

Allergy Prevention

Interventions During Pregnancy and Early Infancy

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Abstract

The increase in allergic disease in western, industrialized countries has been phenomenal. Although the reasons for this increase are unknown, it is clear that the primary management of allergies is prevention. There is now significant epidemiologic data suggesting that a program designed and focused on prevention will have a significant impact on the development of allergies. These include not only environmental, but also dietary restrictions. There remain, however, several major problems. Firstly, it is not clear which subjects will respond best to a program focusing on either pre— or postnatal prevention. Secondly, the immunological mechanisms involved remain unclear. For example, it would be highly desirable to have a readout that monitors the improvement in allergen avoidance, other than clinical response. This review will focus on the immunological background of allergy prevention, including epidemiologic observations and thoughts for future potential allergy prevention.

Index Entries

Allergy prevention; dust mites; environmental exposure; nutrition.

Introduction

There is now an almost universal consensus that the prevalence of allergic diseases has increased considerably in Western industrialized countries, particularly since World War II. The reasons for the increase are unknown. The obvious optimal management of this major public health problem would be primary prevention (i.e., to prevent allergies from ever appearing). Until recently, most of the interest focused on identifying potential risk factors, such as environmental factors that would increase the likelihood for sensitization. Thus, excessive exposure to indoor allergens, particularly house dust mites, tobacco smoke, and poor indoor ventilation, have been suggested as major risk factors, especially when encountered early in life. As a consequence, primary prevention previously focused on reducing exposure to these putative risk factors. However, none of them can more than marginally explain the large regional differences on allergy prevalence, and the results of intervention studies have largely been disappointing.

Over the past few years, novel discoveries related to the maturation of the immune system have resulted in a switch of research interest toward the identification of factors that would protect against the development of manifest allergic disease. An understanding of the development of immune responses to allergens and how this is modified by the environment is a prerequisite for the understanding of why the prevalence of allergy is increasing in developed countries and holds the key for the implementation of primary prevention.

This chapter briefly discusses the development of immune responses to allergens and some aspects of microbial ecology early in life as a background for possible preventive measures in the future. Some results of observational studies and the outcome of prospective intervention studies will be summarized. Finally, some potential future alleys toward allergy prevention will be suggested.

Immunological Background

It is recognized that T-cell function in fetal life is constitutively Th2-based as part of a set of control mechanisms to limit potential damage to the placenta via toxic Th1 cytokines (1). The immune system at birth is not adapted for postnatal life in several aspects, including poor cell-mediated immunity, inability to produce certain immunoglobulin isotypes, poor inflammatory responses, and impaired defenses against intracellular pathogens (2). Thus, the immune system of the newborn baby is characterized by impaired Th1 immune function and a predisposition for type 2 immune responses (summarized in ref. 3). Although the expression of type 2 responses to allergens is the hallmark of allergic diseases and "allergy-type" immunity, such immune responses appear to be normal early in life. A balanced Th1/Th2 immunity is achieved in early childhood. The speed of this process is to some extent genetically determined. But it is probably even more influenced by the environment, notably stimulation by microbial antigens. This is clearly demonstrated by the delayed development of tolerance and other regulatory functions in animals that have been reared under germ-free conditions (4).

Transient, low-level IgE antibody responses to ingested and inhaled allergens are normally encountered during the first years of life and thus seem to be part of the normal maturation process (5). Similarly, low levels of IgE antibodies are more common during infancy in Estonia, a country with a low prevalence of allergic disease, than in Sweden, where childhood allergies are very common (6). Therefore, the differences between allergy-prone and normal infants and between infants in countries with a high vs low prevalence of allergies seem to be a result of faulty downregulation of IgE responses rather than the antibody response in itself. It is also clear from these studies that the mere presence of low levels of IgE antibodies to allergens is neither an indicator of allergy

nor even necessarily associated with subsequently developing disease.

Genetic risk for allergy is associated with delayed postnatal maturation of T-helper cell function (7). Both the cloning frequency of CD4⁺ T cells and the capacity of cloned CD4⁺ T cells to secrete interferon (IFN)- γ and interleukin (IL)-4, were reduced in infants with positive atopic family history, and the reduction was most marked in IFN- γ , indicating an overall Th2 bias in T-cell function in this group (8). Several groups have since reported similar findings in cord blood (9–11). Thus, there is accumulating evidence that the maturation process proceeds at a slower pace than normal in infants with an atopic family history and/or those that develop allergic disease, and that development of tolerance to allergens seems to be more rapid in nonatopic infants.

