, Cahill CK, Schnurr DP. Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clin Infect Dis* 2005;41:262–265.
- Lamson D, Renwick N, Kapoor V, Liu Z, Palacios G, Ju J, Dean A, St George K, Briesse T, Lipkin WI. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. *J Infect Dis* 2006;194:1398–1402.
- Savolainen C, Mulders MN, Hovi T. Phylogenetic analysis of rhinovirus isolates collected during successive epidemic seasons. *Virus Res* 2002;85:41–46.
- Stetson DB, Medzhitov R. Type I interferons in host defense. *Immunity* 2006;25:373–381.
- Sin JJ, Kim JJ, Zhang D, Weiner DB. Modulation of cellular responses by plasmid CD40L: CD40L plasmid vectors enhance antigen-specific helper T cell type 1 CD4⁺ T cell-mediated protective immunity against herpes simplex virus type 2 *in vivo*. *Hum Gene Ther* 2001;12:1091–1102.
- Pinto RA, Arredondo SM, Bono MR, Gaggero AA, Diaz PV. T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. *Pediatrics* 2006;117:e878–e886.
- Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol* 2000;105:692–698.
- Copenhaver CC, Gern JE, Li Z, Shult PA, Rosenthal LA, Mikus LD, Kirk CJ, Roberg KA, Anderson EL, Tisler CJ, *et al.* Cytokine response patterns, exposure to viruses, and respiratory infections in the first year of life. *Am J Respir Crit Care Med* 2004;170:175–180.
- Prescott SL, Macaubas C, Holt BJ, Smallacombe TB, Loh R, Sly PD, Holt PG. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998;160:4730–4737.
- Upham JW, Lee PT, Holt BJ, Heaton T, Prescott SL, Sharp MJ, Sly PD, Holt PG. Development of interleukin-12-producing capacity throughout childhood. *Infect Immun* 2002;70:6583–6588.
- Holt PG, Clough JB, Holt BJ, Baron-Hay MJ, Rose AH, Robinson BW, Thomas WR. Genetic 'risk' for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992;22:1093–1099.
- Rowe J, Macaubas C, Monger T, Holt BJ, Harvey J, Poolman JT, Loh R, Sly PD, Holt PG. Heterogeneity in diphtheria-tetanus-acellular pertussis vaccine-specific cellular immunity during infancy: relationship to variations in the kinetics of postnatal maturation of systemic Th1 function. *J Infect Dis* 2001;184:80–88.
- Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Loh R, Holt PG. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy* 1998;28:39–44. [Discussion: 50–31.]

41. Strachan DP, Seagroatt V, Cook DG. Chest illness in infancy and chronic respiratory disease in later life: an analysis by month of birth. *Int J Epidemiol* 1994;23:1060-1068.
42. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002;288:963-972.
43. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl R, Nowak D, von Mutius E. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001;358:1129-1133.
44. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufe A, *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002;347:869-877.
45. Matricardi PM, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;320:412-417.
46. Sepp E, Julge K, Vasar M, Naaber P, Bjorksten B, Mikelsaar M. Intestinal microflora of Estonian and Swedish infants. *Acta Paediatr* 1997;86:956-961.
47. Bjorksten B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999;29:342-346.
48. Kalliomaki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001;107:129-134.
49. Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, Adams H, van Ree R, Stobberingh EE. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA birth cohort study. *Gut* 2007;56:661-667.
50. Berg RD. The indigenous gastrointestinal microflora. *Trends Microbiol* 1996;4:430-435.
51. Macfarlane GT, Cummings JH. Probiotics, infection and immunity. *Curr Opin Infect Dis* 2002;15:501-506.
52. Novak J, Katz JA. Probiotics and prebiotics for gastrointestinal infections. *Curr Infect Dis Rep* 2006;8:103-109.
53. Weston S, Halbert A, Richmond P, Prescott SL. Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child* 2005;90:892-897.
54. Prescott SL, Dunstan JA, Hale J, Breckler L, Lehmann H, Weston S, Richmond P. Clinical effects of probiotics are associated with increased interferon- γ responses in very young children with atopic dermatitis. *Clin Exp Allergy* 2005;35:1557-1564.
55. Pessi T, Sutas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clin Exp Allergy* 2000;30:1804-1808.
56. Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, Savilahti E. *Lactobacillus* GG effect in increasing IFN- γ production in infants with cow's milk allergy. *J Allergy Clin Immunol* 2004;114:131-136.
57. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, *et al.* Selective probiotic bacteria induce IL-10-producing regulatory T cells *in vitro* by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005;115:1260-1267.
58. Di Giacinto C, Marinaro M, Sanchez M, Strober W, Boirivant M. Probiotics ameliorate recurrent Th1-mediated murine colitis by inducing IL-10 and IL-10-dependent TGF- β -bearing regulatory cells. *J Immunol* 2005;174:3237-3246.
59. Holt PG, Jones CA. The development of the immune system during pregnancy and early life. *Allergy* 2000;55:688-697.
60. Taylor JJ, Mohrs M, Pearce EJ. Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population. *J Immunol* 2006;176:5839-5847.
61. Grindebacke H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergies during birch pollen season. *Clin Exp Allergy* 2004;34:1364-1372.
62. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076-1079.
63. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet* 2003;361:1869-1871.
64. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. Probiotics and prebiotic galactooligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:192-198.
65. Oberhelman RA, Gilman RH, Sheen P, Taylor DN, Black RE, Cabrera L, Lescano AG, Meza R, Madico G. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. *J Pediatr* 1999;134:15-20.
66. Shornikova AV, Casas IA, Mykkanen H, Salo E, Vesikari T. Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. *Pediatr Infect Dis J* 1997;16:1103-1107.
67. Kukkonen K, Nieminen T, Poussa T, Savilahti E, Kuitunen M. Effect of probiotics on vaccine antibody responses in infancy: a randomized placebo-controlled double-blind trial. *Pediatr Allergy Immunol* 2006;17:416-421.
68. Isolauri E, Joensuu J, Suomalainen H, Luomala M, Vesikari T. Improved immunogenicity of oral D $\times$ RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine* 1995;13:310-312.
69. Yasui H, Kiyoshima J, Hori T. Reduction of influenza virus titer and protection against influenza virus infection in infant mice fed *Lactobacillus casei* Shirota. *Clin Diagn Lab Immunol* 2004;11:675-679.
70. Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L, Saxelin M, Korpela R. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ* 2001;322:1327.
71. de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, *et al.* Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. *Vaccine* 2006;24:6670-6674.
72. Wang M, Ahrne S, Jeppsson B, Molin G. Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. *FEMS Microbiol Ecol* 2005;1:219-231.
73. Sakaguchi S. Naturally arising Foxp3-expressing CD25⁺CD4⁺ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005;6:345-352.