The first encounters with ubiquitous allergens occur during the first year of life or even before birth. Thus, allergen-specific immune responses may already be detected in fetal blood after 22 wk of gestation (12), and they are common in T cells from cord blood (11,13). However, it is not known whether such immune responses are associated with a risk for allergy or are part of the normal maturation of immunity and tolerance development.

The immune system of the newborn infant is influenced by maternal immunity, both transplacentally and via the breast milk while the baby is breastfed. The mother can thus be regarded as a major “environmental factor” early in life. During the period of breastfeeding, the mother may provide protective factors and immunity modifying components as well as antigenic stimulation. Besides numerous components that help in the protection against infection, human milk contains components that enhance the maturation of the immune system of the newborn infant. Human milk contains numerous immunologic components, including IgA and other antibodies; various chemokines (14); and cytokines, mainly with anti-inflammatory and IgA stimulatory prop-

erties, such as transforming growth factor (TGF)- β , IL-8, and IL-10 (15,16).

It is well established that human milk often contains food antigens that may induce IgE antibody formation. Less is known regarding the immunologic consequences of introducing foreign antigens while the infant is still breastfeeding. As indicated by studies of immunity to infectious agents, it is possible that this represents a mechanism by which immune responses are modulated (17).

There are considerable individual variations in the composition of human milk. Differences of potential significance for the discussion of possible protection against allergy by breastfeeding include total and specific IgA antibody levels (16), cytokines and chemokines (14,15), and polyunsaturated long-chain fatty acids (PUFAs) (18,19). Such differences may explain the controversy regarding the possible allergy-preventive effects of breastfeeding.

Microbial Ecology

The fact that the development of allergy from an immunological point of view seems to be characterized by delayed maturation of immunity and induction of tolerance to allergens has led to a search for factors that would enhance this process. Some of these factors are infections and other microbial pressures, which are known to have longlasting nonspecific systemic effects on the nature of the immune response to unrelated antigens (20). It has been suggested that recurrent infections during early childhood may protect against allergy (21). However, from an immunological point of view, respiratory infections would not be expected to exert such continuous strong pressure on the maturing immune system as to induce immune deviation, and global differences in the prevalence of such infections do not explain the differences in allergy incidence and prevalence.

The microflora of the large gut may have important roles in both human health and dis-

ease. This perspective is by no means a new concept, as Metchnikoff indicated the importance of the host colonic microflora in maintaining health as long as a century ago (22). Rapid advancements have been made, particularly over the past 10 yr, and the bacterial microflora of the human gut is now accepted as an important influence on the host defense system.

The total mucosal surface area of the adult human gastrointestinal tract is up to 300 m², making it the largest body area interacting with the environment (23). It is colonized with over 10¹⁴ microorganisms, weighing over 1 kg and corresponding to more than 10 times the total number of cells in the body. The gastrointestinal tract of the newborn baby is sterile. However, soon after birth numerous types of microorganisms colonize it. Colonization is complete after approx 1 wk, but the numbers and species of bacteria fluctuate markedly during the first 6 mo of life.

It is recognized that interaction with microbes, especially the normal microbial flora of the gastrointestinal tract, is the principal environmental signal for postnatal maturation of T-cell function (in particular the Th1 component) (7). Recognition of these signals is mediated by a series of Toll-like receptors expressed on cells of the innate immune system and other receptors such as CD14; it is noteworthy that a polymorphism in the CD14 gene has recently been associated with high IgE levels (24). Recent international studies have drawn attention to the wide variations in allergy prevalence between different countries (25,26). On the basis of the findings reviewed above, it has been suggested that variations in patterns of microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in allergy prevalence throughout the world (27). Ongoing cohort studies are focusing in detail on this complex issue. These suggestions are supported by observations that germ-free mice do not develop tolerance in the

absence of a gut flora (4) and by the demonstration of differences in the composition of the gut flora between infants living in countries with a high vs low prevalence of allergy (28,29) and between healthy and allergic infants (30–33). Although all the studies confirm such differences, no particular protective or potentially harmful bacterial species can be identified so far. In the two prospective studies (30,33) and one cross-sectional study (32) the presence of bifidobacteria has associated with less allergy, whereas the presence of *Clostridium difficile* has been linked to allergy (30,31).

Epidemiological Observations

Numerous studies have reported an association between exposure to food and inhalant allergens early in life and sensitization to those allergens. As severely allergic school children were often sensitized at an early age, it was assumed that the exposure early in life was indeed a risk factor for the development of allergic disease. In a German prospective observational study enrolling about 1000 newborn infants who were followed for 11 yr, the relevance of different exposures were assessed, such as mite and cat allergen exposure, environmental tobacco smoke (ETS), early infectious diseases, and vaccinations for the development of childhood asthma up to age 10 yr (34). During the first 3 yr of life, intrauterine tobacco and consistent ETS appeared to have an adjuvant effect on allergic sensitization that was transient and restricted to children with a genetic predisposition for allergy. Children sensitized early in life to any allergen were at a significantly increased risk of being asthmatic at 7 yr. However, there was no relationship between allergen exposure early in life and subsequent development of disease. Although sensitization to indoor allergen was clearly related to exposure, there was no direct association between the levels of indoor allergen in the home environment during early infancy and asthma. Thus, this prospective observational

study did not support the hypothesis that exposure to environmental allergens directly causes asthma in childhood but rather suggests that the induction of IgE antibody responses and the development of childhood asthma are determined by independent factors.

The difference between sensitization (i.e., presence of IgE antibodies to allergens) and the appearance of disease is further indicated by a Swedish study, which was the first to suggest that early exposure to pets may in fact protect against allergic asthma later in life (35). In this study, keeping a cat during the first year of life was associated with a threefold reduction in asthma as well as cat sensitivity at 11 yr, but not at 7 yr. Subsequent studies have yielded similar results (36). These studies are consistent with experimental studies showing that post-natal tolerance is enhanced by high antigen exposure (7).

The conclusion from these studies is that although allergen exposure early in life is associated with temporary IgE antibody formation, it seems to play a marginal role for the appearance of allergic disease.

Intervention Studies

Mothers

The observation that IgE antibodies to foods may occasionally be present in cord blood (37) raised the hypothesis that modifying the exposure to potent food allergens during fetal life could prevent sensitization and subsequent allergic disease. In one study, mothers abstained from eating any cow milk (CM) or eggs during the last trimester of pregnancy (38,39), whereas in another study, the potential of tolerance induction was explored by giving the mothers either one hen egg and about 1 L of cow milk during the last 3 mo of pregnancy (40). The former study comprised 209 mothers who were randomized to either a strict elimination diet or no intervention. The children were followed up at 18 mo and 5 yr. The prevalence of allergic disease could be evaluated in

198 children (95%). Allergic disease was monitored with questionnaires, skin-prick tests (SPTs), serum-IgE determinations, and physical examination. Eczema, allergic rhinoconjunctivitis, and asthma were equally common in groups at both ages. However, persistent food intolerance to egg at 5 yr was significantly more common in children of mothers who had been on the elimination diet, thus suggesting delayed development of tolerance in unexposed children (38).

In the second study, 162 women with respiratory allergy to animal dander and/or pollens were randomly allocated to a diet consisting of either a very low ingestion of hen eggs and CM or a daily ingestion of one hen egg and about 1 L of CM during the last 3 mo of pregnancy (40). At 18 mo, the distribution of atopic disease was similar both in infants of mothers on a restricted diet and those with high intake of the two foods.

It has been known for many years that antigens in foods eaten by lactating mothers are transferred into the breast milk and may sensitize or elicit symptoms in allergic infants. Therefore, it appeared logical to evaluate the possible allergy preventive effect of an elimination diet to breastfeeding mothers of infants with a family history of allergy. This was done in a prospective study comprising 115 mothers and infants (41). The 65 mothers in the intervention group excluded all CM, egg, fish, and nuts from their diet during the first 3 mo of lactation, and 50 mothers maintained an unrestricted diet. The diet of the infants was similar in both groups (i.e., CM was not supplied until age 6 mo and eggs and fish not until age 9 mo). The incidence of atopic dermatitis was significantly lower in the maternal diet group during the first 6 mo postpartum (11 and 28%, respectively) but not after that age. Other atopic/allergic manifestations did not differ and the number of positive SPTs to egg white, CM, or fish at age 9 mo was similar in both groups. Therefore, sensitization was only avoided as long as the infants were exclusively breast-fed

by mothers adhering to the diet. The children were subject to subsequent follow-ups, the last one performed at 10 yr (42). Sensitization to the three foods avoided by the mothers during lactation as well as sensitization to inhaled allergens were similar in the two groups, nor were there any differences with regard to clinical allergy. The authors concluded that no general recommendations should be given to implement prophylactic maternal dietary avoidance during lactation in allergy-prone families.

Observational studies have linked house dust mite (HDM) exposure and dietary fatty acid intake with asthma in childhood. The possible allergy preventive effects of these observations were explored in a study comprising 616 pregnant women who were randomized to an HDM-avoidance intervention, comprising the use of impermeable mattress covers and an acaricide or control and the use of an oil supplement, margarines, and cooking oils containing high levels of omega-3 fatty acids or control (43). Atopic status was measured by SPTs. Symptoms, diagnoses, and medication histories were elicited by means of parental interviews. The diet intervention resulted in a 10% absolute reduction (95% confidence interval [CI], 1.5–18.1; $p = 0.02$) in the prevalence of any wheeze and an 8% absolute reduction (95% CI, 0.5–15.1) asthma. The HDM-avoidance intervention did not affect these outcomes but was associated with a lower use of oral steroids. Thus, the study indicates that dietary omega-3 fatty acids might have a beneficial effect on the prevalence of wheeze during the first 18 mo of life.

Postnatal Diet

Because CM and eggs are the most common allergens associated with allergic disease in infancy strict avoidance has been tried in allergy prevention. As indicated in the previous section, maternal diet during breastfeeding temporarily delayed sensitization to the two allergens and reduced the incidence of infantile dermatitis (41). In a Danish study, infants

with a family history of allergy and elevated cord blood IgE were randomized to one of three blinded formulas when breastfeeding was insufficient (44). In addition, CM products and solid foods were avoided until age 4 mo. Food allergy was confirmed by controlled elimination/challenge procedures. The cumulative incidence of parental-reported CM allergy was significantly higher in children who were fed a partially hydrolyzed formula than in either of two extensively hydrolyzed products at 18 mo (7 vs 3 and 0%). The cumulative incidence of confirmed CM allergy was 1% (3 of 232) in exclusively breast-fed infants and in infants fed extensively hydrolyzed formula (1 of 161), compared to 5% (5 of 85) in infants who were fed partially hydrolyzed formula.

The allergy preventive effect of extensively and partially hydrolyzed CM formulas compared with a regular formula was assessed in a Swedish study, comprising 155 infants with a family history of allergy (45). No CM was given during the first 9 mo of life, and no egg and fish were given up to age 12 mo. Breastfeeding mothers avoided the same foods. At weaning, the infants were randomized to one of the formula groups. There were less cumulative atopic symptoms at 18 mo in the infants receiving extensively hydrolyzed formula compared to infants receiving regular or partially hydrolyzed formula; significantly fewer infants in the former group had a positive SPT to eggs at 9 mo. The findings support a temporary allergy preventive effect of an extensively hydrolyzed formula.

There are ongoing studies in which probiotic lactobacilli are given to the mothers during the last part of pregnancy and then to the newborn babies. The studies are based on observations of differences in the composition of the gut flora between allergic and nonallergic infants (30,31,33) and with the underlying hypothesis that such treatment would modify the neonatal gut flora and through this also affect the development of tolerance. To date, results have been published only from one of

the studies (46,47). This study originally comprised 132 infants with a family history of allergy. Lactobacilli or placebo were given to pregnant women, starting 2–4 wk before delivery and during the lactation period or to the babies if they were not breastfed. The authors reported a 50% reduction in the incidence of atopic eczema during the first 2 yr of life (i.e., 23 vs 46% in the placebo group) (46). At 4 yr, there was still a lower cumulative incidence of eczema (26 vs 46%) (47). However, there was no difference between the groups regarding respiratory symptoms, nor was there specific or total IgE antibody levels and SPT results.

Postnatal Environment

With strenuous avoidance measures, it is possible to decrease and maintain very low levels of HDMs, at least in countries with a temperate climate (48). However, it is much more difficult, or even not feasible, to eradicate cat allergen from the home (49,50). Avoidance measures put a considerable demand on the families. Clearly, they should be worthwhile and not only reduce allergen levels and incidence of sensitization but also reduce the incidence of disease. The efficacy of avoidance measures has been attempted in several continuing prospective intervention studies.

In a randomized controlled study, a birth cohort of 120 infants with a family history of allergy has been followed since 1990. Allergen-avoidance measures were instituted from birth in the prophylactic group. They included a low-allergen diet to the mothers if breastfeeding and alternatively an extensively hydrolyzed formula to the babies. In addition, exposure to HDM was reduced by the use of an acaricide and mattress covers. The control group followed standard advice as normally given by the health visitors. After 1 yr, the prophylactic group reported less total wheezing and eczema. At 2 yr, the reduction in asthma was no longer statistically significant, although a positive SPT was less common in the prophylactic group. At 4 yr, the control group continued to show more

total allergy, and positive SPTs were more common (51). At 8 yr, the prophylactic group was found to be at a reduced risk for current wheeze and sensitization to HDM (52). The authors suggested that the preventive measures might prevent some cases of childhood asthma. However, it should be pointed out that the study groups were small and the results should be confirmed in a larger study. Furthermore, the results cannot readily be extrapolated to other environments.

In 1996, the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study was initiated (53). Children born to allergic mothers were enrolled in a double-blind placebo-controlled trial for evaluating the use of mite-impermeable mattress and pillow covers. Homes were visited before birth, 3 mo after birth, and 12 mo after birth for the collection of dust samples from floors and mattresses. A medical investigation and home visit at age 4 yr are nearing completion. Preliminary results show that mite-allergen levels were lower than in previous Dutch studies and that the intervention measure had a significant effect on mite-allergen levels, without important clinical benefits up to age 2 yr.

A multicenter European, population-based, randomized, controlled study of children at increased atopic risk included 696 newborns (54). Inclusion criteria were a positive history of parental allergy and a positive SPT or specific IgE against at least one out of a panel of common aeroallergens in one or both parents. At age 1 yr, the overall sensitization rate was 6 vs 11% in the control group. The prevalence of sensitization against Der p was 2% in the prophylactic group vs 5% in the control group. Thus, the sensitization rate could be decreased through the use of impermeable mattress encasings and the implementation of easy-to-perform preventive measures. However, there are no data yet on clinical outcomes from this study.

In a randomized intervention British study, a cohort of 1000 children with a family history

of atopy was followed prospectively (55). The intervention group commenced complex avoidance measures from the second trimester of pregnancy, and it was demonstrated that low level of Der p 1 could be achieved and maintained over a long period of time. At 1 yr, the prevalence of sensitization to HDM was low in all groups. Infants in the intervention group, however, had fewer respiratory symptoms in the first year of life, but the authors pointed out that these findings tell us very little about the possible effect of intervention on asthma.

Potential Allergy Prevention in the Future

Future allergy prevention may focus on tolerance induction rather than allergen avoidance and should be based on better knowledge about the normal maturation of immunity. Potentially, both specific and nonspecific routes could be considered for allergy prevention. Specific prevention is based on the observations that, as discussed in previous sections, atopic sensitization to inhalants is associated with development of Th2-associated memory as opposed to weak Th1-like memory in "tolerant" nonatopics and that sensitization to inhalants occurs as a result of failure of normal mucosal tolerance processes, which are initiated during early exposures. Animal studies indicate that the efficiency of this process is directly related to the intensity of antigen stimulation, inferring that protection against sensitization via mucosal tolerance can potentially be enhanced therapeutically (27). Indirect support for this comes from data suggesting that intense cat exposure induces split tolerance involving IgE-selective suppression with concomitant preservation of IgG (56). Based on the experimental and clinical observations (5), it has been suggested that administration of high doses of inhalant allergens early in life would drive the immune system toward tolerance rather than sensitization (57–59).

Nonspecific allergy prevention early in life is based on the possible immune modulating

effects of the intestinal flora. As summarized above, such studies are ongoing and the results so far are encouraging. However, more studies are needed before this attractive form for allergy prevention should be considered as a routine procedure.

Comments

Some of the current confusion regarding the effects of allergen exposure early in life seems to stem from a misconception and the confusion between "sensitization" and "disease." On one hand, as discussed earlier, there is ample evidence that high levels of exposure to indoor allergens is associated with an increased incidence of sensitization early in life. On the other hand, numerous studies over the years have clearly shown that severely allergic and asthmatic children often demonstrated sensitization at an early stage. These two firmly established observations led to the conclusion that high exposure to allergens during the first years of life actually would cause allergic disease and, as a consequence, that reduced exposure to allergen would prevent disease. However, as discussed earlier, the assumed relationship between exposure of healthy infants early in life and subsequent disease (rather than merely demonstrating the presence of IgE antibodies) has not been confirmed, nor have any of the intervention studies summarized above given any experimental support for the assumption.

Few authors suggesting various allergy preventive measures have discussed the potential ethical issues arising in connection with giving advice, which might interfere with the lives of families subject to the recommendations. One could argue that advice in medical issues should be based solidly on research, as is the case with pharmacotherapy, for example. There is also confusion between primary and secondary prevention especially among the general public. Secondary prevention refers to the obvious need to reduce exposure to relevant allergens for those individuals who

manifest clinical disease triggered by allergen exposure. In these cases, allergen avoidance is part of the treatment, similar to the avoidance of certain foods, which is part of the treatment of, for example, diabetes and celiac disease. This should not be confused with primary prevention, which refers to intending to prevent the onset of allergic disease.

The conclusion from the various efforts to prevent allergy is that although it is possible to reduce the incidence of cow milk allergy in infancy by avoiding exposure, the total incidence of allergy is only marginally affected, even by strenuous efforts to modify the home environment and diet. Ethical concerns can be raised against giving advice with such low efficacy as documented until now. Future efforts toward allergy prevention may potentially be based on various means to enhance induction of tolerance toward allergens, rather than by avoidance regimens.

References

- Wegmann, T.G., Lin, H., Guilbert, L., et al. (1993), *Immunol Today* **14**, 353–356.
- Björkstén, B. (1999), *J Allergy Clin Immunol* **104**, 1119–1127.
- Prescott, S.L. (2003), *Curr Opin Allergy Clin Immunol* **3**, 125–132.
- Sudo, N., Sawamura, S., Tanaka, K., et al. (1997), *J Immunol* **159**, 1739–1745.
- Hattevig, G., Kjellman, B., and Björkstén, B. (1993), *Pediatr Allergy Immunol* **4**, 182–186b.
- Julge, K., Vasar, M., and Björkstén, B. (2001), *Clin Exp Allergy* **31**, 1854–1861.
- Holt, P.G., Sly, P.D., and Björkstén, B. (1997), *Pediatr Allergy Immunol* **8**, 53–58.
- Holt, P.G., Clough, J.B., Holt, B.J., et al. (1992), *Clin Exp Allergy* **22**, 1093–1099.
- Martinez, F.D., Stern, D.A., Wright, A.L., et al. (1995), *J Allergy Clin Immunol* **96**, 652–660.
- Tang, M.L., Kemp, A.S., Thorburn, J., et al. (1994), *Lancet* **344**, 983–985.
- Prescott, S.L., Macaubas, C., Smallacombe, T., et al. (1999), *Lancet* **353**, 196–200.
- Jones, A.C., Miles, E.A., Warner, J.O., et al. (1996), *Pediatr Allergy Immunol* **7**, 109–116.
- Warner, J.A., Jones, A.C., Miles, E.A., et al. *Allergy* **51**, 447–451.
- Böttcher, M.F., Jenmalm, M.C., Björkstén, B., et al. (2000), *Pediatr Res* **47**, 592–597.
- Böttcher, M.F., Jenmalm, M.C., Garofalo, R.P., et al. (2000), *Pediatr Res* **47**, 157–162.
- Böttcher, M.F., Jenmalm, M.C., and Björkstén, B. (2003), *Pediatr Allergy Immunol* **14**, 35–41.
- Hanson, L.A., Silfverdal, S.A., Korotkova, M., et al. (2002), *Adv Exp Med Biol* **503**, 99–106.
- Duchén, K. and Björkstén, B. (2001), *Lipids* **36**, 1033–1042.
- Duchen, K., Casas, R., Fageras-Bottcher, M., et al. (2000), *Pediatr Allergy Immunol* **11**, 29–39.
- Rook, G.A. and Stanford, J.L. (1998), *Immunol Today* **19**, 113–116.
- Gerrard, J.W., Geddes, C.A., Reggin, P.L., et al. (1976), *Ann Allergy* **37**, 91–100.
- Metchnikoff, E. *The Prolongation of Life*. London: William Heinemann, 1907.
- Midtvedt, T. (1985), *Microecol Ther* **15**, 295–302.
- Baldini, M., Lohman, I., Halonen, M., et al. (1999), *Am J Resp Cell Mol Biol* **20**, 976–983.
- (1996), Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* **9**, 687–695.
- (1998), Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* **351**, 1225–1232.
- Holt, P.G. and Macaubas, C. (1997), *Curr Opin Immunol* **9**, 782–787.
- Sepp, E., Naaber, P., Voor, T., et al. (2000), *Microbiol Ecol Health Dis* **12**, 22–26.
- Sepp, E., Julge, K., Vasar, M., et al. (1997), *Acta Paediatr* **86**, 956–961.
- Kalliomäki, M., Kirjavainen, P., Eerola, E., et al. (2001), *J Allergy Clin Immunol* **107**, 129–134.
- Böttcher, M.F., Nordin, E.K., Sandin, A., et al. (2000), *Clin Exp Allergy* **30**, 1590–1596.
- Björkstén, B., Naaber, P., Sepp, E., et al. (1999), *Clin Exp Allergy* **29**, 342–346.
- Björkstén, B., Sepp, E., Julge, K., et al. (2001), *J Allergy Clin Immunol* **108**, 516–520.
- Lau, S., Nickel, R., Niggemann, B., et al. (2002), *Paediatr Respir Rev* **3**, 265–272.
- Hesselmar, B., Aberg, N., Aberg, B., et al. (1999), *Clin Exp Allergy* **29**, 611–617.
- Ownby, D.R., Johnson, C.C., and Peterson, E.L. (2002), *JAMA* **288**, 963–972.
- Croner, S., Kjellman, N.I., Eriksson, B., et al. (1982), *Arch Dis Child* **57**, 364–368.
- Fälth-Magnusson, K. and Kjellman, N.I.M. (1992), *J Allergy Clin Immunol* **89**, 709–713.
- Fälth-Magnusson, K. and Kjellman, N.I.M. (1987), *J Allergy Clin Immunol* **80**, 868–875.
- Lilja, G., Dannaeus, A., Foucard, T., et al. (1989), *Clin Exp Allergy* **19**, 473–479.

41. Hattevig, G., Kjellman, B., Sigurs, N., et al. (1989), *Clin Exp Allergy* **19**, 27–32.
42. Hattevig, G., Sigurs, N., and Kjellman, B. (1999), *Acta Paediatr* **88**, 7–12.
43. Mührshahi, S., Peat, J.K., Marks, G.B., et al. (2003), *J Allergy Clin Immunol* **111**, 162–168.
44. Halken, S., Hansen, K.S., Jacobsen, H.P., et al. (2000), *Pediatr Allergy Immunol* **11**, 149–161.
45. Oldaeus, G., Anjou, K., Björkstén, B., et al. (1997), *Arch Dis Child* **77**, 4–10.
46. Kalliomäki, M., Salminen, S., Arvilommi, H., et al. (2001), *Lancet* **357**, 1076–1079.
47. Kalliomäki, M., Salminen, S., Poussa, T., et al. (2003), *Lancet* **361**, 1869–1871.
48. Custovic, A., Simpson, B.M., Simpson, A., et al. (2001), *Lancet* **358**, 188–193.
49. Custovic, A., Hallam, C.L., Simpson, B.M., et al. (2001), *J Allergy Clin Immunol* **108**, 537–539.
50. Munir, A.K., Björkstén, B., Einarsson, R., et al. (1995), *Allergy* **50**, 55–64.
51. Hide, D.W., Matthews, S., Tariq, S., et al. (1996), *Allergy* **51**, 89–93.
52. Arshad, S.H., Bateman, B., and Matthews, S.M. (2003), *Thorax* **58**, 489–493.
53. Koopman, L.P., van Strien, R.T., Kerkhof, M., et al. (2002), *Am J Respir Crit Care Med* **166**, 307–313.
54. Halmerbauer, G., Gartner, C., Schierl, M., et al. (2003), *Pediatr Allergy Immunol* **14**, 10–17.
55. Gore, C. and Custovic, A. (2003), *Paediatr Respir Rev* **4**, 213–224.
56. Platts-Mills, T., Vaughan, J., Squillace, S., et al. (2001), *Lancet* **357**, 752–756.
57. Holt, P.G. (1994), *Lancet* **344**, 456–458.
58. Holt, P.G., Macaubas, C., and Sly, P.D. (1998), *Allergy* **53**, 72–76.
59. Holt, P.G. and Sly, P.D. (2000), *Thorax* **55**, 700–703.